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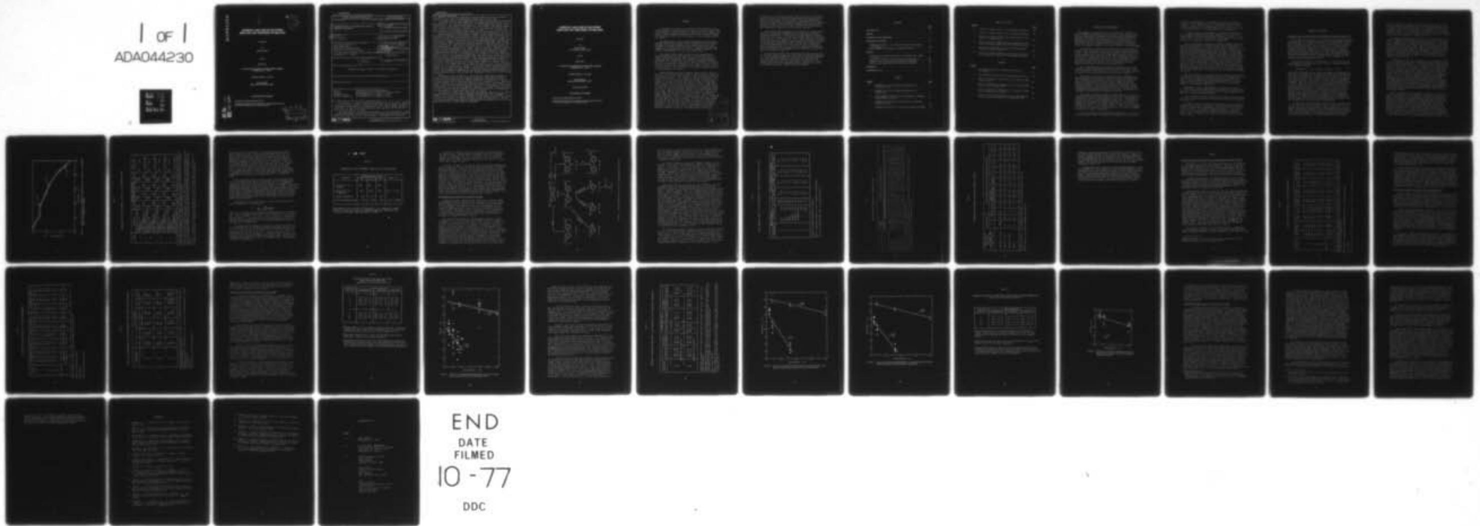

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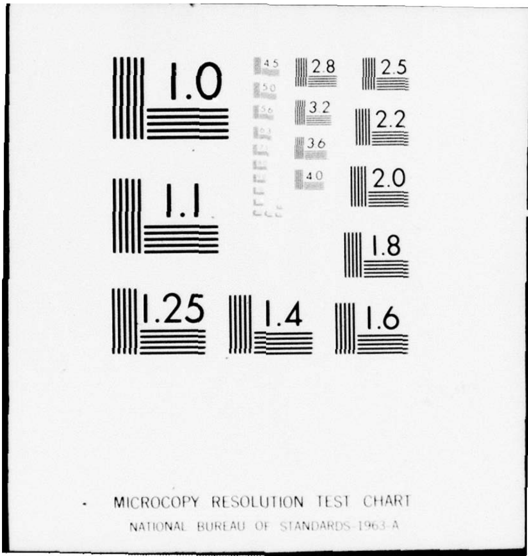
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CHEMICAL ANALYSIS OF SULFONES USED FOR THE TREATMENT OF MALARIA

Final Report

By

JOHN H. PETERS

July 1977

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D.C. 20314

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

To determine if a causal relationship existed between the occasional incidence of agranulocytosis in subjects receiving dapsone (DDS) and the amount of sulfone contaminants in DDS tablets, we compared the levels of these compounds and DDS in several lots of tablets returned from overseas with the levels in the same lots that had been stored in U.S. depots.

Spectrophotometric, thin-layer chromatographic (TLC) and high-pressure liquid chromatographic (HPLC) procedures were used to determine DDS and sulfone contaminants both in DDS tablets and in DDS standards. Contaminants were identified

20. Abstract (Continued)
by comparison of their mass fragmentation patterns (MFP) with those of authentic samples of four isomeric diaminodiphenyl sulfones and 14 derivatives.

Six diphenyl sulfone (DS) contaminants were identified as 4-amino-4'-chloro-DS (I), 4-amino-DS (II), 2,4'-diamino-DS (III), 4-amino-4'-methylamino-DS (IV), 3,4'-diamino-DS (V), and 4-amino-4'-hydroxy-DS (VI). The highest total sulfone contamination found in any tablet lot was 2.2%. Four lots of tablets selected for potential clinical use were found to conform to U.S.P. specifications. However, they also contained the contaminants listed above. The sum of I, II, III, and VI was 1.1 to 1.9% of the total sulfones present in addition to trace quantities of IV and V.

Photodecomposition studies of DDS in organic solvents revealed that the rate of disappearance depended on concentration, solvent, and the method of analysis. The high rate of loss in ethylene dichloride resulted from the effect of (a) products from the irradiation of the solvent and (b) direct photodecomposition of DDS. Pre-irradiation of ethanol or water did not cause decomposition of DDS. Photodecomposition rates of DDS in ethanol and ethanolic extracts of DDS tablets were the same. No relationship was found between the storage history of tablets and the rate of photodecomposition. Saturation of ethanol with O_2 or N_2 had no significant influence on the photodecomposition rate of DDS. HPLC of irradiated ethanolic solutions of DDS revealed a major peak, identified as 2-amino-4'-nitro-DS by its chromatographic properties and MFP. It accounted for 25 to 50% of the DDS lost during irradiation. In water, the photodecomposition rate of DDS was ten times faster than that in ethanol. Two peaks were found by HPLC; the major one was identified by its MFP as 4-amino-4'-(2-phenylhydrazino)-DS. The minor peak exhibited the same parent ion and was considered to be an isomer. Rates of disappearance ($T\frac{1}{2}$) of DDS in water were essentially the same for analytic DDS or aqueous or ethanolic extracts of tablets. They ranged from 0.23 to 0.58 hr (10 μg/ml), 1.6 to 2.5 hr (100 μg/ml), and 3.2 to 4.6 hr (170 μg/ml). Shorter $T\frac{1}{2}$ values were obtained in aqueous solutions purged with N_2, suggesting that removal of O_2 resulted in more rapid photodecomposition. No autocatalytic decomposition of DDS was observed in aqueous solutions of DDS or aqueous extracts of DDS tablets that had been irradiated for sufficient time to cause a 50-70% loss of DDS and that were subsequently stored at 4^0 or 37^0 for up to 35 days.

The absolute light intensities of the ultraviolet lamps used for photolysis studies were determined with the chemical actinometer, potassium ferrioxalate. Studies with this light-sensitive compound showed that the intensity of light in the sample chamber of the fluorometer was 7.91×10^{16} quanta cm^{-2} min^{-1}. When thin layers of DDS or tablet extracts were prepared on glass slides from ethanolic solutions or slurries and irradiated with light (254 nm) of this intensity, 5 and 50 μg disappeared at rates of 3.2 and 0.5% hr^{-1}, respectively. However, when thin layers of 5 μg of analytic DDS were prepared from water and irradiated, the disappearance rate decreased to a significantly lower value of 0.7% hr^{-1}.

One photolysis product from irradiating solid DDS was identified by TLC, HPLC, diborane reduction, and its MFP as 4-amino-4'-formamido-DS. This compound accounted for 0.8-1.3% of the unrecovered DDS in 50 μg irradiated for 16-64 hr; layers deposited from aqueous or ethanolic solutions yielded the same results. The MFP of a second product before and after trimethylsilylation suggested that it was the formamide derivative of DDS (4-NH_2-4'-ureido-DS).

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SUMMARY

To determine if a causal relationship existed between the occasional incidence of agranulocytosis in subjects receiving dapsone (DDS) and the amount of sulfone contaminants in DDS tablets, we compared the levels of these compounds and DDS in several lots of tablets returned from overseas with the levels in the same lots that had been stored in U.S. depots.

Spectrophotometric, thin-layer chromatographic (TLC) and high-pressure liquid chromatographic (HPLC) procedures were used to determine DDS and sulfone contaminants both in DDS tablets and in DDS standards. Contaminants were identified by comparison of their mass fragmentation patterns (MFP) with those of authentic samples of four isomeric diaminodiphenyl sulfones and 14 derivatives.

Six diphenyl sulfone (DS) contaminants were identified as 4-amino-4'-chloro-DS (I), 4-amino-DS (II), 2,4'-diamino-DS (III), 4-amino-4'-methylamino-DS (IV), 3,4'-diamino-DS (V), and 4-amino-4'-hydroxy-DS (VI). The highest total sulfone contamination found in any tablet lot was 2.2%. Four lots of tablets selected for potential clinical use were found to conform to U.S.P. specifications. However, they also contained the contaminants listed above. The sum of I, II, III, and VI was 1.1 to 1.9% of the total sulfones present in addition to trace quantities of IV and V.

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INTRODUCTION AND BACKGROUND

The purpose of these studies was to determine the contents of dapsone (4,4'-diaminodiphenyl sulfone, DDS) and closely related trace chemical contaminants in pharmaceutical preparations of DDS employed previously for prophylaxis against malaria. The need for such information was based on the possibility that some contaminant in the DDS preparations used for mass prophylaxis of military personnel in Vietnam may have been responsible for the development of agranulocytosis in a number of subjects, with fatal outcome in eight persons[1]

As pointed out in greater detail previously[2] other antimalarial drugs or drug combinations used earlier have never produced agranulocytosis. Also, inadvertent retreatment with DDS in Japan of a patient who had recovered from an episode of DDS-induced agranulocytosis in Vietnam did not again cause this effect. This observation would seem to rule out an idiosyncratic response to DDS. The agranulocytosis in this patient could have been due to some contaminant unique to the DDS used in Vietnam that resulted from the intense heat and high humidity characteristic of the tropical climate there.

Other cases of DDS-induced reversible agranulocytosis have been reported in Vietnam[3,4] and in other parts of the world[5] Thus, this toxic side effect of DDS therapy is not exclusive to Vietnam, although no cases with fatal consequences have been reported elsewhere.

Our interest in the problem of measuring trace contaminants in DDS preparations began when we learned that chemists at SRI had shown that a pharmaceutical preparation of DDS contained small amounts of several sulfone derivatives closely related to DDS[6] We obtained analytical samples of these derivatives and established procedures for their qualitative detection and quantitative measurement. These methods were applied to DDS preparations used by us as standards and to pharmaceutical preparations used by our clinical collaborators for treatment of leprosy patients throughout the world[7]

The sulfone contaminants found by the SRI chemists in one lot of commercial DDS (Sterling Winthrop Research Institute, Lot 8L-2223) were identified as 4-amino-4'-chlorodiphenyl sulfone (I), 4-aminodiphenyl sulfone (II), and 2,4'-diaminodiphenyl sulfone (III). In four pharmaceutical and two commercial preparations of DDS, we did not detect Compound I[7] Relative to the amount of DDS, we found in the commercial preparations 0.70 to 2.7% of Compound II and $<$ 0.05 to 3.3% of Compound III; and in the pharmaceutical preparations, 0.27 to 0.99% of Compound II and 0.16 to 0.80% of Compound III.

