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Assessment of the Ready Biodegradability of Bisphenol A

R. J. West, P. A. Goodwin, G. M. Klecka

Toxicology and Environmental Research and Consulting, The Dow Chemical Company, 1803 Building, Midland, MI 48674, USA

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Many papers have been published in recent years which have evaluated, in the laboratory, the potential for Bisphenol A to impact the endocrine systems of a variety of environmental species. Bisphenol A (BPA, CAS #00080-05-7) is a widely used chemical intermediate and is produced globally in quantities exceeding 640,000 metric tons per year. Accordingly, the environmental fate, effects, and exposures of BPA have recently been reviewed (Staples et al 1998). This review concluded, based on the physical properties and predominantly captive manufacture/use patterns for this chemical, that releases to the environment are most likely to occur through permitted discharges of treated industrial wastewaters. Therefore, the potential for exposure to BPA in the environment will be influenced largely by its fate and lifetime in aquatic environments, such as in wastewater treatment operations and associated receiving waters.

Of all processes effecting the environmental fate and lifetime of BPA in the aquatic environment, biodegradation is expected to be the most important. It can be difficult and costly to measure the rates of chemical biodegradation in actual or simulated environmental matrices. However, laboratory tests of "ready biodegradability", such as those described by OECD, have been used as conservative surrogates to indicate the propensity for a chemical to be biodegraded in the aquatic environment. The OECD defines these tests as "stringent tests which provide limited opportunity for biodegradation and acclimatization to occur. It may be assumed that a chemical giving a positive result in a test of this type will rapidly biodegrade in the environment and, therefore, may be classified as readily biodegradable" (OECD 1996).

MATERIALS AND METHODS

The ready biodegradability of BPA was evaluated using the Manometric Respirometry procedure described by OECD Test Guideline No. 301 F (OECD 1996). The performance of BPA in this test was examined in two iterations of the test, using microbial inocula collected from the same source on two separate days. An initial test was conducted to evaluate the suitability of the respirometric apparatus, test chemical concentrations, and incubation conditions for performing the test in accordance with the OECD guideline. A subsequent test was conducted, based on results of the initial test. Briefly, both iterations of the test

Correspondence to: R. J. West

procedure used reaction mixtures (500 mL) composed of a dilute activated sludge inoculum (30 mg/L suspended solids) in a defined mineral medium. BPA was added to duplicate reaction mixtures as the sole added organic carbon source, at nominal concentrations of 7 and 25 mg/L. The 25 mg/L BPA concentration was selected to provide a theoretical oxygen demand (ThOD) which fell within the required range specified in the OECD test guideline. The 7 mg/L concentration was selected to evaluate biodegradation of BPA at the lowest concentration at which the respirometer could distinguish biodegradation of the test chemical from background respiration. The biodegradation reactions were incubated in darkness in a circulated air incubator, and continuously stirred at 200 rpm.

The microbial inoculum consisted of activated sludge mixed liquor collected from the City of Midland Wastewater Treatment Plant (Midland, Michigan, USA) on May 28 and August 25, 1997. This facility treats approximately 1.1 x 10^4 cubic meters (3.0 x 10^6 gal) of wastewater daily, of which > 90% is from domestic sources. The inocula were collected one day prior to initiation of the tests, and continuously aerated in the laboratory to allow minimization of residual biological oxygen demand (BOD).

The test chemical, a white crystalline solid, was provided by The Dow Chemical Company, Freeport Texas, and was identified as lot # 95112111C-705. The purity of this material was determined to be 99.7% by HPLC, and structural identity was confirmed by infrared spectroscopy. To aid solubilization in the reaction mixtures, the material was ground to a uniform fine powder prior to use. Sodium benzoate (99% pure, Aldrich Chemical), was used as a biodegradability reference material. This material was added to the reaction mixtures as a concentrated aqueous solution to give 100 mg/L as benzoate.

