# Aquatic Photodegradation of Albendazole and Its Major Metabolites. 1. Photolysis Rate and Half-Life for Reactions in a Tube

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Aquatic photodegradation of albendazole (ABZ) and its three major metabolites, albendazole sulfoxide (ABZSO), albendazole sulfone ($ABZSO_2$), and 2-aminoalbendazole sulfone ($ABZ2NH_2$), was studied under natural sunlight. The sunlight exposures were conducted in solution at pH 5, 7, and 9 to encompass conditions of reversible ionization or protonation of the test substances. The photodegradation rate constants and half-lives were determined for the test substances. An HPLC method was used to measure the disappearance of chemical in solution with time. The substances degraded rapidly with photolytic half-lives ($t_{1/2}$) for ABZ, ABZSO, $ABZSO_2$, and $ABZ2NH_2$ of 0.012–0.031, 0.096–0.33, 0.041–0.91, and 0.35–0.81 days, respectively. The reaction quantum yield ($\Phi$) was measured at each pH and will be presented in part 2 of this presentation.

Albendazole is a broad spectrum anthelmintic which is effective against all classes of helminths—gastrointestinal roundworms, lungworms, tapeworms, and liver flukes—that commonly infect domestic animals. The extent of ecological and environmental impact of this substance is minimal owing to its use as a therapeutic agent under environmentally controlled conditions. This study was undertaken as part of a requirement for New Animal Drug Application of albendazole by the U.S. Food and Drug Administration.

There is potential for entry of the test substance into the environment from its usage in cattle, sheep, and broiler and from manufacturing. Aquation photodegradation is a possible degradation pathway for test substances that reach the water body as a result of runoff from soil. Measuring the rate of photolysis as a means of predicting persistence of chemicals in water began with the widespread use of pesticides and herbicides in agriculture (Wolfe et al., 1978). Earlier studies were of a qualitative nature, providing essentially reaction pathways rather than dealing with reaction kinetics. Later studies were developed with more emphasis on providing a quantitative framework for reaction kinetics (Zepp and Cline, 1977; Miller and Zepp, 1979; Wolfe et al., 1977) and reaction quantum yield measurements (Zepp, 1978). This study was conducted under the guidelines specified in the U.S. FDA *Environmental Assessment Technical Handbook* (FDA, 1987).

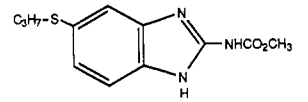
## EXPERIMENTAL PROCEDURES

**Preparation of Exposure Solutions.** The exposure solutions were prepared in buffer solutions of pH 5, 7, and 9: pH 5, 0.001 M $NaH_2PO_4$ adjusted to pH 5 with 0.01 M NaOH; pH 7, 0.10 M $KH_2PO_4$ adjusted to pH 7 with 0.10 M NaOH; pH 9, 0.25 M $Na_2B_4O_7$ adjusted to pH 9 with 0.10 M HCl.

All buffers were prepared using ASTM type II A water. The glassware used in the experiment was sterilized by autoclaving. All laboratory work was performed under subdued light.

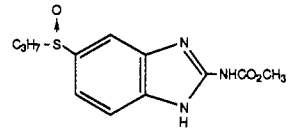
Triplicate weights (<1 mg) of $^{14}C$-labeled ABZ, ABZSO, $ABZSO_2$, and $ABZ2NH_2$ were placed in 1-L volumetric flasks followed by 1 mL each of acetonitrile and the appropriate buffer.

ABZ



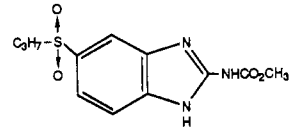
Methyl [5-(propylthio)-1 H-benzimidazol-2-yl] carbamate

ABZSO



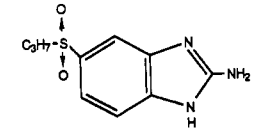
Methyl [5-(propylsulfinyl)-1 H-benzimidazol-2-yl] carbamate

$ABZSO_2$



Methyl [5-(propylsulfinyl)-1 H-benzimidazol-2-yl] carbamate

$ABZ2NH_2$



5-(Propylsulfonyl)-1 H-benzimidazol-2-amine

Dissolution was achieved by sonication for 30 min, adding more buffer solutions as necessary, and bringing the final volume up to 1000 mL.

The exposure solutions were aerated with air filtered through a 0.22-$\mu$m membrane filter (Millex-$FG_{50}$, Millipore Corp.) until saturation. The aeration was done after aliquots were removed for determining the UV–vis absorption spectra. Aliquots (3 × 100 $\mu$L) were removed from each solution for radioassay to determine the final concentration.