Later, we examined two additional DDS preparations. The first, an Imperial Chemical Industries pharmaceutical preparation being used by

a clinical collaborator, Dr. D. Elizondo of San Jose, Costa Rica, yielded 0.15% of Compound II and 0.04% of Compound III but < 0.02% of Compound I. These results essentially agreed with levels of these materials found earlier in another sample from this manufacturer that was obtained from Dr. L. Levy, USPHS Hospital, San Francisco.[7]

The second sample was a pharmaceutical preparation (Parke-Davis, Lot No. GFJ751, September 1966) kindly supplied to us by Dr. R. S. Rozman, WRAIR. This material was found to contain Compound I to the extent of 0.27% relative to DDS content (96% of theory). It also contained 1.0% of Compound II and 0.39% of Compound III.

Also, we subsequently learned that Lofberg at WRAIR[8] had qualitatively identified 4-amino-4'-hydroxydiphenyl sulfone (Compound VI) in addition to Compounds I, II, and III in some tablet preparations of DDS. However, no quantitative data were available. Our application of Lofberg's thin-layer chromatographic (TLC) procedure to the two DDS preparations described above yielded weak ultraviolet (UV)-absorbing spots at R_f 0.39. An authentic sample of Compound VI prepared at SRI for earlier studies[9] exhibited an R_f of 0.33. Without further studies, we could not be certain that Compound VI was present in the preparations we examined, but Lofberg's finding of this compound in six different lots (same manufacturer?) is strong evidence that his observations are valid. In addition, he also found the same contaminants in DDS before admixture with fillers and compaction into tablets. These observations indicate that the impurities are in the manufactured DDS and are not introduced by the excipients or in the process of preparing the tablets.

Our major emphasis in this work has been on comparative qualitative and quantitative analyses of samples of the same lots of DDS tablets that have been returned from overseas and that were stored in U.S. depots. By these comparisons, we examined the possibility that the agranulocytosis observed overseas was attributable to contaminants unique to those samples of DDS tablets.

Following the above investigations, we examined the effects of UV irradiation on analytic DDS and DDS from tablets in various solvents and in the solid state to determine the rates of photodecomposition of DDS and the products produced therefrom.

This final report covers in detail only the work of the third contract year. Results obtained in the first two years have been reported previously.[10,11]

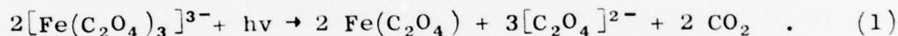
The primary means of identifying contaminants in analytic DDS and in DDS tablets was mass spectroscopy. This technique was also used to identify compounds found in DDS samples subjected to UV irradiation in the solid state or in ethanolic or aqueous solutions. An extensive library of mass fragmentation patterns was accumulated during the project, and a section has been included that gives a detailed summary of the results.

MATERIALS AND METHODS

Preparation and Use of the Chemical Actinometer Potassium Ferrioxalate

To evaluate the stability of the UV mercury lamp (General Electric, No. G4T4/1) used in our photolysis studies, we had periodically measured the fluorescence of a standard solution of quinine sulfate.[11] Because these determinations did not give a measure of the light intensity at 254 nm, which is the major emission wavelength of this lamp, we determined the absolute intensity by using the chemical actinometer, potassium ferrioxalate [$K_3Fe(C_2O_4)_3$]. Several chemical actinometers are available to measure the intensity of light, but potassium ferrioxalate, as described by Parker[12] and Hatchard and Parker,[13] is simple to use and has yielded results comparable to data obtained with the standard thermopile-galvanometer system.

Potassium ferrioxalate is light-sensitive. When irradiated with light varying in wavelength from 200 to 500 nm, it degrades according to the following equation:



$$2[Fe(C_2O_4)_3]^{3-} + h\nu \rightarrow 2\ Fe(C_2O_4) + 3[C_2O_4]^{2-} + 2\ CO_2 \quad . \qquad (1)$$

The ferrous ions formed can be readily determined colorimetrically by measuring the absorption of the complex they form with 1,10-phenanthroline. Then the percentage of degradation of the actinometer can be calculated by reference to a standard curve for ferrous ions prepared from ferrous sulfate. Over the 200- to 500-nm range of wavelengths, the quantum efficiency falls from 1.25 at 254 nm to approximately 0.9 at 500 nm. Thus, for each quantum of light absorbed by the actinometer at 254 nm, 1.25 ferrous ions are formed, whereas only 0.9 ions are formed per quantum at 500 nm. To summarize, the following factors make potassium ferrioxalate a reliable general-purpose chemical actinometer[12,13]: the slight decrease in quantum efficiency over the wide wavelength range, the minimal influence of small temperature changes on the quantum efficiency, the constant rate of degradation throughout the range of 0.02 to 72% decomposition, the formation of reaction products that do not significantly absorb the incident light, and the sensitivity of the ferrous ion determination.

To prepare the ferrous sulfate calibration curve, we mixed 1.5 ml of 0.5 to 3.0 × 10^{-4} M ferrous sulfate in 0.1 N H_2SO_4 with 0.5 ml of a solution that was 1.8 N with regard to sodium acetate and 1.1 N with regard to H_2SO_4 and contained 1.0 mg of 1,10-phenanthroline per milliliter. The color was allowed to develop for 30 min at room temperature, and the absorbance of each solution was determined at 510 nm. To utilize the full range of the spectrophotometer (3.0 absorbance

units), we reduced the volume of ferrous sulfate from 3.0 ml (as described by Hatchard and Parker[13]) to 1.5 ml, as described above. The calibration curve was nonlinear above 1.0 absorbance units if 3.0 ml of ferrous sulfate were used, whereas it was linear over the entire absorbance range if 1.5 ml were used. The sensitivity of this modified method was 0.006 ± 0.001 (SD) absorbance units per nanomole of ferrous sulfate, with a coefficient of variation of 2.4%.

We prepared the potassium ferrioxalate by the method described by Parker.[12] Because this actinometer is light-sensitive, all the steps for its preparation and crystallization were carried out in a photographic darkroom under a safelight (Wratten Series 6B). After three crystallizations from water, green crystals of potassium ferrioxalate were obtained that decomposed over a range of 230 to 240^0 (literature decomposition temperature, 230^0).[14] The product, dissolved in 0.1 N H_2SO_4, exhibited the UV-visible spectrum shown as the solid line in Figure 1. The broken line in Figure 1 is the spectrum of this compound reported by Parker.[12] As shown, the spectra are essentially identical from 200 to 450 nm.

We studied the effect of UV irradiation of the actinometer under various illumination conditions. We prepared the actinometer solution by dissolving the $K_3Fe(C_2O_4)_3$ in 0.1 N H_2SO_4 to make a final solution that was 6.78×10^{-3} M (the solution was prepared in the darkroom and stored in glass bottles in the dark). Aliquots of 4 ml were irradiated in 10 x 75-mm quartz tubes situated 3.5 cm from an unfiltered 4-watt low-pressure mercury lamp (General Electric, No. G4T4/1) in the Turner Model 110 fluorometer. This lamp emits 95% of its energy at 254 nm. At various times, the quantity of ferrous ions formed was determined in 1.5 ml of the irradiated sample by the procedure described above for the ferrous sulfate standard curve. We allowed the ferrous-phenanthroline color to develop in the dark to prevent further degradation of the actinometer. To correct for the degradation caused by stray light during the processing of the samples, we determined the ferrous ion content of unirradiated samples subjected to the same handling procedures and subtracted the value from the experimental samples.

Table 1 presents the total nanomoles of ferrous ions formed, the rate of their formation, the percentage of degradation of the actinometer, and the rate of its degradation. Trials 1 and 2 were performed on different days and after the lamp had been on for 40 min. The rate of formation of ferrous ions during the 20-min irradiation period was 30.4 and 28.5 nmoles min^{-1}, respectively; the rate of degradation of the actinometer was 0.112 and 0.105% min^{-1}, respectively, for Trials 1 and 2. The stability of the lamp when it was operated continuously for several hours is shown by the results in Trial 3. After 26 hr of continuous operation, the rate of formation of ferrous ions was 29.0 nmoles min^{-1}, and the rate of degradation of the actinometer was 0.107% min^{-1}; these values are nearly identical to those obtained when the lamp was operated for short periods. In the

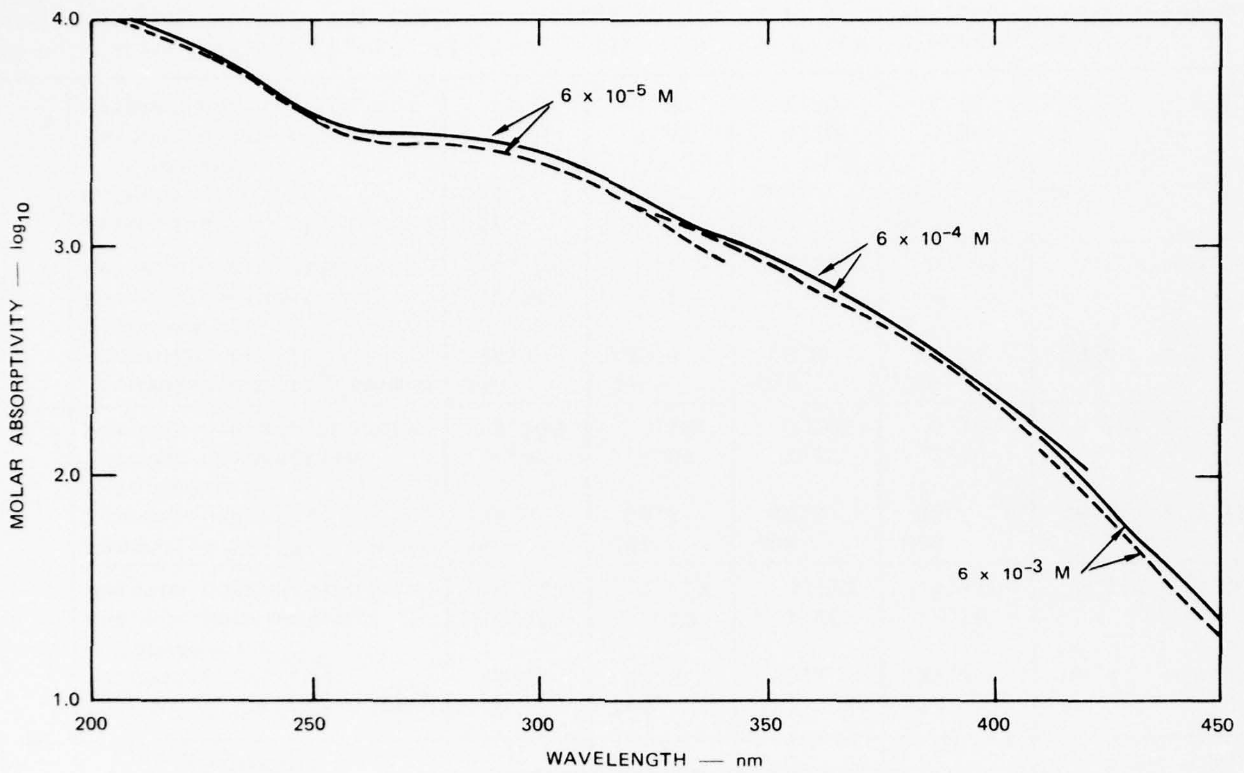


FIGURE 1 ABSORPTION SPECTRUM OF POTASSIUM FERRIOXALATE IN 0.1 N H_2SO_4: (——) PREPARED AT SRI: (– – –) REPORTED BY PARKER