Using these materials, the following series of reaction mixtures was prepared:

1) Inoculum Blanks (2)- inoculated mineral medium (MM) only
2) Test Chemical (2 each)- 7 or 25 mg/L BPA + MM
3) Reference Chemical (2)- 100 mg/L benzoate + MM
4) Tox Controls (1 each)- 100 mg/L benzoate + 7 or 25 mg/L BPA + MM
5) Killed Controls (1 each)- 7 or 25 mg/L BPA + MM + 2% (wt.) formaldehyde

Immediately after preparation of the reaction mixtures, the reaction vessels (1L, Pyrex glass) were connected to a Columbus Instruments Micro-Oxymax™ respirometer system (Columbus, Ohio, USA). This instrument measured oxygen and CO_2 concentrations in the headspace of each reaction vessel at 4-hour intervals over the 28-day test period. The average percent biodegradation and mineralization of BPA were determined from the net cumulative oxygen consumption and CO_2 evolution (respectively) in duplicate reaction mixtures containing the test chemical. The net cumulative oxygen consumption and CO_2 evolution in these reaction mixtures were determined by subtracting the average cumulative oxygen consumption and CO_2 evolution determined in duplicate blank reaction mixtures containing only the inoculated mineral medium.

Dissolved organic carbon (DOC) measurements were performed on the biodegradation reaction mixtures at the time of test initiation and at day 28 to confirm removal of the added BPA. DOC concentrations in filtered samples (Whatman 13 mm, 0.7μm GF/F) were determined using a Shimadzu model TOC-5000 carbon analyzer. The filtration procedure was demonstrated to provide complete recovery of dissolved BPA in the filtrate, without introduction of measurable foreign DOC.

RESULTS AND DISCUSSION

The OECD specifies several criteria for demonstrating the validity of results from its tests of ready biodegradability. These criteria are based on parameters such as inoculum viability, precision among replicate reaction mixtures, maintenance of reaction mixture pH, and maintenance of incubation temperatures (OECD 1996). The results of the preliminary suitability test indicated that the microbial inoculum, respirometric analysis parameters, and selected BPA concentrations were suitable for determining the ready biodegradability of BPA according to the OECD 301 F test guideline. However, the average recorded reaction mixture temperature during the initial test (27.1 ± 0.6 °C, ± 1 SD) indicated that refinement of temperature control in the circulated air incubator was necessary to maintain the reaction mixture temperature at the required 22 ± 1 °C. Using improved temperature control measures, the reaction mixture incubation temperature averaged 22.5 ± 0.2 °C (± 1 SD) over the 28-day duration of the subsequent test. Hereafter, the initial test conducted at 27 °C is referred to as "Test I" and the subsequent test conducted at 22 °C is referred to as "Test II".

Both iterations of the manometric respirometry test showed rapid and extensive biodegradation of BPA at concentrations of 7 mg/L and 25 mg/L. The time required to achieve 10% biodegradation (i.e. lag time) ranged from 4.7 to 6.1 days for both concentrations of BPA in both iterations of the test. Only 2.5 to 5.0 additional days were required to reach the required 60% ready biodegradability criterion (Table 1). As shown in Figure 1, the rate and extent of BPA biodegradation in Test II, based on net oxygen consumption, were sufficient to classify this material as "readily biodegradable" according to the current OECD guidelines. The slightly elevated incubation temperature of Test I (27 °C) resulted in little or no enhancement in the rate or extent of BPA biodegradation relative to that observed in Test II at 22 °C (Table 2). Therefore, the results of both Test I and Test II can be considered as independent tests confirming the ready biodegradability of this material. Reaction mixtures containing 7 mg/L BPA showed slight declines in net cumulative oxygen consumption and CO_2 production with extended incubation to 28 days (Fig. 1A). This apparent decrease in net biodegradation can be attributed to subtraction of endogenous respiration in the inoculum blanks which was of slightly greater magnitude than endogenous respiration in the test chemical reactions after complete utilization of the added BPA. Killed control reactions prepared with 2% formalin showed no

net oxygen consumption or CO_2 production, thereby attributing the net consumption and production of these gases solely to biodegradation of BPA.

The extent of BPA biodegradation determined from net oxygen consumption was confirmed by measurement of similar extents in mineralization and DOC removal (Table 2). The close agreement in percent biodegradation by these three metrics confirms that BPA was not merely partially degraded, but completely mineralized in the ready biodegradability test.