From each exposure solution, triplicate 7-mL aliquots were transferred into quartz (11 × 100 mm) exposure tubes for each sampling period. Controls (dark controls) were prepared in a similar manner except that the tubes were covered with aluminum

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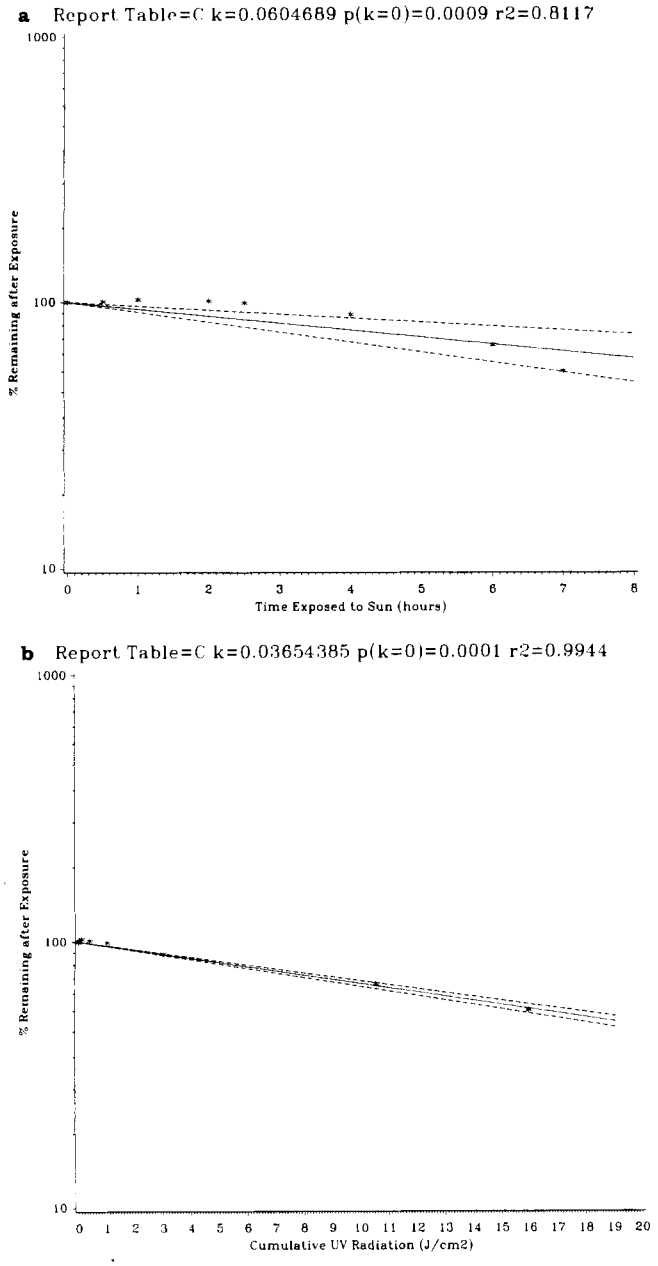


**Figure 1.** Amount of *p*-nitroacetophenone remaining after exposure to sunlight.

foil to prevent exposure. Finally, the tubes were capped with sterilized silicone stoppers to prevent losses by evaporation.

**Preparation of Actinometer Solution.** The actinometer solution consisted of a mixture of *p*-nitroacetophenone (PNAP) and pyridine (Dulin and Mill, 1982). On the basis of degradation rates obtained from a preliminary study, actinometers were prepared containing two different concentrations of pyridine to accommodate the full range of sampling time points. The actinometers contained PNAP ($2 \times 10^{-5}$ M) in acetonitrile and either 0.55 or 0.11 M pyridine. The solutions were prepared by placing 0.5 mL of 0.02 M PNAP in a 500-mL volumetric flask and adding an appropriate volume of pyridine to bring its concentration to the desired value (0.55 or 0.11 M) after the solution was diluted to 500 mL with reagent grade water.

**Determination of UV–Vis Absorption Spectra.** The UV–vis absorption spectrum (290–800 mn) of each exposure solution in 1-cm quartz cells was measured using a Varian 2290 spectrophotometer. A scan rate of 2 nm/s and a bandwidth of 3 mm were used. Exposure solutions with absorbance >0.05 au (within 290–800 nm) were either diluted or freshly prepared prior to exposure.