Table 1

DEGRADATION OF THE ACTINOMETER $K_3Fe(C_2O_4)_3$ BY ULTRAVIOLET LIGHT

Trial[a]	Parameter	Irradiation Time (min)				Mean ± SE
		5	10	15	20	
1[b]	Nanomoles Fe (II) formed	156	305	451	595	-
	Nanomoles Fe (II) formed/min	31.2	30.5	30.1	29.8	30.4 ± 0.30
	Percent degradation	0.58	1.12	1.66	2.19	-
	Percent degradation/min	0.116	0.112	0.111	0.110	0.112 ± 0.0013
2[b]	Nanomoles Fe (II) formed	145	281	426	569	-
	Nanomoles Fe (II) formed/min	29.0	28.1	28.4	28.5	28.5 ± 0.19
	Percent degradation	0.53	1.04	1.57	2.10	-
	Percent degradation/min	0.106	0.104	0.105	0.105	0.105 ± 0.0004
3[b]	Nanomoles Fe (II) formed	146	294	433	569	-
	Nanomoles Fe (II) formed/min	29.2	29.4	28.9	28.5	29.0 ± 0.20
	Percent degradation	0.54	1.08	1.60	2.10	-
	Percent degradation/min	0.108	0.108	0.107	0.105	0.107 ± 0.0007
4[c]	Nanomoles Fe (II) formed	257	497	732	966	-
	Nanomoles Fe (II) formed/min	514	497	488	483	496 ± 6.8
	Percent degradation	0.95	1.83	2.70	3.56	-
	Percent degradation/min	1.90	1.83	1.80	1.78	1.83 ± 0.026

[a] Four ml of 6.78×10^{-3} M $K_3Fe(C_2O_4)_3$ in 0.1N H_2SO_4 was irradiated in quartz tubes (10 × 75 mm) in a Turner Model 110 fluorometer. The tubes were situated 3.5 cm from an unfiltered 4-watt low-pressure mercury lamp (General Electric No. G4T4/1; 95% emission at 254 nm).

[b] Trials 1 and 2 were performed 24 hours apart. Each trial was started after the lamp had been on for 40 min. Trial 3 was performed 26 hours after Trial 2, and the lamp remained on during this entire time period. The aperture between the lamp and the sample tube was set at 1X, which is a round hole, area 9.6 mm^2.

[c] Trial 4 was performed with the aperture between the lamp and the sample tube set at 30X, which is a rectangle, area 301 mm^2. Irradiation times were 0.5, 1.0, 1.5, and 2.0 min, respectively.

first three trials, the aperture of illumination between the sample and the lamp was 9.6 mm^2. This area was increased to 301 mm^2 in Trial 4, and the irradiation times were one-tenth those of the first three trials. The rate of formation of ferrous ions increased approximately 17-fold, to 496 nmoles min^{-1}, with a similar increase in the rate of degradation of the actinometer to 1.83% min^{-1}. This latter aperture setting is the one that we have used for all our irradiation studies on DDS, both when it was in solution and in the solid state. In all four trials, the linearity of the rate of the photolytic reaction was shown by the fact that the correlation coefficients of the linear regressions of time versus nanomoles formed or percentage of degradation were equal to or greater than 0.9998. In addition, the coefficients of variation for all rates were equal to or less than 2.9%.

Table 2 shows the effect of visible light on the actinometer solution. We obtained these results by exposing 6.5 ml of the actinometer solution in a quartz tube (10 X 75 mm) on a bench top in a laboratory illuminated by 24 40-w fluorescent lamps (Sylvania, No. F40CW). The minimum distance from the plane of the fluorescent lamps to the sample was 156 cm. Under these conditions, the rate of ferrous ion formation (34.4 nmoles min^{-1}) and the rate of actinometer degradation (0.144% min^{-1}) were very similar to the results obtained from the first three trials (Table 1). The relatively large surface area of the quartz tube exposed to the visible irradiation and the combined wattage of the lamps probably account for the similar photolysis rates.

The intensity of the incident light in terms of quanta per unit of time is quantitated from the equation:

$$I_o^i = \frac{Np}{\Phi_{Fe}\ t\ (1-10^{-\epsilon[A]\ell})}\ , \qquad (2)$$

where Np is the number of ions of product formed during time (t) in minutes; Φ_{Fe} is the quantum efficiency of the actinometer at the wavelength used; and $(1-10^{-\epsilon[A]\ell})$ is the fraction of the incident light absorbed by the actinometer. In this term, ϵ is the molar absorptivity of the actinometer at the wavelength used; $[A]$ is its molar concentration; and ℓ is the light path in centimeters.

The published value[15] for Φ_{Fe} of potassium ferrioxalate at 254 nm is 1.25. The value for ϵ at 254 nm determined experimentally was 3407 (Figure 1); $[A]$ was 6.78×10^{-3}, and ℓ was 1.0 cm. Using these values, the exponential term $10^{-\epsilon[A]\ell}$ equals 10^{-23}, yielding a value of $1-10^{-23}$ or 1, for the fraction of light absorbed. As stated previously, potassium ferrioxalate absorbs essentially 100% of the incident light. Thus, if Np is expressed as the number of ferrous ions formed per minute, the denominator of Eq. (2) equals 1.25.

Table 2

DEGRADATION OF THE ACTINOMETER $K_3Fe(C_2O_4)_3$ BY VISIBLE LIGHT

Parameter	Irradiation Time (min)[a]			Mean ± SE
	10	20	30	
Nanomoles Fe (II) formed	360	651	1041	-
Nanomoles Fe (II) formed/min	36.0	32.6	34.7	34.4 ± 0.99
Percent degradation	1.50	2.71	4.34	-
Percent degradation/min	0.150	0.136	0.145	0.144 ± 0.0040

[a] Approximately 6.5 ml of 6×10^{-3} M $K_3Fe(C_2O_4)_3$ was exposed in a quartz tube (10 × 75 mm) on a bench in a laboratory that was illuminated with 24 40-watt fluorescent lamps (Sylvania No. F40CW).

In Trial 1 of Table 1, the rate of formation of ferrous ions was 30.4 nmoles min^{-1}. Using Avogadro's constant of 6.02×10^{23} molecules per mole, we calculate that 1.83×10^{16} ferrous ions were formed per minute. I_0^1 then equals 1.83×10^{16} divided by 1.25, or 1.46×10^{16} quanta min^{-1}. The area of exposure was 9.6 mm^2, or 0.096 cm^2, yielding 1.52×10^{17} quanta cm^{-2} min^{-1}.

In Trial 4 of Table 1, the aperture area was increased approximately 30-fold to 301 mm^2, or 3.01 cm^2, and the rate of formation of ferrous ions was 496 nmoles min^{-1}. Using the same calculations as above, we obtain 2.98×10^{17} ferrous ions formed per min, 2.38×10^{17} quanta min^{-1}, and 7.91×10^{16} quanta cm^{-2} min^{-1}. These results indicate that at the larger aperture the I_0^1 was approximately one-half that obtained using the small aperture. This probably resulted from the varying intensities of light that struck the sample when the larger aperture was used. Parker[12] found, by means of a traversing micro slit, that variations from the mean intensity for a 35 × 45 mm aperture ranged from about 1.5 times the mean at the center to about 0.5 times the mean at the edge of the aperture. Parker reported that the mean intensity of the 125-w mercury lamp situated 18 cm from the actinometer solution was 2.4×10^{17} quanta cm^{-2} min^{-1}. Numerous factors contribute to this parameter; nevertheless, under standard conditions, the ferrioxalate actinometer can serve as a useful standard to calibrate light sources throughout the range of 200 to 500 nm.

Mass Fragmentation Patterns of Diphenyl Sulfones

During this project, numerous mass spectra were obtained of known diaminodiphenyl sulfones and of several compounds isolated from pharmaceutical preparations of DDS before and after they had been subjected to UV irradiation. The identity of many of these compounds was confirmed by comparing their mass spectra with the fragmentation pattern of the authentic compounds. We have included most of this information in this section. All spectra were obtained under the direction of Dr. D. Thomas, Senior Mass Spectroscopist, SRI, using an LKB 9000 GC-MS equipped with a DEC Model PDP-12 computer.

The fragmentation pattern for diaminodiphenyl sulfones is shown in Figure 2. It is also a general pattern that is common to diphenyl sulfones.[16] The most probable structures of the fragments are shown, but in some cases only the m/e of the fragment is indicated. As shown in Figure 2, the sulfone parent ion rearranges to the aminodiphenyl sulfinate ester (A) followed by cleavage of the S-O ester bond to yield an aminophenylsulfoxy ion (G) and the aminophenyloxy ion (H). In the parent sulfone ion, the oxygen in the ester bond may migrate toward either phenyl group and therefore, in asymmetric diphenyl sulfones, two different phenylsulfoxy and two different phenyloxy fragments can be produced. For ortho-substituted diphenyl sulfones, the relative abundance of fragments B, D, E and I is considerably greater than for other isomers. To form B, the parent ion can lose H_2O and B can then lose SO to form E. Fragment D can be derived from the parent ion by the loss of HSO_2 and I

