

# Photo-enhanced Biodegradation of a Test Substance using Artificial Sunlight with a Ready Biodegradation Test Design.

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## Abstract

An experiment has been conducted to measure the influence of sunlight on the ultimate biodegradation (i.e., conversion to CO<sub>2</sub>) on a test substance that has not previously been known to be readily biodegradable. This experiment is meant to evaluate the ultimate biodegradation potential of a non-readily biodegradable test substance in the natural aqueous environment that is also exposed to sunlight. This situation would be applicable to pharmaceuticals entering receiving streams from waste water treatment facilities and to pesticides entering surface water due to run-off. Current testing guidelines tend to evaluate photo-degradation and biodegradation separately. The study is comprised of two steps: the first step is to expose the test substance to artificial sunlight under sterile conditions in a neutral aqueous environment to break chemical bonds and the second step is to inoculate the test solution and incubate it under similar conditions as described in the OECD 310 Guideline 'Ready Biodegradability - CO<sub>2</sub> in sealed vessels (Headspace Test)'. The results of this combined test design show that compounds that have the propensity to absorb sunlight can achieve higher mineralization rates to CO<sub>2</sub> when exposed to sunlight and an inoculum rather than exposed to solely to an inoculum. The results and further conclusions are presented below.



Figure 1: Photos showing the quartz photolysis dish alone and under artificial sunlight.

## Methods: Conduct of the Photo-enhanced Biodegradation Test

- Test Design:** conduct of the photo-enhanced biodegradation test combined elements of the OECD 316 Aqueous Photolysis Test followed by the OECD 310 Headspace Biodegradation Test using Glipizide-dosed sterile mineral media at 10 mg Carbon/L during the light exposure, followed by inoculation of the dosed solutions with secondary effluent at an inoculum concentration of 10%. Enough volume of the dosed mineral medium was exposed to light to have at least 4 time points for the biodegradation portion of the test (8 vials). Inoculated mineral medium vials served as blank controls and 8 vials of inoculated mineral medium dosed with sodium benzoate served as a positive control.
- Matrix:** 301B Inorganic Mineral Medium
- Inoculum source:** Secondary effluent derived from settled activated sludge (Wareham, MA).
- CO<sub>2</sub> Trapping solutions:** 2 M KOH solution; 0.125 mL added per 13.5 mL of test solution..
- Temperature:** 22 +/- 2°C
- Sampling and Analysis:** At each time point, 0.125 mL of 2 M KOH solution was injected into each sealed vial to stop biodegradation and to convert any CO<sub>2</sub> into CO<sub>3</sub> before shaking and analyzing for total inorganic carbon using a Shimadzu TOC Instrument equipped with an Infrared Detector (calibrated using sodium bicarbonate).
- Test substance:** Glipizide (supplied by Acros Organics)
- Reference substance:** Sodium Benzoate (preliminary and definitive testing)
- Negative substance:** Metformin (supplied by Sigma Aldrich)
- Test concentration:** 10 mg carbon/L

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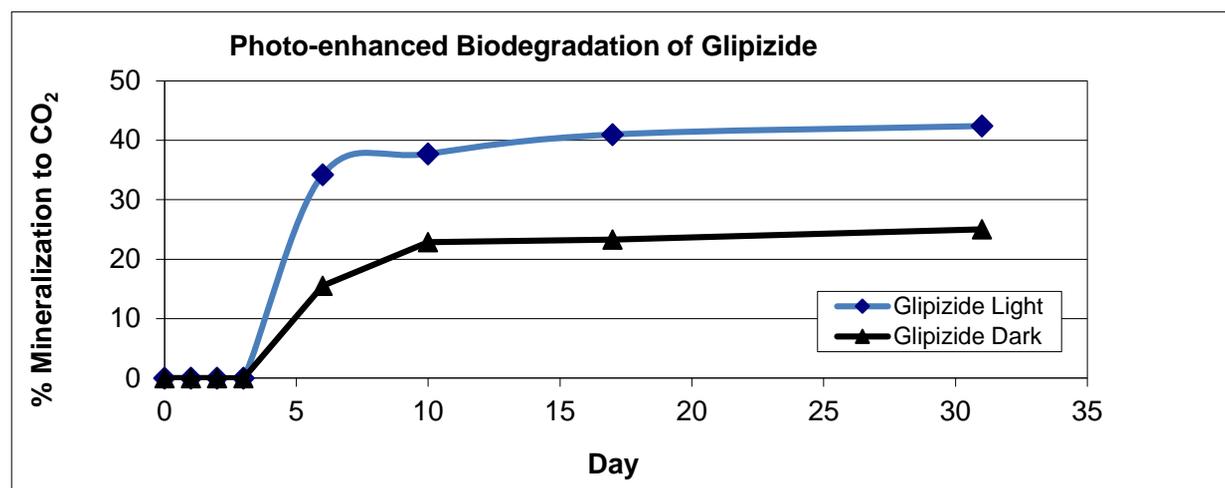


Figure 3: Percent biodegradation of Glipizide over Time in the Photo-enhanced Biodegradation Test showing conversion to CO<sub>2</sub>. (Photolysis took place between day 0 and day 3.)



Example of the OECD 310 test vessel

## Results & Conclusions

- The UV-vis absorption scans showed that Glipizide had the propensity to absorb sunlight above 290 nm. UV-vis scans showed that Metformin, used as a negative control, did not absorb sunlight above 290 nm and did not show an increase in degradation in the light exposed samples. Sodium benzoate, the positive control already known to be readily biodegradable was tested in the headspace portion of the study and showed over 60% biodegradation by day 3 of the biodegradation test.
- The carbon concentration of Glipizide did not change after the exposure to light.
- Upon exposure to an inoculum, the light samples of Glipizide converted 42% of its carbon content to CO<sub>2</sub>. Whereas the dark samples of Glipizide only converted 25% of its carbon content to CO<sub>2</sub> after 28 days of incubation.
- The increase in conversion to CO<sub>2</sub> in the light-exposed Glipizide samples is nearly a 70% increase of what is measured in the dark control Glipizide samples.
- This study shows that compounds with light-absorbing characteristics when exposed to sunlight may photodegrade into photoproducts that are more susceptible to not only to biodegradation but to ultimate biodegradation (conversion to CO<sub>2</sub>) than the intact parent compound. By considering pre-exposure or simultaneous exposure to sunlight in certain environmental circumstances couple with biodegradation, the results could provide a more favorable risk assessment.