**Exposure to Sunlight.** The quartz tubes filled with exposure solutions were placed outdoors on an exposure rack with a black, nonreflective background. The tubes were secured at an inclination of 30° from the vertical with the upper end point due north. The exposure site was at a latitude of 35° 54′. The dark controls were placed alongside with the respective exposure solutions. The exposure took place between October 13 and 22, 1988.

**Meteorological Measurements.** *A. Cumulative Ultraviolet Solar Radiation.* Cumulative ultraviolet solar radiation was measured using an International Light Model 1L1745 UV curing radiometer system. The instrument had a dynamic range of $10^{-6}$–$10^{0}$ W/cm$^2$ for ultraviolet light flux.

The output from the radiometer was transmitted to an AT&T personal computer which calculated 10-min mean ultraviolet solar radiation flux values and cumulative ultraviolet energy flux values at 10-min intervals. A hard-copy output was generated continuously.

*B. Ambient Air Temperature.* A Climatronics Model EWS electronic weather station was used to measure ambient air temperature. The output from the instrument was recorded by a Hewlett-Packard Model 7132 strip chart recorder.

**Selection of Sampling Periods.** A preliminary study was conducted to determine the rate of degradation of ABZ, ABZSO, $ABZSO_2$, and $ABZ2NH_2$ in solutions exposed to natural sunlight. The results were used to select the sampling periods for the definitive study. The sampling periods were selected to accommodate at least six sampling points between 20 and 80% degradation of each test compound. Actinometer samples were removed with test samples at each sampling period. At each sampling point, nine tubes of exposure solutions (triplicates for pH 5, 7, and 9), nine tubes of dark controls, and six tubes of actinometer (three exposed and three dark controls) were removed for analysis. The samples were protected from light and prepared immediately for analysis.

**Quantitative Assessment of Photolysis.** *A. Sample Preparation.* Test solutions were processed using a solid-phase extraction cartridge (Sep-Pak) prior to analysis to remove buffer salts. Cartridges were preconditioned by flushing with 2 mL of methanol followed by 5 mL of distilled water. After removal of aliquots ($3 \times 200$ μL) for radioassay, the remainder from the quartz tube was transferred to 5-mL Glaspak syringes (Becton, Dickinson and Co., Rutherford, NJ) to which the Sep-Pak cartridge had been attached. The solution was forced through the cartridge followed by 1 mL of distilled water rinse of the quartz tube. Finally, the test material and the possible degradation products were removed from the cartridge dropwise using 3 mL of acetonitrile. The acetonitrile eluent was evaporated to dryness with dry nitrogen at 30 °C and reconstituted in 300 μL of acetonitrile/acetic acid (5/1 v/v) prior to HPLC analysis.

*B. HPLC.* Two HPLC systems were used to analyze ABZ, ABZSO, $ABZSO_2$, $ABZ2NH_2$, and the PNAP/pyridine actinometer.

ABZ, $ABZSO_2$, and $ABZ2NH_2$ were analyzed using a Waters Associates liquid chromatograph with a Model 721 system controller and a Model 6000A pump. A 250 mm × 4.6 mm Alltech Econosphere 5-μm $C_{18}$ column was used. The mobile phase consisted of acetonitrile/0.01 M $KH_2PO_4$ (40/60 v/v) at a flow rate of 2 mL/min. A Waters Model 712 WISP autosampler was used for sample injection. The detector used was a Kratos Analytical Instruments FS 970 LC fluorometer equipped with a 300-nm cutoff filter and a GM 970 monochromator set at an excitation wavelength of 280 nm. The signal was stored and integrated using a Raytest Radio-Chromato-Graphic-System IBM, version 7.7 AT.

ABZSO and PNAP in the actinometer were analyzed using a Waters liquid chromatograph equipped with a Model 510 pump. For ABZ, a 250 mm × 4.6 mm Alltech Econosphere 5-μm $C_{18}$ column was used with a mobile phase consisting of acetonitrile/0.01 M $KH_2PO_4$ (40/60 v/v) at a flow rate of 2 mL/min. A Waters Model 710B WISP autosampler was used for sample injection. The detector used was a McPherson Instruments PL-750 HPLC.PLUS spectrofluorescence detector at excitation and emission wavelengths of 285 (bandwidth 2 nm) and 345 nm (bandwidth 16 nm), respectively. The data handling (signal integration), pump, and autosampler control were handled by a Waters Maxima 820 chromatography data system. PNAP was chromatographed on a 250 mm × 4.6 mm Du Pont Zorbax $C_{18}$