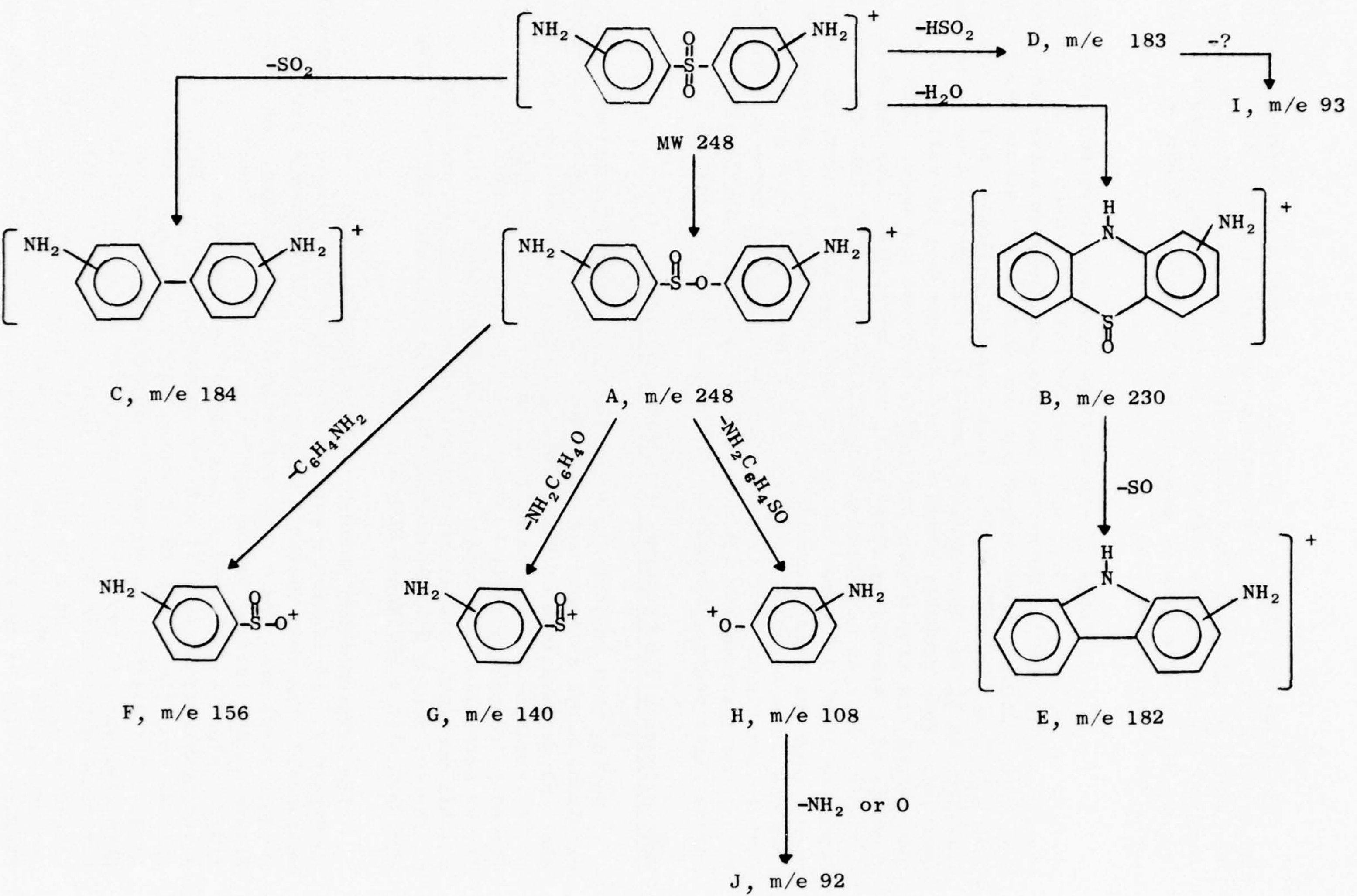


Figure 2. Mass fragmentation pattern of diaminodiphenyl sulfones.

can then form by a further loss of 90 mass units. Other fragments common to all diaminodiphenyl sulfones are C, F, and J, which arise from the loss of SO_2, $C_6H_4NH_2$, and either NH_2 or O from fragment H (m/e 108). The last fragment that was common to all the diaminodiphenyl sulfones, but is not shown in Figure 2, was one with a m/e of 65, which is probably the HSO_2^+ ion.

The relative abundance of the above fragments found in the mass spectra of four isomeric diaminodiphenyl sulfones is summarized in Table 3. In all cases, the relative abundance of the fragments is expressed as a percentage of the parent ion. The effect of ortho-substitution is readily apparent from the fragmentation pattern of 2,4'-DDS. Fragments B, D, E, and I occur with much greater relative abundance than do these fragments from the other isomers. This ortho-effect follows a similar pattern previously described for isomeric methylated diphenyl sulfones.[16] Although the m/e of each fragment is the same for all isomers, each one exhibits its own fragmentation pattern that is quite distinct from that of other isomers.

Numerous derivatives of 4,4'-DDS were also included in the mass spectral studies. The fragmentation patterns of the acetamido, formamido, monomethyl, and phenylhydrazino derivatives are summarized in Table 4. Wherever possible, the letter designations of Figure 2 were used to identify fragments. Thus, the fragment R_1R_2B (Column 5) would be the parent ion (Column 1) less water and R_1R_2C (Column 6) would be the parent ion less SO_2. Except for the phenylhydrazino and the methylamino compounds, the relative abundance of fragments resulting from the loss of H_2O, SO_2, HSO_2, and H_2SO_2 was very low or not measurable. For the disubstituted acetamido and formamido compounds, intense peaks were observed that corresponded to the respective monosubstituted compounds. In all compounds, there was an abundant fragment that represented the substituted aminophenylsulfoxy ion (R_2G), but it was most prominent for the phenylhydrazino, the diformamido, and the methylamino compound. These same three compounds contributed the most to the substituted aminophenyloxy ion (R_2H). That the acetyl and formyl groups are labile is shown by the abundant fragments (R_2) that corresponded to the acetyl and formyl ions. This was also accompanied by a prominent fragment (A) that corresponded to the parent ion of DDS. This latter fragment occurred to a much smaller extent for the phenylhydrazino and methylamino compounds. The remainder of the fragmentation pattern for each compound was similar to that for DDS, but each compound exhibited its own unique relative abundance pattern.

The last group of diphenyl sulfone compounds studied were substituted in the 4-position with NH_2 or $NHCOCH_3$ and in the 4'-position with NO_2, Cl, or H. Table 5 summarizes the mass fragmentation patterns of these compounds. None of the compounds lost any groups bonded directly to the phenyl ring, but the acetyl group (R_1) of the acetamido compound was cleaved. The loss of SO_2 occurred to an appreciable extent from the 4-amino-4'-chloro and from the 4-amino compound. Only the 4-amino compound exhibited any appreciable loss of HSO_2 or H_2SO_2. In all

Table 3

MAJOR FRAGMENTS FOUND BY MASS SPECTROMETRY OF ISOMERIC DIAMINODIPHENYL SULFONES

Major Fragments			Relative Abundance (% of Parent)			
Designation[a]	By Loss Of[b]	m/e	4,4'-DDS	3,4'-DDS	2,4'-DDS	3,3'-DDS
A	—	248	100	100	100	100
B	H_2O (18)	230	< 0.09	0.1	14.1	< 0.1
C	SO_2 (64)	184	5.7	35.0	24.8	13.2
D	HSO_2 (65)	183	3.6	5.0	146	6.2
E	H_2SO_2 (66)	182	1.1	2.4	55.6	3.9
F	$C_6H_4NH_2$ (92)	156	2.4	4.0	9.9	3.8
G	$NH_2C_6H_4O$ (108)	140	52.6	31.8	14.4	30.8
H	$NH_2C_6H_4SO$ (140)	108	95.6	48.8	23.9	2.7
I	? (155)	93	7.1	5.8	62.2	3.8
J	? (156)	92	27.9	24.1	27.2	21.6
K[c]	? (183)	65	34.0	28.2	41.8	26.0

[a] Probable structures are shown in Figure 2.

[b] Mass of structural element(s) lost is indicated in parenthesis.

[c] This ion is probably HSO_2^+ and is not shown in Figure 2.

Table 4

FRAGMENTATION PATTERNS OF SUBSTITUTED 4,4'-DIAMINODIPHENYL SULFONES

Diphenyl Sulfone Derivative	4,4'-Diamino-diphenyl Sulfone Substituent		Major Fragments and Relative Abundances (% of Parent)[a]															
	R_1	R_2	R_1R_2A	R_1R_2B	R_1R_2C	R_1R_2D	R_1R_2E	R_2A[b]	R_2G	R_2H	R_2	A	F	G	H	I	J	K
4-amino-4'-(2-phenylhydrazino)-	-H	$-NHC_6H_5$	100	< 0.2	4.4	5.3	1.0	-	11.2	23.0	4.1	0.3	2.4	5.1	4.9	2.7	4.1	7.0
4,4'-diacetamido-	$-COCH_3$	$-COCH_3$	100	< 0.2	< 0.2	< 0.2	< 0.2	97.0	4.8	< 0.2	160	120	5.3	67.4	93.9	24.3	16.7	0.8
4,4'-diformamido-	-CHO	-CHO	100	< 0.5	1.0	1.0	0.5	252	36.7	20.0	111	21.0	27.6	151	280	35.7	109	120
4-amino-4'-acetamido-	-H	$-COCH_3$	100	< 0.2	0.2	< 0.2	< 0.2	-	7.4	< 0.2	53.9	80.4	8.7	74.1	98.0	35.2	27.8	40.9
4-amino-4'-formamido-	-H	-CHO	100	< 0.3	1.0	0.3	< 0.3	-	5.0	0.8	9.4	105	10.8	96.1	163	13.6	58.3	63.3
4-amino-4'-methylamino-	-H	$-CH_3$	100	< 0.1	5.7	7.2	1.1	-	32.9	77.7	-[c]	4.4	3.9	18.7	18.8	3.3	11.2	30.3

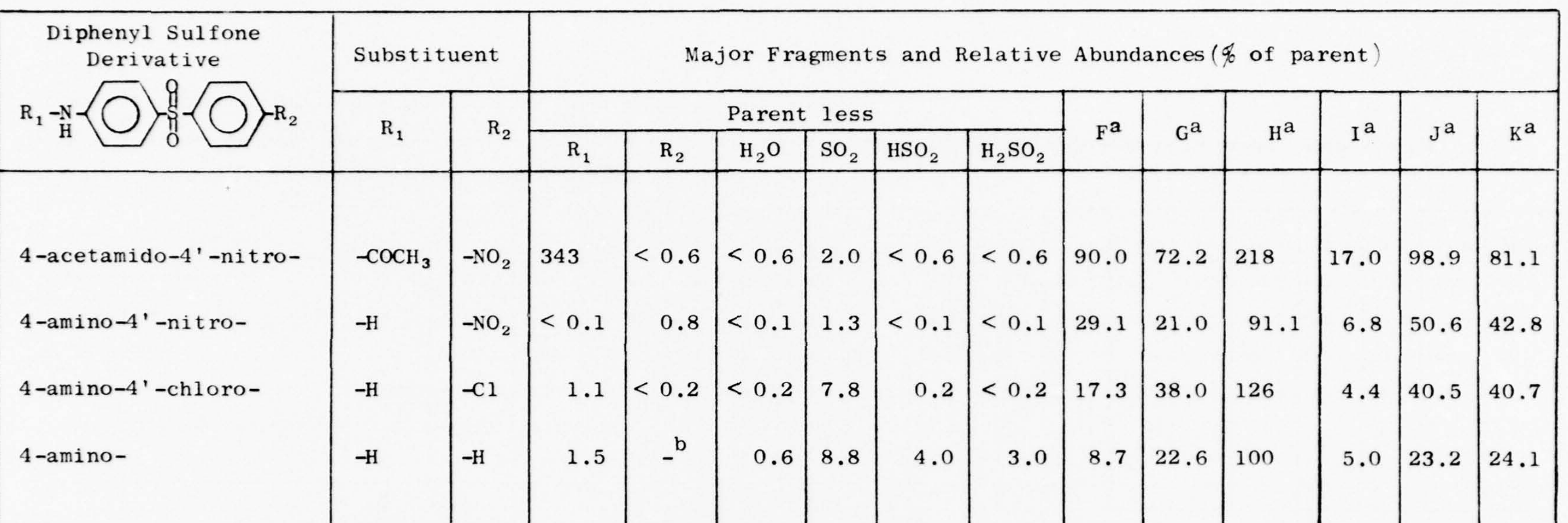
[a] Letters used refer to the fragmentation pattern of the 4,4'-isomer of Table 3.

[b] In the disubstituted compounds, R_2A is not distinguishable from R_1A. In the monosubstituted compounds, R_2A corresponds to the parent compounds, R_1R_2A.

[c] The range of the fragmentation spectrum did not include fragments with m/e of $\leq$ 15.