The ready biodegradability of BPA has been investigated previously using various test procedures. Our present investigation was deemed necessary in light of conflicting results among previous tests, and the use of procedures in previous tests which did not meet regulatory requirements for classification of ready biodegradability. For example, Stone and Watkinson (1983) showed that biodegradation of BPA in both the Closed Bottle Test (OECD 301 D) and Modified Sturm Test (OECD 301 B) was insufficient to meet ready biodegradability criteria. BPA was later shown by Mobil Corporation (1993) to meet biodegradation levels sufficient for classification as readily biodegradable, using an EPA test procedure similar to the OECD 301B: Modified Sturm Test. Mineralization of BPA was shown to exceed 60% of the theoretical CO_2 yield within a 10-day window, and reached 83.6% after 28 days. However, since their test procedure was not conducted in strict accordance with the OECD Principles for Good Laboratory Practice guidelines, the results of the Mobil test could not be used for regulatory classification of BPA as "readily biodegradable". The results presented here using the OECD 301 F test procedure are in good agreement with the Mobil Corp. study, and bring final resolution to the issue of BPA ready biodegradability. The test results reported here were generated in accordance with the OECD Principles of Good Laboratory Practice guidelines (OECD 1996), and followed the most current OECD guidelines for testing of ready biodegradability. In addition, our second iteration of the 301 F test procedure fully met the criteria for "validity of results" and "ready biodegradability" as currently defined in the OECD guidelines. The results of each independent iteration of the 301 F test, which included supplemental measurements of extensive mineralization and DOC removal, clearly demonstrate the ready biodegradability of BPA.

Tests of ready biodegradability are not intended to provide estimates of actual chemical biodegradation rates or half-lives in aquatic environments. However, the stringent nature of these tests allows their results to be used as a conservative predictor of chemical persistence. By OECD definition, a chemical which is classified as "readily biodegradable" may be assumed to rapidly biodegrade in the environment. In the case of BPA, its classification as a "readily biodegradable" compound does indeed correspond with rapid biodegradation in samples from actual aquatic environments. Dorn et al (1987) examined biodegradation of 3 mg/L BPA in laboratory reaction mixtures containing water from a treated industrial wastewater outfall, its associated receiving stream, and a downstream