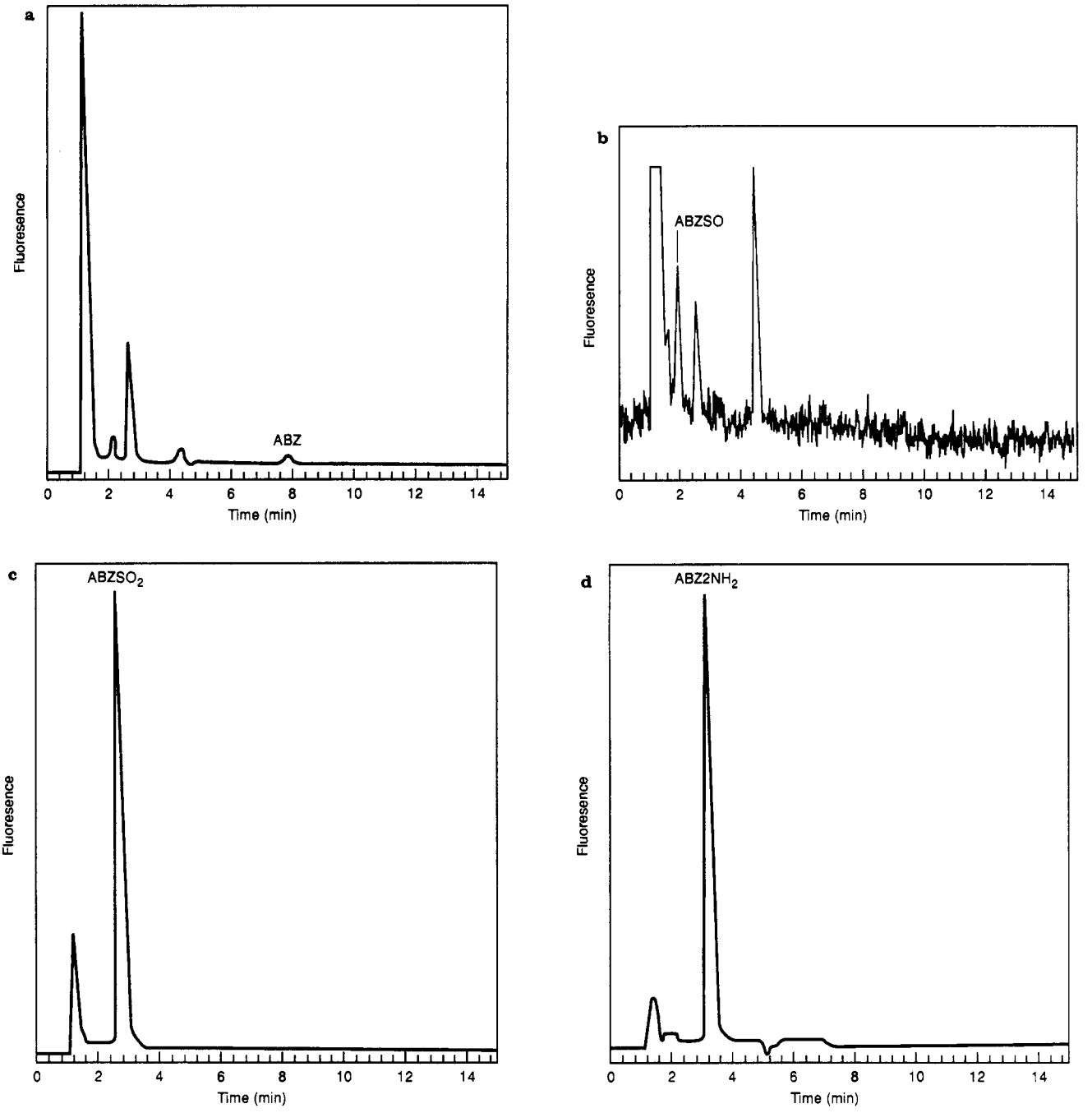


**Figure 2.** (a) HPLC fluorescence chromatogram of an albendazole sample in pH 9 buffer exposed to sunlight for 4.0 h. (b) HPLC fluorescence chromatogram of an albendazole sulfoxide sample in pH 9 buffer exposed to sunlight for 3.0 h. (c) HPLC fluorescence chromatogram of an albendazole sulfone sample in pH 9 buffer exposed to sunlight for 2.0 h. (d) HPLC fluorescence chromatogram of an albendazole-2-aminosulfone sample in pH 9 buffer exposed to sunlight for 40 h.

column with a mobile phase consisting of acetonitrile/water (36/65 v/v) pumped at a flow rate of 1 mL/min. A Kratos Analytical Instruments Spectroflow-773 UV–vis detector set at 288 nm was used to detect PNAP.