Table 5

FRAGMENTATION PATTERNS OF SUBSTITUTED DIPHENYL SULFONES



Diphenyl Sulfone Derivative (R_1-NH-C_6H_4-SO_2-C_6H_4-R_2)	Substituent		Major Fragments and Relative Abundances (% of parent)											
			Parent less											
	R_1	R_2	R_1	R_2	H_2O	SO_2	HSO_2	H_2SO_2	F[a]	G[a]	H[a]	I[a]	J[a]	K[a]
4-acetamido-4'-nitro-	$-COCH_3$	$-NO_2$	343	< 0.6	< 0.6	2.0	< 0.6	< 0.6	90.0	72.2	218	17.0	98.9	81.1
4-amino-4'-nitro-	-H	$-NO_2$	< 0.1	0.8	< 0.1	1.3	< 0.1	< 0.1	29.1	21.0	91.1	6.8	50.6	42.8
4-amino-4'-chloro-	-H	-Cl	1.1	< 0.2	< 0.2	7.8	0.2	< 0.2	17.3	38.0	126	4.4	40.5	40.7
4-amino-	-H	-H	1.5	-[b]	0.6	8.8	4.0	3.0	8.7	22.6	100	5.0	23.2	24.1

[a] Letters used refer to the fragmentation pattern of the 4,4'-diamino isomer of Table 3.

[b] Parent less R_1 or R_2 yields a fragment with same m/e.

compounds, including 4-acetamido-4'-nitrodiphenyl sulfone after loss of the acetyl group, one-half of the molecule was identical to DDS, and thus all compounds exhibited the characteristic fragments representing the aminophenylsulfoxy and aminophenyloxy ions. The respective nitro- or chlorophenylsulfoxy or oxy ions were not detected. The final fragment common to all the sulfones was one of m/e 65 (K), which probably represents the HSO_2^+ ion.

Confirmation of the identity of 4-amino-4'-hydroxydiphenyl sulfone was performed after this compound had been trimethylsilylated to yield a disubstituted derivative wherein the proton on the hydroxyl group and one proton on the amino group were replaced with a trimethylsilyl group. Fragmentation patterns were obtained following gas chromatography but were determined using a low electron beam energy of about 12 electon volts. As a result, only the parent ion (m/e 393) was observed, with a small amount of the monosilyl derivative (m/e 321).

RESULTS

Studies on the Possible Autocatalytic Decomposition of DDS in Water

Previously[11] we observed that DDS was decomposed by UV irradiation of the compound dissolved in various solvents. The rate of decomposition of DDS, expressed as the half-time of disappearance ($T\frac{1}{2}$), depended on the solvent employed. Thus, $T\frac{1}{2}$ values for DDS at 100 μg/ml were 1.6 hr in ethylene dichloride, 6.4 hr in ethyl acetate, 4.5 hr in ethanol, and 1.6 hr in water. The rate of decomposition of DDS was also concentration-dependent, being more rapid at lower concentrations.

To examine the possibility that DDS decomposition, once started, may proceed autocatalytically, we performed further studies on aqueous solutions of DDS. To ensure that the water employed was completely free of traces of organic matter, we redistilled previously deionized and distilled water from an alkaline potassium permanganate solution. The water was stored in low-alkali glass bottles.

Concentrations of DDS of 10, 100, and 170 μg/ml water were prepared from DDS tablets of Lots 111 CC and 590 CD that had been triturated in water. Approximately 2 ml of these solutions and the same concentrations of the DDS standard were irradiated, as previously described,[11] in the Turner fluorometer for 0.4, 2, and 4 hr, respectively. These irradiation times had been found previously to cause decomposition of approximately 50% of the starting concentrations of the DDS standard in water. Following irradiation, samples were split and stored in the dark at 4° or 37°. Solutions containing initial concentrations of 100 and 170 μg/ml were sampled at various times during storage by taking 10- and 5-μl aliquots, respectively. After diluting the aliquots to 1.0 ml with ethyl acetate, we measured the DDS content by high-pressure liquid chromatography (HPLC) on a silica gel column.* The amount of DDS in each stored solution containing a concentration of 10 μg/ml before irradiation was determined after evaporating a 25-μl aliquot to dryness under a stream of nitrogen on a water bath (48°). The residue was dissolved in 250 μl of ethyl acetate, and a 100-μl aliquot was subjected to HPLC. Identical concentrations of nonirradiated samples stored under the same conditions served as controls.

The results of storage at 4° are shown in Table 6. Immediately after irradiation and before storage, the recovery of DDS ranged from 30.7% in the DDS standard at 10 μg/ml to 56.3% in Lot 111 CC at 170 μg/ml.

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*LiChrosorb SI-60, 5 μm, 3.2 × 250 mm column (Altex Scientific, Inc.) using ethyl acetate as the developing solvent.

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Table 6

STABILITY OF DDS IN WATER AT 4° FOLLOWING IRRADIATION

Storage Day[a]	DDS Standard (μg/ml)[b]			Lot 111 CC (μg/ml)[b]			Lot 590 CD (μg/ml)[b]		
	10	100	170	10	100	170	10	100	170
0	30.7	53.7	52.5	41.9	53.8	56.3	34.3	53.5	33.6
1	43.6	54.5	53.7	—[c]	58.9	60.0	—[c]	52.6	36.2
2	32.7	53.2	56.1	37.2	61.2	60.1	36.1	55.2	35.9
7	28.7	46.8	54.1	49.6	58.6	60.4	28.7	51.1	37.5
14	25.7	54.8	61.0	40.3	62.8	60.5	38.9	54.7	37.6
21	25.7	—[c]	—[c]	55.8	—[c]	—[c]	23.1	—[c]	—[c]
28	22.8	48.2	56.0	42.6	45.9	42.7	31.5	52.9	33.5
35	—[c]	—[c]	—[c]	—[c]	52.9	53.0	—[c]	51.1	35.4
$T_{\frac{1}{2}}$ (days)[d]	43.6	224	-292	-136	150	111	98.6	772	984
r	-0.8179	-0.4897	0.4848	0.3798	-0.6005	-0.6841	-0.4151	-0.4183	-0.2115
p	< 0.05	NS	NS	NS	NS	NS	NS	NS	NS

[a] Aqueous concentrations of DDS of 10, 100, and 170 μg/ml were irradiated 0.4, 2, and 4 hr, respectively. The DDS concentrations were immediately determined and then the samples were stored in the dark at 4° and assayed on the indicated days.

[b] The values are percentages of DDS found relative to unirradiated samples.

[c] Not assayed.

[d] Values are calculated from the regression lines of the logarithmic disappearance of DDS.

The samples were stored up to 35 days and the percent recovery of DDS on the various days was used to calculate a $T\frac{1}{2}$ using nonlinear regression analysis. Of all the samples, only the DDS standard at a concentration of 10 μg/ml demonstrated a significantly progressive loss of DDS, with an apparent $T\frac{1}{2}$ of 43.6 days. These results do not indicate that irradiated solutions of DDS derived from tablets undergo autocatalytic decomposition at 4^0.

Table 7 summarizes the results obtained from samples stored at 37^0. Only the solution of the DDS standard and that of DDS from Lot 590 CD at an initial concentration of 10 μg/ml demonstrated further decomposition, with statistically significant $T\frac{1}{2}$ values of 17.6 and 40.5 days, respectively. Some samples stored at either 4^0 or 37^0 showed apparent increases in DDS concentration during the prolonged storage period, but these were probably due to slight evaporative changes that occurred in the small volumes of the samples. Thus, the destructive effect of UV irradiation on aqueous solutions of DDS seemed to occur only during the time of actual exposure. Little significant autocatalytic decomposition of DDS was observed in samples stored at either 4^0 or 37^0.

Tests on the Stability of DDS in Pre-Irradiated Water

During previous irradiation studies of DDS in ethylene dichloride,[11] we found that pre-irradiating the solvent for only 0.5 hr resulted in essentially a rapid and complete loss of fluorescence of DDS when this pre-irradiated solvent was used to prepare dilute solutions (10 μg/ml) of DDS. The loss of fluorescence, although not necessarily a specific indicator of chemical destruction because of the possibility of fluorescent quenching, was verified as a loss of DDS by HPLC. Thus, prior irradiation of ethylene dichloride formed products that decomposed DDS.

We have examined the possible occurrence of this effect in water. Water was pre-irradiated for 0.5 to 16 hr in the Turner fluorometer and then used to make solutions of DDS by diluting 0.2 ml of stock aqueous solutions (100 μg/ml) of the DDS standard and of DDS tablets (Lots 111 CC and 590 CD) with 1.8 ml of the pre-irradiated water. Immediately after mixing, 25 μl of each aqueous sample was evaporated to dryness as previously described. The residue was dissolved in 250 μl of ethyl acetate, of which 100 μl was subjected to HPLC. The remainder of each aqueous sample was then stored in the laboratory at room temperature under ambient conditions of light and temperature. The concentration of DDS in the samples was determined at various times over a period of 35 days.

The results obtained are summarized in Table 8. In contrast to the results using pre-irradiated ethylene dichloride, we found no initial loss of DDS or any progressive loss of DDS upon storage, regardless of the length of time the water was pre-irradiated. Rates of change were calculated using nonlinear regression. Only two samples from Lot 111 CC that were mixed with water that had been pre-irradiated 0.5 and 1 hr showed statistically significant $T\frac{1}{2}$ values of 96 and 115 days,

Table 7

STABILITY OF DDS IN WATER AT 37° FOLLOWING IRRADIATION

Storage Day[a]	DDS Standard (μg/ml)[b]			Lot 111 CC (μg/ml)[b]			Lot 590 CD (μg/ml)[b]		
	10	100	170	10	100	170	10	100	170
0	30.7	53.7	52.5	41.9	53.8	56.3	34.3	53.5	33.6
1	43.6	53.5	56.9	—[c]	60.6	59.3	—[c]	50.3	36.7
2	38.6	51.2	57.0	41.1	62.5	59.7	41.7	56.4	37.9
7	28.7	57.2	57.9	45.0	59.9	62.9	36.1	53.4	34.0
14	16.8	60.6	67.2	43.4	65.5	56.9	38.9	54.7	34.8
21	15.8	—[c]	—[c]	44.2	—[c]	—[c]	25.0	—[c]	—[c]
28	13.9	64.9	67.4	7.8	54.7	59.0	24.1	52.0	29.4
35	—[c]	—[c]	—[c]	—[c]	62.0	66.9	—[c]	49.7	39.1
$T_{\frac{1}{2}}$ (days)[d]	17.6	-88.9	-83.9	15.9	-3103	-288	40.5	428	878
r	-0.9333	0.9478	0.8870	-0.6871	0.0437	0.5648	-0.8197	-0.5044	-0.1170
p	< 0.005	< 0.02	< 0.02	NS	NS	NS	< 0.05	NS	NS

[a] Same as footnote **a** in Table 6, except that samples were stored at 37°.

[b] Same as footnote **b** in Table 6.

[c] Not assayed.

[d] Same as footnote **d** in Table 6.