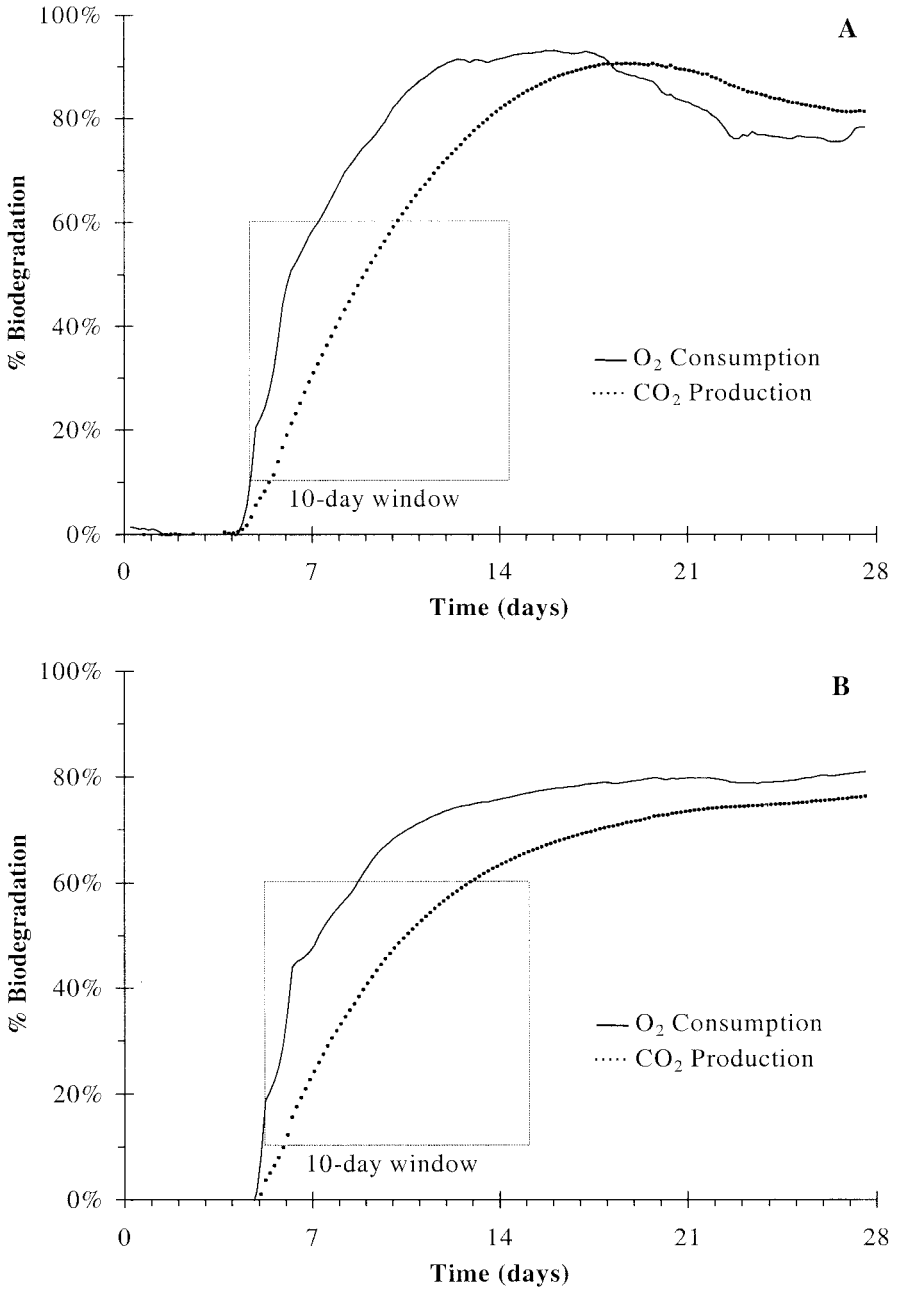

Figure 1. Biodegradation of 7 mg/L (A) and 25 mg/L (B) BPA using the OECD 301 F: Manometric respirometry procedure (Test II, 22 °C). Plots show the average percent biodegradation based on net oxygen consumption (solid line) and net CO_2 production (dotted line) for duplicate reaction mixtures.

Table 1. Summary of BPA biodegradation based on O_2 consumption in the OECD 301 F test. (*Test I at 27 °C, Test II at 22 °C*)

Reaction Mixtures	Time to achieve (days) 10% Biodeg.	60% Biodeg.	% Biodegradation at 10-day window	Day 28
Test Chemical:				
7 mg/L BPA	5.6, 4.7	9.1, 7.2	77.6, 92.3	74.7, 78.2
25 mg/L BPA	6.1, 5.2	11.1, 8.7	73.7, 77.1	81.4, 81.0
Reference Chemical:				
100 mg/L Benzoate	0.6, 0.9	2.1, 2.5	94.3, 93.3	96.8, 98.3
Toxicity Controls:				
Benzoate + 7 mg/L BPA	0.6, 0.8	2.3, 2.6	91.6, 85.6	96.8, 95.0
Benzoate + 25 mg/L BPA	0.6, 0.8	6.1, 5.1	82.8, 67.9	93.1, 92.2

Table 2. Comparison of BPA biodegradation based on O_2 consumption, mineralization, and DOC removal in the OECD 301 F test. (*Test I at 27 °C , Test II at 22 °C*)

Reaction Mixtures	Ave. % Biodegradation after 28 days based on Net O_2 Consumption	Net CO_2 Production	DOC Removal
Test Chemical:			
7 mg/L BPA	74.7, 78.2	76.2, 81.2	79.3, 77.1
25 mg/L BPA	81.4, 81.0	75.9, 76.3	81.2, 93.5
Reference Chemical:			
100 mg/L Benzoate	96.8, 98.3	75.8, 77.0	97.1, 99.5
Toxicity Controls:			
Benzoate + 7 mg/L BPA	96.8, 95.0	76.5, 77.0	99.5, 99.4
Benzoate + 25 mg/L BPA	93.1, 92.2	73.0, 74.8	97.4, 95.8

shipping channel. They determined biodegradation half-lives for BPA of 3.0, 2.5, and 4.0 days in the effluent, receiving stream, and downstream channel, respectively. More recently, the respirometric test procedure described here has been adapted for measuring biodegradation and mineralization of BPA (5 mg/L) in numerous surface water samples collected from around the world (Klecka et al. *unpublished data*). The half-life for BPA biodegradation in seven different freshwater and estuarine water samples averaged (± 1SD) 1.2 ± 0.2 days, with an average biodegradation lag time of 3.4 ± 0.7 days. The respirometric determination of surface water biodegradation kinetics at 5 mg/L BPA was validated using parallel biodegradation studies containing radiolabeled [^{14}C]-BPA. In these radiolabeled studies, similar lag times and half-lives were observed for BPA biodegradation at initial concentrations ranging from 5.0 x 10^{-5}

mg/L to 5.5 mg/L. The ready biodegradability test results presented here, in combination with measured rapid biodegradation in samples from actual aquatic environments, provide a clear weight of evidence that BPA does not persist when introduced to the aquatic environment.

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