*C. Quantification.* ABZ, its metabolites, and PNAP in the exposed solutions were quantitated using an external standard method. Appropriate standards (six) encompassing 10 and 100% of the starting test concentration of exposed solutions were prepared. The unknowns were run bracketed with the standard samples. Quality control standards and solvent blanks (acetonitrile/water, 5/1) were run after every sixth sample. Samples exposed for the longest period of time were analyzed first except for PNAP, where the analysis was performed in the order in which they were removed from exposure. The quality control samples were prepared separately from the standards, and their concentration range was within 10–100% of the starting test concentrations of the exposed solutions.

*D. Recovery Validation.* The method for recovery of $^{14}$C-labeled ABZ and its metabolites from buffer solutions was validated. Test solutions fortified with ca. 15 and 100% of the target exposure concentrations of $^{14}$C test material (ABZ and its metabolites) were prepared and subjected to the solid-phase extraction procedure described under Sample Preparation. The initial and final concentrations were determined by radioassay methods.

*E. Statistical Analysis.* Photolysis of a chemical in an optically thin aqueous solution follows the first-order rate expression

$$C = C_0 e^{-k_p t} \quad (1)$$

where $C_0$ is the initial concentration, $C$ is the concentration at exposure duration $t$ (in hours), and $k_p$ is the sunlight direct aqueous photolysis rate constant measured in a tube.

In contrast to a water body, the tubes (11 × 100 nm) used for exposure received radiation from all directions, because of

**Table I. Photolysis Rate Constants ($k_p$) of ABZ and Its Metabolites Based on Time of Exposure to Sunlight**

| compd | pH | $k_p$, $h^{-1}$ | SE | 95% conf limit lower | 95% conf limit upper | $r^2$ |
|---|---|---|---|---|---|---|
| ABZ | 5 | 0.428 | 0.0765 | 0.216 | 0.641 | 0.8868 |
| | 7 | 0.314 | 0.0661 | 0.131 | 0.498 | 0.8500 |
| | 9 | 0.828 | 0.167 | 0.365 | 1.29 | 0.8603 |
| ABZSO | 5 | 0.235 | 0.0165 | 0.195 | 0.274 | 0.9664 |
| | 7 | 0.269 | 0.00941 | 0.246 | 0.292 | 0.9927 |
| | 9 | 0.516 | 0.0488 | 0.396 | 0.635 | 0.9490 |
| $ABZSO_2$ | 5 | 0.0733 | 0.00288 | 0.0665 | 0.0801 | 0.9893 |
| | 7 | 0.188 | 0.00929 | 0.166 | 0.209 | 0.9808 |
| | 9 | 1.16 | 0.160 | 0.771 | 1.55 | 0.8976 |
| $ABZ2NH_2$ | 5 | 0.0318 | 0.00322 | 0.0244 | 0.0393 | 0.9245 |
| | 7 | 0.0625 | 0.00326 | 0.0550 | 0.0701 | 0.9787 |
| | 9 | 0.0726 | 0.00532 | 0.0603 | 0.0849 | 0.9588 |

**Table II. Photolysis Rate Constants ($k_p$) Based on UV Cumulative UV Radiation**

| compd | pH | $k_p$, $h^{-1}$ | SE | 95% conf limit lower | 95% conf limit upper | $r^2$ |
|---|---|---|---|---|---|---|
| ABZ | 5 | 0.685 | 0.0271 | 0.607 | 0.758 | 0.9937 |
| | 7 | 0.511 | 0.00839 | 0.487 | 0.534 | 0.9989 |
| | 9 | 1.33 | 0.0397 | 1.22 | 1.44 | 0.9965 |
| ABZSO | 5 | 0.0481 | 0.00299 | 0.0410 | 0.0552 | 0.9737 |
| | 7 | 0.0588 | 0.00233 | 0.0531 | 0.0645 | 0.9906 |
| | 9 | 0.164 | 0.00451 | 0.153 | 0.175 | 0.9955 |
| $ABZSO_2$ | 5 | 0.0172 | 0.000301 | 0.0165 | 0.0179 | 0.9979 |
| | 7 | 0.0408 | 0.000879 | 0.0388 | 0.0428 | 0.9963 |
| | 9 | 0.380 | 0.0149 | 0.344 | 0.417 | 0.9909 |
| $ABZ2NH_2$ | 5 | 0.0193 | 0.00238 | 0.0135 | 0.0251 | 0.9167 |
| | 7 | 0.0344 | 0.000828 | 0.0324 | 0.0364 | 0.9965 |
| | 9 | 0.0447 | 0.00273 | 0.0380 | 0.0514 | 0.9781 |

