Biodegradation of DBNPA

General

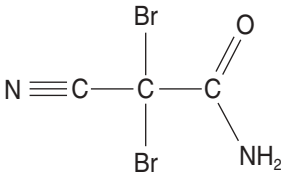
The biodegradation of 2,2-dibromo-3-nitriloproplonamide (DBNPA), the active ingredient in Antimicrobial 7287, Antimicrobial 8536, DBNPA 100 Powder, DBNPA 100 PTECH, and Dow Time-Release Antimicrobial CT, was evaluated in an OECD ready biodegradation test. The test procedure, "CO_2 Evolution Test" OECD Method 301B, was modified to accommodate the particular testing needs for an antimicrobial compound. The use of radiolabeled DBNPA (uniform ^{14}C-labeling) and sensitive radioanalytical techniques permitted low test concentrations of DBNPA to be run to avoid potential inhibitory effects common to the biodegradation testing of antimicrobial compounds.

Extensive mineralization of 0.06 mg/L [^{14}C]DBNPA to $^{14}CO_2$ was observed, reaching 78% of the theoretical $^{14}CO_2$ yield after 28 days. Mineralization at a 10-fold higher concentration (0.6 mg/L) reached only 11% after 28 days, suggesting some inhibition of biodegradation at the higher concentration. This slower degradation was consistent with the antimicrobial activity of DBNPA. Though the strict criteria of a ready biodegradation classification were not totally met, the results of this study demonstrate that DBNPA will rapidly biodegrade at environmentally realistic concentrations.

Experimental Procedure

Test Compound

The test compound was uniformly labeled with carbon-14. The radiochemical purity was greater than 98%.



Modified "CO_2 Evolution Test," OECD Method 301B

Test mixtures containing two concentrations of [^{14}C]DBNPA, 0.06 and 0.6 mg/L, were prepared in one-liter shake flasks. in addition, a killed control (chemical sterilization with formaldehyde) was included to confirm that any degradation observed was biologically mediated. The microbial inoculum was obtained from a municipal wastewater treatment plant and added to a mineral medium at the concentration specified in the test procedure 30 mg/L suspended solids). The flasks were sealed with rubber stoppers fitted with CO_2 traps containing sodium hydroxide. The test mixtures were incubated in the dark on a gyrator shaker for 28 days at 22 ± 1°C.

Test mixtures were analyzed on days 0 and 28 to measure the total amount of radioactivity present. The CO_2 traps were routinely replaced with fresh traps and analyzed to determine the amount of $^{14}CO_2$ produced in the test mixtures.

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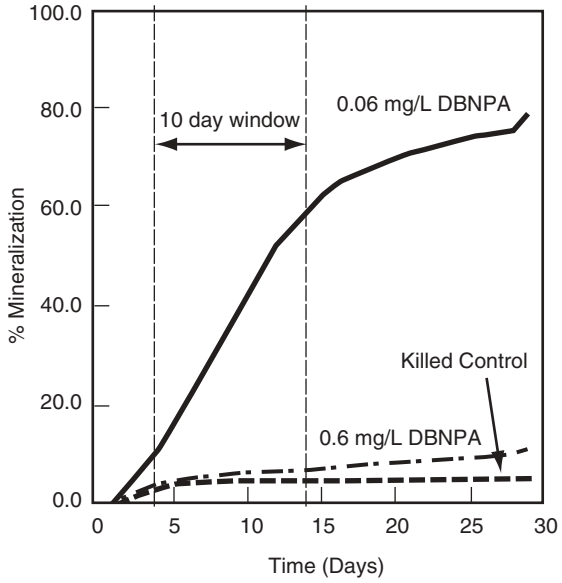
Results

Extensive mineralization of [^{14}C]DBNPA to $^{14}CO_2$ was observed at the low test concentration (0.06 mg/L DBNPA), reaching 78% of the theoretical $^{14}CO_2$ yield after 28 days (see Figure 1). For a compound to be classified as "ready biodegradable," 60% mineralization to CO_2 must be achieved within 10 days of the start of biodegradation. DBNPA did not meet this criteria, although approximately 58% mineralization of DBNPA occurred during this "10-day window." Mineralization of [^{14}C]DBNPA at 0.6 mg/L reached only 11% after 28 days, suggesting some inhibition of biodegradation at the higher concentration. Little $^{14}CO_2$ was measured in the killed controls prepared with 2% formalin (5% mineralization), indicating that most of the mineralization of [^{14}C]DBNPA at the lower test concentration was biologically mediated.

Figure 1. Mineralization of [^{14}C]DBNPA to $^{14}CO_2$ in a Modified OECD Ready Biodegradability Test



At the conclusion of the study, 84 to 98% of the radioactivity added to the test mixtures as [^{14}C]DBNPA was recovered. Radioactivity incorporated into $^{14}CO_2$ accounted for 11 to 78% of the radioactivity added to viable test mixtures. The balance of the radioactivity remained in solution or was associated with biomass.

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