Table 8

EFFECT OF PRE-IRRADIATION OF WATER AND STORAGE ON THE STABILITY OF DDS SOLUTIONS

Pre-irradiation Time (hr)[a]	Days of Storage[b]					
	0	1	7	14	28	35
DDS Standard						
0.5	99.0	103	109	99.0	94.1	104
1	92.0	106	106	97.0	87.0	93.0
8	103	114	103	103	101	101
16	106	93.5	96.3	99.1	88.9	107
Lot 111 CC						
0.5	102	—[c]	108	100	82.4	81.5
1	101	—[c]	99.1	101	88.9	79.6
8	95.5	91.1	111	92.0	100	—[c]
16	103	119	124	115	107	—[c]
Lot 590 CD						
0.5	105	—[c]	109	115	99.1[d]	98.2[e]
1	101	—[c]	94.8	102	87.1[d]	104[e]
8	99.2	94.4	96.0	94.4	79.2[d]	92.8[e]
16	100	108	97.4	98.3	89.7[d]	97.4[e]

[a] Water was irradiated in the Turner fluorometer for the indicated times and then used to make DDS solutions of 10 μg/ml.

[b] Samples were stored in the laboratory at 22°. Values are the percent recovery of DDS on the various days.

[c] Not assayed.

[d,e] Assayed on days 21 and 28, respectively.

respectively. Thus, in contrast to the very short $T\frac{1}{2}$ of 0.4 hr when DDS was irradiated in water at the same concentration, we observed that pre-irradiation of water did not cause an immediate loss of DDS or any consistent progressive loss on storage.

Studies on the Ultraviolet Irradiation of Solid DDS

To examine the effects of UV irradiation on solid DDS, we designed a system in which DDS, deposited on glass rectangles (9 × 13 mm) cut from 0.25-mm thick microscope slides, was exposed to UV irradiation in the Turner fluorometer. A 50-μl aliquot of ethanol containing 5 or 50 μg of analytic DDS was allowed to air-dry on these glass rectangles. An adaptor was fabricated that allowed two such rectangles to be held perpendicular to the light path and 3.5 cm from the fluorometer lamp. The entire area of each slide was exposed. Recoveries of DDS were initially determined by eluting each slide with 4.5 ml of ethyl acetate at 50^0 and analyzing each eluate by HPLC.[17] However, subsequent elutions with methanol demonstrated that ethyl acetate did not recover all of the DDS remaining after irradiation.[18] All recoveries reported here were determined by eluting each slide with 2 ml of methanol at 50^0 for 15 min and analyzing each eluate by our chromatographic-fluorometric HPLC method.[19]

Table 9 shows the mean (± SE) recoveries of analytic DDS from unirradiated and irradiated slides. Unirradiated slides containing DDS stored for the same length of time served as controls, and the recoveries from these slides were used to correct the recoveries of irradiated slides. When 5 μg of DDS was irradiated, the mean recovery at 4 hr was 52%; when 50 μg was irradiated for the same length of time, 93% was recovered. The recovery of DDS from nonirradiated slides ranged from a low of 82% to a high of 102%. No apparent relationship was observed between the percentage of recovery of unirradiated DDS and the length of time DDS remained on the slide.

That the recovery of analytic DDS decreased with the length of the irradiation time is shown clearly in Figure 3, a plotting of the individual corrected percentages of recovery from which were generated the means in Table 9. Linear regression analysis was used to calculate the slopes of the regression lines. When 5 μg DDS was irradiated, the slope of the regression line indicated that the loss of DDS occurred at a rate of 3.2% hr^{-1}. Similar analysis of the data when 50 μg DDS was irradiated showed that DDS disappeared at a rate of only 0.5% hr^{-1}.

To measure the effect of UV irradiation on DDS tablets, samples from Lots 111 CC and 590 CD were used because tablets from these lots (stored at Tracy, CA) were previously evaluated for their stability to UV irradiation and for autocatalysis.[17] Slurries were prepared by triturating a tablet in absolute ethanol. They were then sonicated for about 2 min and 50-μl aliquots containing 5 or 50 μg of DDS were placed on glass slides as described above. Irradiated and nonirradiated slides were eluted with methanol, and the DDS content of each eluate was determined by HPLC.

Table 9

RECOVERY OF ANALYTIC DDS FROM GLASS SLIDES
BEFORE AND AFTER IRRADIATION
(Application from Ethanolic Solution)[a]

Irradiation Time (hr)[b]	Percent Recovery[c]		
	Unirradiated	Irradiated	Corrected
5 μg irradiated			
2	85 ± 1.9 (7)	53 ± 2.6 (8)	63 ± 3.1
4	82 ± 2.5 (8)	42 ± 2.7 (8)	52 ± 3.6
6	101 ± 7.8 (7)	47 ± 3.4 (8)	45 ± 3.8
7	95 ± 4.0 (2)	36 ± 1.5 (2)	38 ± 2.0
8	84 ± 0.5 (2)	46 ± 0.0 (2)	55 ± 0.0
50 μg irradiated			
4	92 ± 3.6 (6)	84 ± 2.7 (7)	93 ± 5.0
7	102 ± 0.4 (5)	93 ± 0.9 (4)	91 ± 0.9
16	93 ± 3.3 (11)	89 ± 3.9 (12)	88 ± 2.4
24	91 ± 2.6 (4)	74 ± 0.5 (4)	81 ± 0.6

[a]DDS was added in 50 μl of ethanol to glass slides 9 mm × 13 mm and allowed to air-dry. DDS was recovered before and after irradiation by heating the slide for 15 min in 2 ml of methanol at 50°.

[b]Slides were irradiated 3.5 cm from the unfiltered 4-watt mercury lamp (GE No. G4T4/1) of the Turner fluorometer.

[c]Unirradiated slides were stored in the dark during each irradiation period and the percent recovery from these slides was used to calculate a corrected percent recovery for irradiated slides. The recoveries are means ± SE of the number of slides indicated in parentheses.

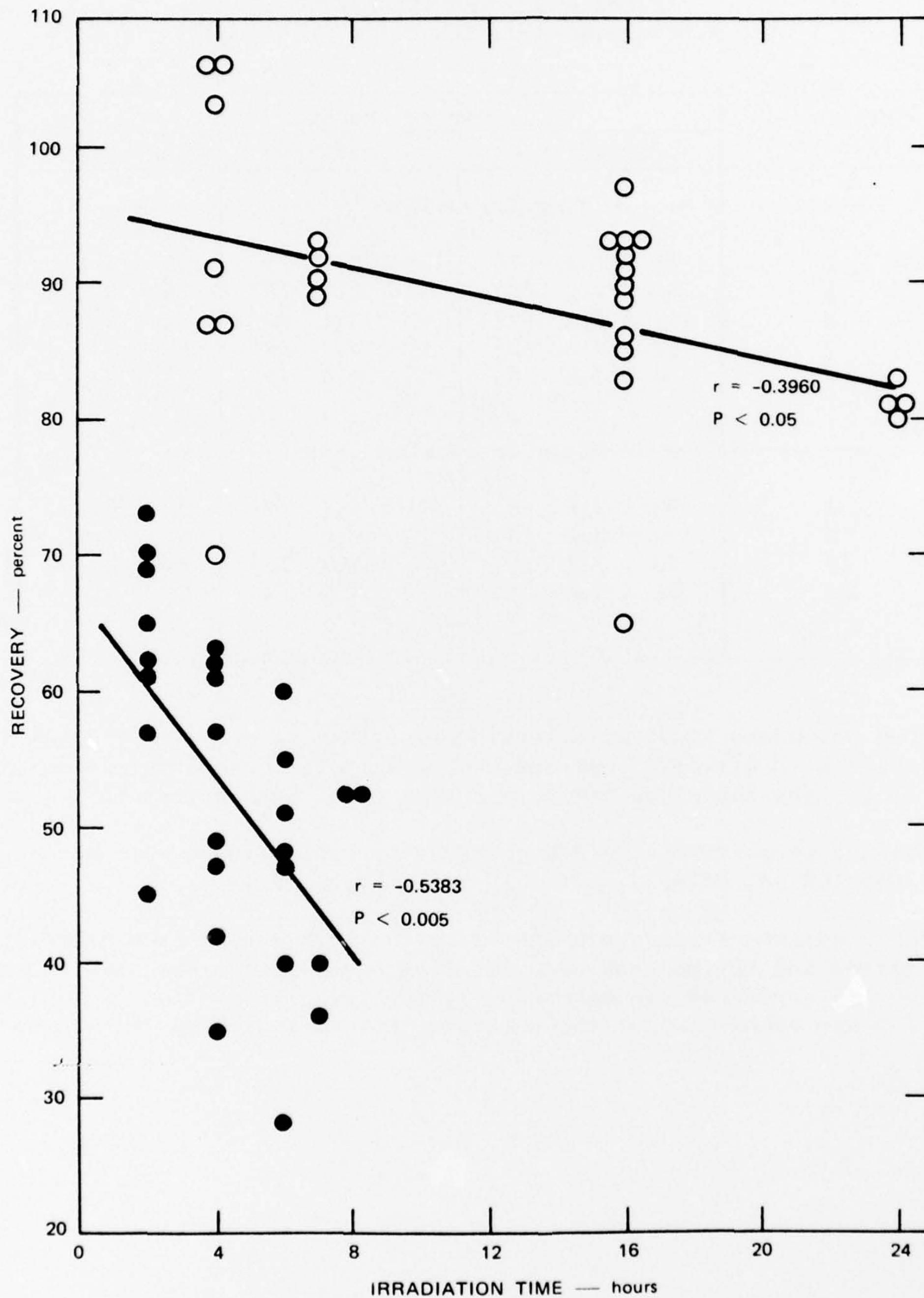

FIGURE 3 EFFECT OF ULTRAVIOLET IRRADIATION OF 5 μg (SOLID CIRCLES) AND 50 μg DDS (OPEN CIRCLES) ON GLASS SLIDES

Table 10 shows the mean (± SE) recoveries of tablet DDS from unirradiated and irradiated slides. When 5 μg of DDS was irradiated, the rate of loss of DDS by linear regression analysis was 3.2 and 2.6% hr^{-1} for Lots 111 CC and 590 CD, respectively. The 95% confidence limits for the degradation rates of these two samples were 2.3 to 4.1% hr^{-1} and 2.0 to 3.2% hr^{-1}, respectively, indicating that the rate of degradation of DDS in the two lots was not different. Also, these rates were not significantly different from the degradation rate obtained for 5 μg of analytic DDS.

The lower portion of Table 10 shows the results when 50 μg of DDS from the same tablets was irradiated. The rate of loss for both lots was 0.5% hr^{-1}, a value that was not different from the rate of degradation of 50 μg of analytic DDS. In all cases, unirradiated slides containing DDS, which were stored for the same amount of time, served as controls. Mean recovery of DDS from the control slides was 96% (range, 86 to 102%). Only small differences were noted among the recoveries from the different lots or from slides to which different quantities of DDS had been applied.

Figures 4 and 5 show the linear relationships between the corrected recovery of DDS and the irradiation time for 5 and 50 μg of DDS from Lots 111 CC and 590 CD, respectively. In all cases, the linear correlation coefficients indicated that the slopes of the regression lines were significantly different from zero.

All of the above studies were performed on DDS deposited on the glass slides from an ethanolic solution or slurry. It is possible that trace amounts of ethanol, which may have remained on the slide after evaporation, affected the rate of destruction of the DDS. To investigate this possibility, 5 μg of analytic DDS in 50 μl of distilled water was air-dried on 9 x 13-mm glass slides. The slides were then desiccated in vacuo over a mixture of activated silica gel (Central Scientific Co., indicating, 6-16 mesh) and anhydrous $CaSO_4$ (W. A. Hammond Drierite Company, indicating, 8 mesh) for 24 hr prior to being stored or irradiated. The irradiations and the determination of the recoveries of DDS were performed as described above.