**Table III. Photolytic Half-Lives[a] Based on Total Length of Exposure to Sun**

| compd | pH | $t_{1/2}$,[a] days | $t_{1/2}$,[b] days |
|---|---|---|---|
| ABZ | 5 | 0.144 | 0.023 |
| | 7 | 0.196 | 0.031 |
| | 9 | 0.0745 | 0.012 |
| ABZSO | 5 | 0.263 | 0.33 |
| | 7 | 0.229 | 0.27 |
| | 9 | 0.120 | 0.096 |
| $ABZSO_2$ | 5 | 0.842 | 0.91 |
| | 7 | 0.328 | 0.39 |
| | 9 | 0.0531 | 0.041 |
| $ABZ2NH_2$ | 5 | 1.94 | 0.81 |
| | 7 | 0.987 | 0.46 |
| | 9 | 0.850 | 0.35 |

[a] Average day length during exposure (Oct 13–23) was 11.22 h. [b] Based on UV radiation day.

scattering of the light off the walls. As a consequence, the photoreactions in tubes are expected to be faster than those under natural conditions on the flat water body (Dulin et al., 1982; Mill et al., 1982). The photolytic rate constant in a tube ($k_p$) is related to that in a water body ($k_{pE}$) by

$$k_p = 2.2k_{pE} \quad (2)$$

Equation 1 can be rearranged to give

$$\ln (C_0/C) = k_p t \quad (3)$$

The photolytic half-life can be calculated using

$$t_{1/2} = 0.693/k_p \quad (4)$$

Average concentrations at each sampling point were fitted to eq 3 using REG procedure available in SAS version 5.16, running on either a VAX 8550 or a VAX 8650 computer (VMS 4.7).

**Table IV. Total Time of Experiment, Total Time of Sunlight Exposure, and Cumulative UV Radiation**

| compd | pH | total time, h | sunlight, h | cum UV, $J/cm^2$ |
|---|---|---|---|---|
| ABZ | 5 | 29.0 | 15.0 | 34.7 |
| | 7 | 34.0 | 20.0 | 41.8 |
| | 9 | 10.0 | 10.0 | 21.6 |
| ABZSO | 5 | 9.5 | 9.5 | 43.1 |
| | 7 | 57.5 | 28.3 | 117 |
| | 9 | 9.5 | 9.5 | 43.1 |
| $ABZSO_2$ | 5 | 57.5 | 28.33 | 117 |
| | 7 | 30.0 | 15.5 | 71.4 |
| | 9 | 5.0 | 5.0 | 26.1 |
| $ABZ2NH_2$ | 5 | 82.0 | 40.0 | 41.8[a] |
| | 7 | 34.0 | 20.0 | 41.8[a] |
| | 9 | 34.0 | 20.0 | 41.8[a] |

[a] The UV sensor failed 34 h into the experiment, and the value given here was taken at 34 h.

The REG procedure was used to calculate the simple least-squares estimate of the degradation rate constant ($k_p$), its standard error ($SEM_{kp}$), a $t$ statistic based upon the null hypothesis that $k_p$ was equal to zero, the probability of this $t$ statistic given the degrees of freedom for error (df), and the coefficient of determination ($r^2$) for each set of data fit. Since the fitted function did not include a term representing the intercept (i.e., forced through zero), $r^2$ is limited to represent the ratio of the model sum of squares to the total sum of squares and should only be used as a relative measure of goodness-of-fit to compare the three different independent measures of exposure. In this case, it is not appropriate to calculate a coefficient of correlation ($r$). Upper and lower confidence limits were determined for each estimate of $k_p$ using

$$\text{upper limit} = k_p + ({}^t0.05, \text{df} \times \text{SEM}_{k_p}) \quad (5)$$

$$\text{lower limit} = k_p + ({}^t0.05, \text{df} \times \text{SEM}_{k_p}) \quad (6)$$

where $k_p$ is the estimated value, $t_{0.05}$, df is the two-tailed Students' $t$ statistic given $a = 0.05$ and df, degrees of freedom, and $SEM_{k_p}$ is the standard error of $k_p$ plots of the observed and calculated concentrations (expressed as $100C/C_0$) were plotted using the SAS/GRAPH procedure GPLOT. The upper and lower bounds of $k_p$ were used to generate lines representing the lower and upper bound of the fitted line, respectively.

## RESULTS AND DISCUSSION

**Meteorological Observations.** Clear weather prevailed during the exposure of ABZSO and $ABZSO_2$. However, overcast skies prevailed through the exposure of ABZ and $ABZ2NH_2$.