Table 11 shows the mean (± SE) recoveries from unirradiated and irradiated slides of analytic DDS applied from an aqueous solution. Figure 6 shows the linear relationship between the corrected recovery of DDS and the irradiation time. The rate of loss of DDS determined by linear regression analysis was 0.7% hr^{-1}. This is more than four times slower and significantly different ($P < 0.01$) from the rate of 3.2% hr^{-1} determined above for 5 μg of analytic DDS applied from an ethanolic solution. The former rate was measured approximately six months after the latter rate. To determine whether a long-term decline in the output of the mercury lamp used for the irradiations was responsible for this difference in rate of loss, the quantum flux of

Table 10

RECOVERY OF TABLET DDS FROM GLASS SLIDES BEFORE AND AFTER ULTRAVIOLET IRRADIATION

Irradiation Time (hr) [a]	Percent Recovery [b]					
	Lot 111 CC			Lot 590 CD		
	Unirradiated	Irradiated	Corrected	Unirradiated	Irradiated	Corrected
5 μg irradiated [d]						
2	102 ± 2.9 [c]	86 ± 1.7	85 ± 1.5	96 ± 1.6	76 ± 2.0	78 ± 2.1
4	95 ± 0.7 [c]	60 ± 3.1	63 ± 3.1	98 ± 1.4	63 ± 2.6	64 ± 2.7
16	99 ± 0.9 [c]	34 ± 2.8	35 ± 2.9	98 ± 1.1	38 ± 2.0	39 ± 2.2
Degradation rate (% hr^{-1})			3.2			2.6
50 μg irradiated [d]						
4	94 ± 2.5 [c]	94 ± 2.6	100 ± 2.6	97 ± 0.9 [c]	96 ± 1.0	100 ± 1.0
16	86 ± 1.4	80 ± 0.9	93 ± 1.2	95 ± 0.6 [c]	88 ± 0.6	92 ± 0.8
40	98 ± 0.9	81 ± 1.8	82 ± 1.8	91 ± 1.0 [c]	74 ± 1.5	81 ± 1.5
Degradation rate (% hr^{-1})			0.5			0.5

[a] Slides were irradiated 3.5 cm from the unfiltered 4-watt mercury lamp (GE No. G4T4/1) of the Turner fluorometer.

[b] Unirradiated slides were stored in the dark during each irradiation period and the percent recovery from these slides was used to calculate a corrected percent recovery for irradiated slides. The recoveries are means ± SE of 4 slides unless otherwise indicated.

[c] Mean ± SE of 3 slides.

[d] From an ethanolic slurry of a tablet, 50 μl was added to glass slides 9 mm × 13 mm and allowed to air-dry. DDS was recovered before and after irradiation by heating the slide for 15 min in 2 ml of methanol at 50°.

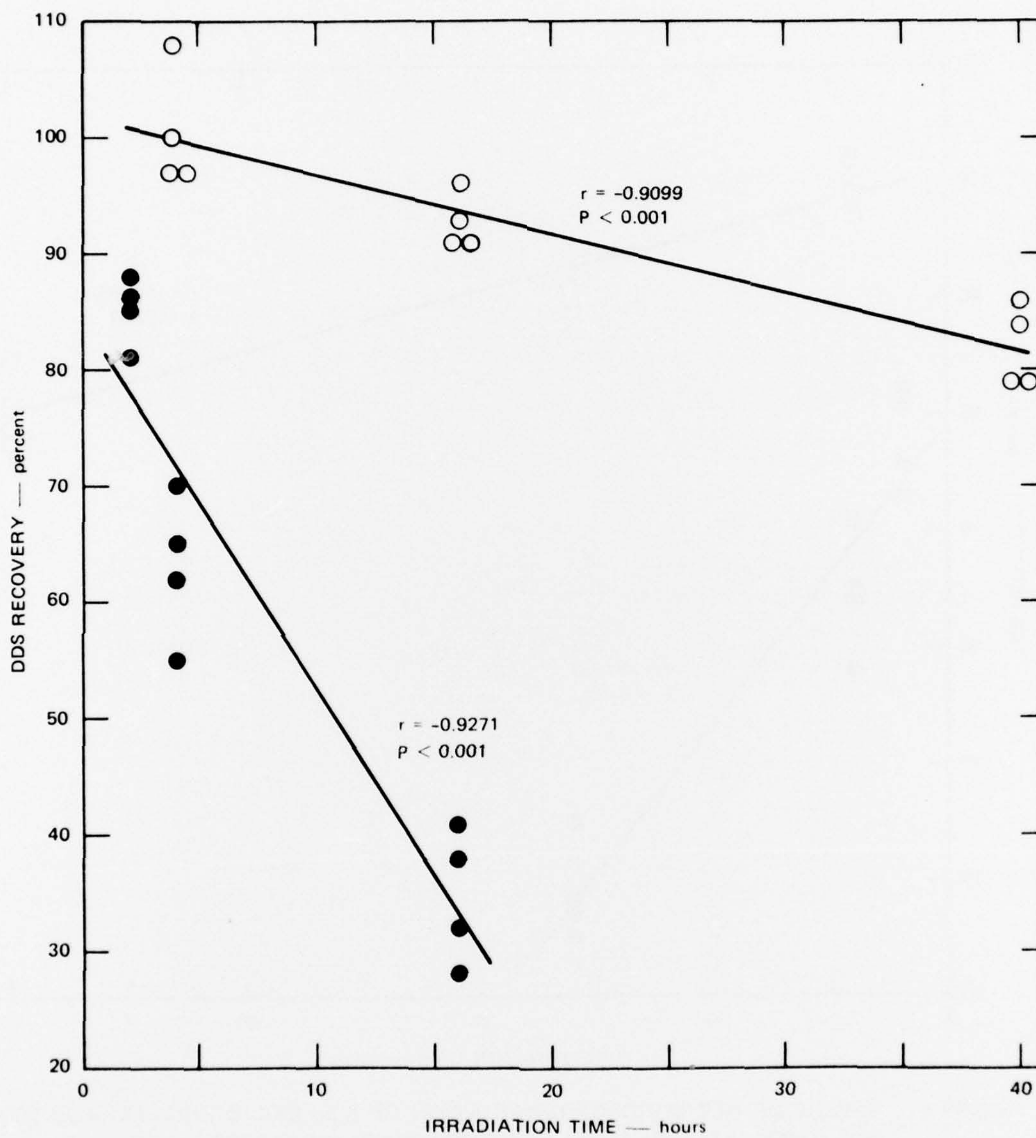

FIGURE 4 EFFECT OF ULTRAVIOLET IRRADIATION OF 5 μg (SOLID CIRCLES) AND 50 μg DDS (OPEN CIRCLES) FROM LOT IIICC ON GLASS SLIDES

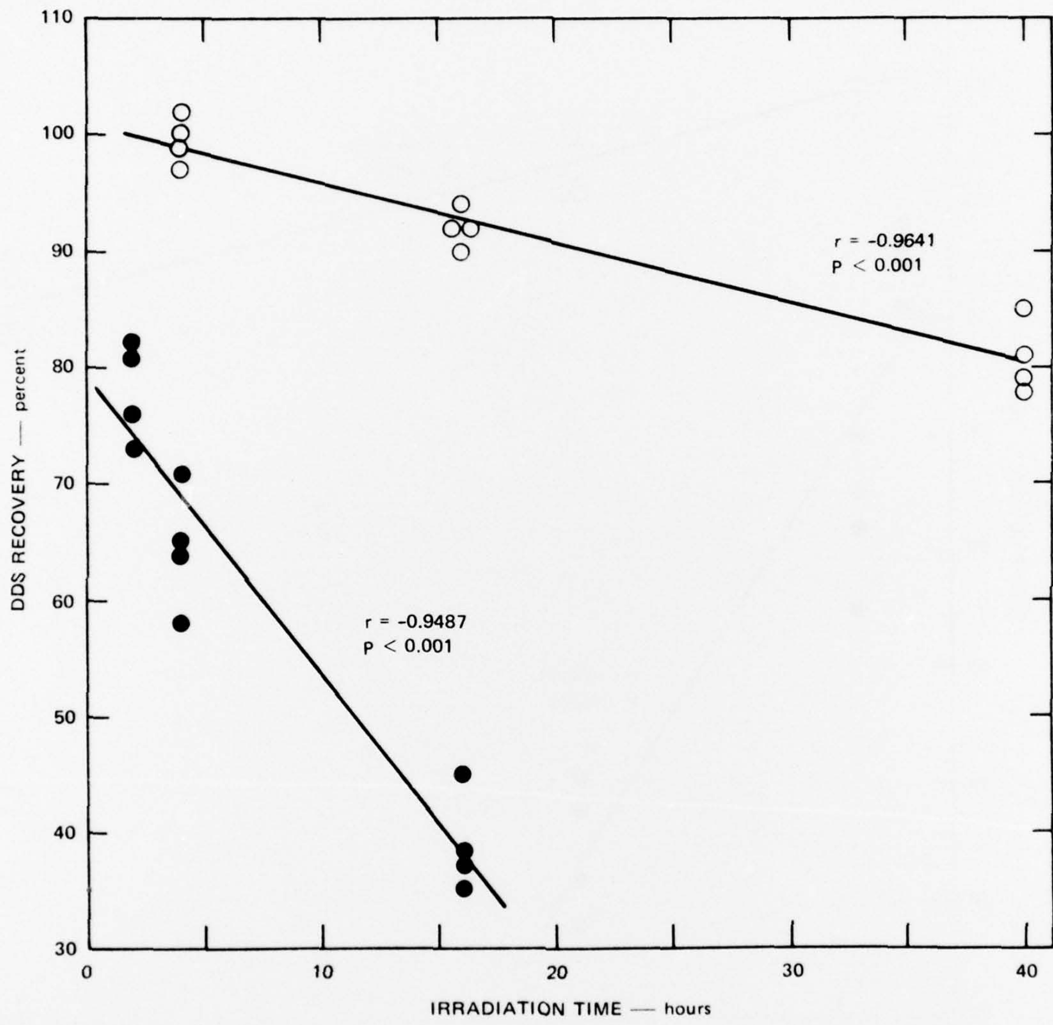

FIGURE 5 EFFECT OF ULTRAVIOLET IRRADIATION OF 5 μg (SOLID CIRCLES) AND 50 μg DDS (OPEN CIRCLES) FROM LOT 590CD ON GLASS SLIDES

Table 11

RECOVERY OF ANALYTIC DDS FROM GLASS SLIDES BEFORE AND AFTER IRRADIATION
(Application from Aqueous Solution)[a]

Irradiation Time (hr)[b]	Percent Recovery[c]		
	Unirradiated	Irradiated	Corrected
2	97 ± 0.8 (8)	91 ± 1.4 (7)	94 ± 1.2 (7)
4	96 ± 1.4 (8)	85 ± 1.7 (7)	89 ± 1.7 (7)
15.5	103 ± 0.8 (4)	84 ± 2.1 (4)	81 ± 2.0 (4)
16	100 ± 0.4 (4)	84 ± 0.8 (3)	85 ± 0.8 (3)

[a]Five μg of DDS in 50 μl of water was applied to 9 x 13-mm glass slides. The slides were air-dried and desiccated for 24 hr. DDS was recovered by heating the slide for 15 min in 2 ml of methanol at 50°.