**Recovery Validations.** Nearly quantitative recoveries were obtained after sample workup for ABZ and its metabolites fortified at 100% of the target concentration. At 15% level of fortification, the recoveries ranged upward from ca. 80%.

Recoveries of the radiolabel from the exposed solutions were variable and lower than those from freshly prepared solutions, reflecting the action of sunlight and buffer solutions on the test material. With the exception of $ABZ2NH_2$, recoveries of unexposed controls averaged between ca. 70 and 100%. The appearance of polar degradation products in the HPLC chromatogram suggested that the lower recoveries may be due to poor retention of these products on the solid-phase extraction cartridge.

**Actinometry.** If solar irradiance is monitored concurrently with the exposure of the test chemical, an estimate of $k_p$ and $\Phi$ can be made under varying weather

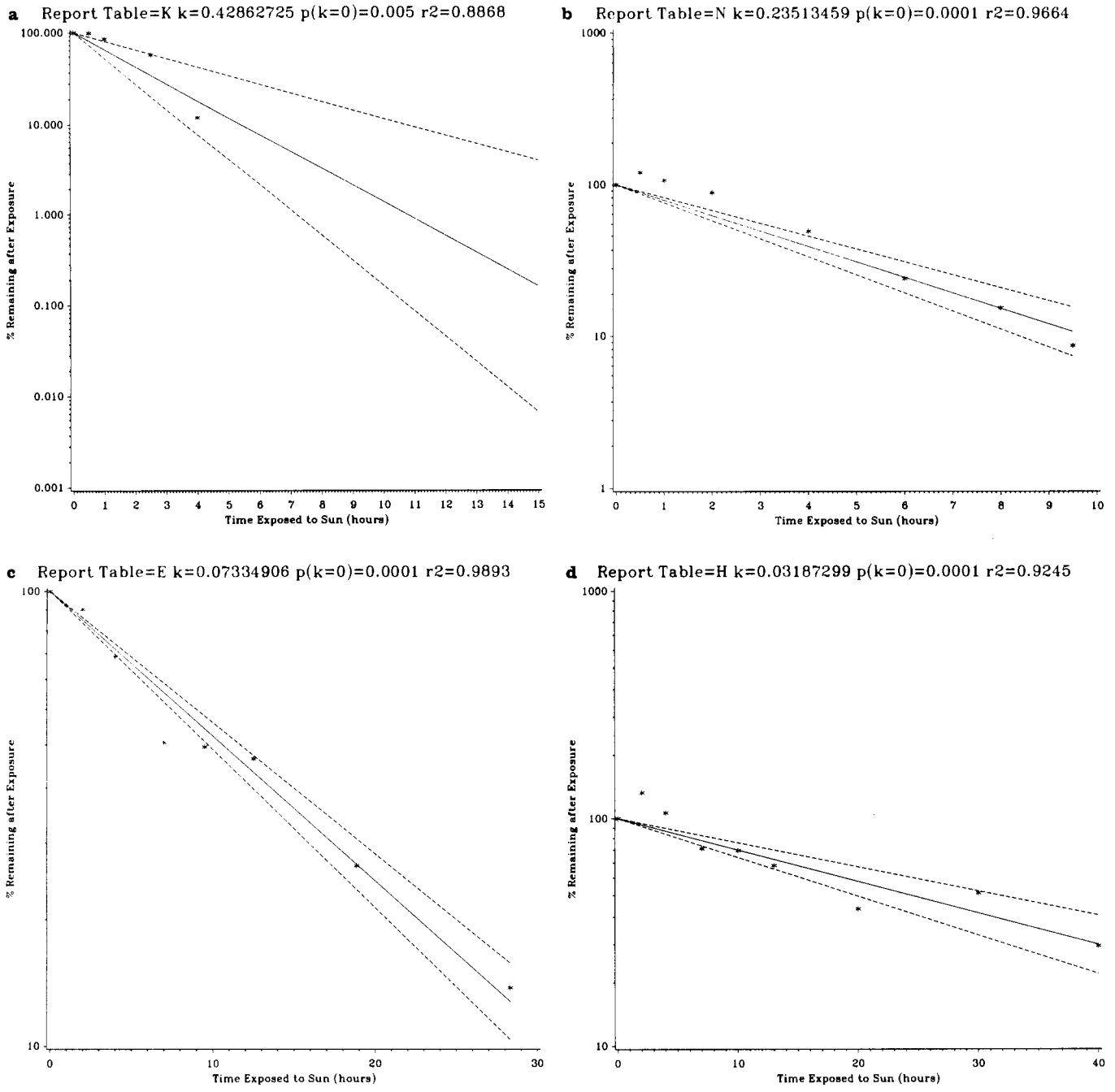


**Figure 3.** Amount of albendazole (a), albendazole sulfoxide (b), albendazole sulfone (c), and albendazole-2-aminosulfone (d) remaining in solutions at pH 5 exposed to sunlight.

conditions, diurnal cycling, and seasonal changes. An actinometer with similar absorption properties and a known Φ value exposed alongside with the test chemical can be used to correct photolysis rate constants measured outdoors under clear sky conditions. The correlation between rate of disappearance of PNAP and cumulative UV radiation was excellent ($r^2$ = 0.994); however, the relationship between the PNAP disappearance and the time of exposure to sun was less dramatic ($r^2$ = 0.812), owing to diurnal changes in light intensity and intermittent inclement weather conditions during the exposure period (Figure 1). The same actinometer with 0.55 M pyridine exposed to sunlight on two different days showed drastically different rate constants when the loss of PNAP was correlated with the time of exposure. On October 14, under clear sky conditions, the PNAP in the actinometer degraded with a rate constant of 0.199/h, whereas on October 19, under overcast skies, the PNAP in the actinometer decayed with a rate constant of 0.060/h. However, the difference of the two rate constants is much smaller when the rate of disappearance was correlated with the cumulative UV radiation, assuming October 14 with clear sky as a "UV radiation day" with a cumulative irradiance of 44 J/cm$^2$ on which the rates of decomposition were normalized.

**Photolysis of ABZ and Its Metabolites.** The HPLC chromatograms of exposed test solutions revealed several products of photolysis, the majority of which are well resolved from their respective parent compounds and eluted near the void volume (see Figure 2).

The photolysis rate constants and half-lifes were based on the time of exposure to sunlight and on the cumulative UV radiation, normalized to UV radiation day, and are presented in Tables I and II. There was good to excellent correlation between the rates of decomposition of test chemicals and the cumulative UV radiation.

A clear day, under October sunlight, represents an estimated average of 44 J/cm$^2$ of cumulative UV radiation. As evident from Table IV, the exposure of ABZSO and $ABZSO_2$ took place under clear skies, and expectedly the $t_{1/2}$ values calculated based on sunlight exposure time and UV radiation day are quite comparable. For ABZ and $ABZ2NH_2$, overall $t_{1/2}$ values based on UV radiation day were much shorter than those calculated on the the basis of total time exposed to sunlight (Table III), reflecting the overcast conditions of exposure (Table IV).

Among the four compounds tested, ABZ was the most susceptible to photolysis followed by ABZSO. $ABZSO_2$ and $ABZ2NH_2$ degraded at a slower rate than the other two compounds. The rates of degradation of ABZ and its metabolites at pH 5 are illustrated in Figure 3. The HPLC peak profiles of the exposed solutions indicated that photolysis proceeded with a stepwise oxidation at sulfur (ABZ–ABZSO–$ABZSO_2$), but the strongest peaks of degradation products were present at virtually void volume retention times, indicating the presence of polar photolytic products. The shortest half-lives for all substances were obtained at pH 9, indicating the increased susceptibility to photolysis under alkaline conditions (see Table III).

There was no appreciable decomposition in dark controls, indicating that, hydrolytically, the compounds were stable during the length of experiment.

**Conclusions.** Albendazole and its major metabolites are steadily degraded by natural sunlight at the surface of a flat water body. The degradation appears to proceed through stepwise oxidation at sulfur (ABZ–ABZSO–$ABZSO_2$), leading to polar photolytic products. The degradation was predominant at pH 9. It is evident that, under clear sky conditions, albendazole and its three major metabolites, ABZ, ABZSO, and $ABZSO_2$, will undergo rapid degradation to 50% of their starting concentration at the surface of a flat water body within a day between late summer and early fall. The photolytic rates measured in a quartz tube may be faster than that at the flat water surface owing to the internal reflections of the incident light inside the tube. Nevertheless, these compounds pose a minimal threat to aquatic species owing to their lack of persistence in the aquatic environment.

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ABBREVIATIONS USED

ABZ, albendazole; ABZSO, albendazole sulfoxide; $ABZSO_2$, albendazole sulfone; $ABZ2NH_2$, 2-aminoalbendazole sulfone; PNAP, *p*-nitroacetophenone; PYR, pyridine.

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**Registry No.** ABZ, 54965-21-8; ABZSO, 54029-12-8; $ABZSO_2$, 75184-71-3; $ABZ2NH_2$, 80983-34-2.