[b]Slides were irradiated 3.5 cm from the unfiltered 4-watt mercury lamp (GE No. G4T4/1) of the Turner fluorometer.

[c]Unirradiated slides were stored in the dark during each irradiation period and the percent recovery from these slides was used to calculate a corrected percent recovery for irradiated slides. The recoveries are means ± SE of the number of trials in parentheses.

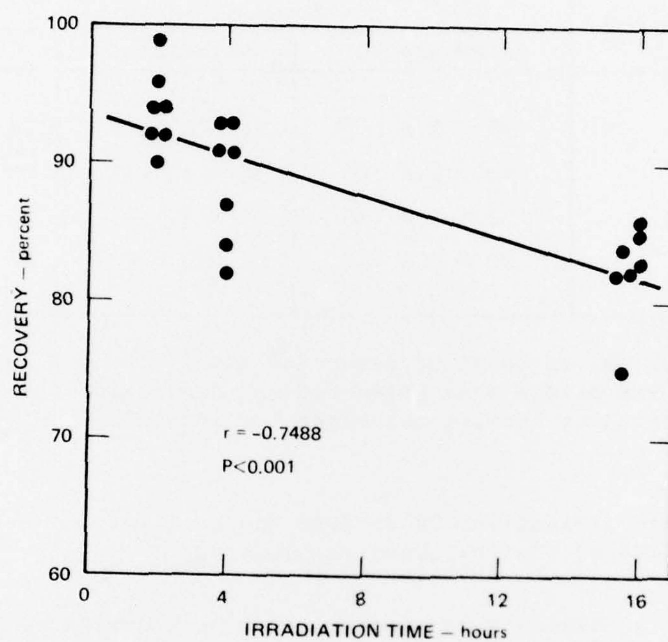

FIGURE 6 EFFECT OF ULTRAVIOLET IRRADIATION ON SOLID DDS APPLIED TO GLASS SLIDES BY EVAPORATION OF AQUEOUS SOLUTIONS

the Turner fluorometer was redetermined using the chemical actinometer, potassium ferrioxalate, described above. A value of 8.03×10^{16} quanta cm^{-2} min^{-1} was obtained. This is almost identical to the value of 7.91×10^{16} quanta cm^{-2} min^{-1} determined at the time that the rate of loss of solid analytic DDS applied from ethanol was measured. Thus, the slower rate of loss of DDS applied from an aqueous solution is not explained by a less intense source of irradiation. The difference appears to be related to the different solvents used to deposit the DDS on the glass slides.

Isolation and Identification of Photolysis Products

To prepare sufficient quantities of photolysis products for purification and identification, we employed a commercial photochemical reactor* to irradiate large amounts of DDS. The absolute light intensity of this reactor was evaluated using the chemical actinometer, $K_3Fe(C_2O_4)_3$, as previously described. A 4-ml aliquot of the actinometer solution was irradiated in a quartz tube (10×75 mm) that was completely wrapped with aluminum foil. An oblong window (5.5×33 mm, 1.84 cm^2) was cut in the foil to provide a small area for illumination so that the evaluation of this reactor would be similar to our previous tests on the Turner fluorometer. Separate aliquots of the actinometer solution were irradiated for 0.5, 1.0, 1.5, and 2.0 min. The mean rate ($\pm$ SE) of formation of ferrous ions was 527 ± 27.5 nmoles min^{-1} and the rate of degradation of the actinometer was calculated to be $1.69 \pm 0.089\%$ min^{-1}. This rate was constant during the 2-min period and exhibited a linear regression coefficient of 0.9984 ($P < 0.005$). From these results, the absolute light intensity was calculated to be 2.84×10^{17} quanta min^{-1}, or 1.54×10^{17} quanta cm^{-2} min^{-1}, which is almost identical to the light intensity calculated above for samples of this actinometer exposed in the Turner fluorometer with the aperture set at 1X.

To irradiate large quantities of DDS in the dry state, we evaporated 17 ml of absolute ethanol containing 16 mg of analytic DDS on a glass plate (20×20 cm). Two of these plates were then placed (back to back) in a vertical position in the reactor and irradiated for 24 hr. A third plate of the DDS served as a control and was wrapped with foil and stored in the dark at room temperature for 24 hr. Each plate was eluted by submerging it for 15 min in a shallow pan of methanol maintained at 50^0. After the initial elution, each plate was rinsed with fresh methanol and the combined eluate and wash were diluted to a final volume of 250 ml with methanol. Aliquots of each plate eluate were diluted 1:100 with ethyl acetate and analyzed for DDS by our fluorometric HPLC method. Recoveries of DDS from the irradiated plates were 70 and 73%; recovery from the unirradiated plate was 99%.

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* Rayonet Photochemical Reactor, The Southern N.E. Ultraviolet Co., Middletown, CT. The cylindrical irradiation chamber (38 cm high $\times$ 25.5 cm diameter) was illuminated by 16 mercury lamps (No. RPR-2537A) arranged around its circumference.

To isolate photolysis products, we concentrated the combined methanolic eluate of the irradiated plates under vacuum. The total concentrate was streaked on silica gel plates* which were then developed with ethyl acetate. Under UV light (254 nm), fluorescent bands at R_f 0.11 and 0.30 were observed in addition to DDS (R_f 0.50). The two lower bands were very faint or not seen at all when the eluate of the control plate was subjected to TLC. The band exhibiting an R_f of 0.30 was scraped off and the silica was extracted with methanol at 50° for 15 min. The eluate was centrifuged and the clear supernatant was subjected to TLC, using ethyl acetate. The fluorescent spot with an R_f of 0.33 was scraped off and eluted with methanol. The methanol was concentrated and the resulting concentrate was subjected to HPLC.** A large peak eluting at 21 min was collected and evaporated to dryness. The UV spectrum of this material in methanol exhibited a major absorption peak at 297 nm and a minor peak at 257 nm. These absorptions are characteristic of a diphenylsulfone. The mass spectrum of this material (Compound XIV) exhibited an intense parent ion at m/e 276, with intense peaks at m/e 108 (aminophenyloxy ion) and m/e 140 (aminophenylsulfoxy ion). As discussed in the section on mass fragmentation patterns, these last two fragments were found to be characteristic of diaminodiphenyl sulfones or N-substituted derivatives, which readily lose their substituent. They were also observed in the spectrum of 4-amino-4'(2-phenylhydrazino)diphenyl sulfone (Compound XIII), which we identified as a photodegradation product of DDS in water.[11] Compound XIV was tentatively identified as the monoformyl derivative of DDS (MFD) by comparison with the authentic compound, which yielded an identical mass spectrum.

In addition, the UV spectrum, R_f on TLC, and retention time after HPLC of Compound XIV were identical to those for MFD. Further tests were performed by subjecting Compound XIV and MFD to the diborane reduction procedure previously used to prepare microquantities of 4-amino-4'-methylaminodiphenyl sulfone from MFD.[10] The main spectra of the reduced MFD and Compound XIV both yielded intense peaks at m/e 262 (4-amino-4'-methylaminodiphenyl sulfone) and several less intense fragment peaks, which were also identical in the two spectra. These results combined with the other evidence prove conclusively that the isolated photolysis product of DDS was MFD.

To determine the quantitative significance of MFD in the photolysis of DDS, 50-μg samples of solid analytic DDS applied in 50 μl of ethanol to glass slides were irradiated for 16 and 64 hr in the Turner

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* Silica gel 60, 0.25-mm thick on 20 × 20-cm glass plates, E. Merck, Darmstadt, Germany.

** The residue was chromatographed on a prepacked column (10 × 250 mm) of 5-μm Lichrosorb SI-60 silica, Altex Scientific Inc., Berkeley, CA., using ethyl acetate at a flow rate of 1.5 ml min^{-1}. The absorbance of the effluent was monitored at 290 nm.

fluorometer and eluted as described above. When approximately 1 μg of irradiated DDS was subjected to our fluorometric-HPLC method (290/380 activation/emission monitoring wavelengths), a peak was observed on the trailing edge of the DDS peak that was quantitated by reference to a similar response obtained from adding 0.1 to 10 ng of authentic MFD to approximately 1 μg of DDS. Using this method, we found that MFD in samples of DDS irradiated for 16 hr accounted for only 0.8% of the unrecovered DDS. This value increased to 1.3% in samples irradiated for 64 hr.

To investigate the possibility that trace amounts of ethanol remaining on the slides after evaporation of this solvent might be responsible for the formation of MFD, 89-μg samples of DDS in 50 μl of distilled water at 95^0 were deposited onto glass slides.

The slides were air-dried and vacuum-dessicated as described above. After irradiation for 72 hr in the Turner fluorometer, analysis of MFD and DDS showed that MFD accounted for 0.7% of the unrecovered DDS. This is slightly lower than, though not drastically different from, the values for DDS applied from an ethanolic solution. Thus, the formation of MFD from DDS does not appear to be dependent on residual traces of ethanol or its photodegradation products.

Large quantitites of the compound with R_f 0.11 (Compound XV) were prepared by irradiating 20 mg of analytic DDS evaporated from 20 ml of absolute ethanol on glass plates as described above. Recoveries of DDS from irradiated plates were 80 and 82%; recovery from the unirradiated plate was 96%. After multiple-development TLC analysis with ethyl acetate, the band of silica corresponding to Compound XV was scraped off, extracted with methanol and subjected to HPLC (same conditions as those used for Compound XIV). A large peak eluting at 50 min was collected. The UV spectrum of this material in methanol exhibited a major absorption peak at 297 nm and a minor peak at 259 nm, which is characteristic of a diphenyl sulfone. A similar procedure carried out with large amounts (16 mg) of analytic DDS evaporated from 16 ml of distilled water at 95^0 led to isolation of a substance that had TLC, HPLC, and UV absorption properties indistinguishable from those of Compound XV. Thus, formation of Compound XV does not appear to be dependent on trace amounts of ethanol remaining on the glass plates after evaporation of this solvent.

Compound XV was subjected to mass spectroscopy, and fragmentation patterns were obtained after direct introduction of the underivatized sample into the mass spectrometer as well as after trimethylsilylation. No parent ion could readily be seen in the underivatized sample, but a peak of m/e 274 was readily seen that yielded an elemental formula of $C_{13}H_{10}N_2O_3S$ by high-resolution analysis. In addition, fragments corresponding to DDS (m/e 248) and fragments G, H, J, and K (Figure 2 and Table 3) were readily seen. These results suggested that a labile group was being lost from a substituted DDS derivative. The sulfone $NH_2\text{-}C_6H_4\text{-}SO_2\text{-}C_6H_4\text{-}NH\text{-}CO\text{-}NH_2$ (mol. wt. 291) could lose NH_3 to give a

fragment of m/e 274 or lose CONH to yield DDS. The trisilyl and disilyl derivatives of this compound would have molecular weights of 507 and 435, respectively. Parent ions of m/e 507 and 435 were observed in the mass spectrum, suggesting that Compound XV is the formamide derivative of DDS, 4-amino-4'-ureidodiphenyl sulfone.

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