to inactive compounds. A series of phenyl derivatives containing various electron-donating or -withdrawing substituents in the 4 position was prepared to evaluate the electronic contribution to the sterilizing properties of the molecule. However, a comparison of the activities of ENT 62758 and ENT 62586 to those of ENT 62585 and ENT 62689 plainly indicated that changes in electronic effects were not related to the activity of the sulfonamides.

Selected groups of compounds, designed to correlate changes in activity with changes in location of various substituents on the benzene ring, gave conflicting results. For example, in the two isomeric pairs of chloro-substituted derivatives (ENT 52766, ENT 62756 and ENT 62684, ENT 62714) each isomer in a pair had essentially the same sterilizing activity. However, in the nitrobenzene series, the ortho (ENT 62821) and meta (ENT 62822) isomers were inactive whereas the para isomer (ENT 62689) was moderately active.

The second modification of ENT 52766 concerned the naphthyl portion of the molecule. Inactive compounds resulted from eliminating one of the two nitro groups (ENT 62664, ENT 62788), but when the entire 2,4-dinitro-1-naphthyl group was replaced with the 2,4-dinitrophenyl group (ENT 62775), moderate activity was obtained when both sexes were treated, and male sterility increased about tenfold. However, addition of another nitro substituent to the benzene ring (ENT 62785) gave a highly toxic picryl derivative with no apparent sterilizing activity. Similar results were encountered for ENT 62786, a trifluoromethyl analog of ENT 62785.

The importance of an unsubstituted amidic nitrogen as a prerequisite for activity became apparent when methylation of the nitrogen in ENT 52766 gave inactive ENT 62697 and replacement of the amidic function in ENT 62586 with oxygen gave the inactive 2,4-dinitro-1-naphthol-*p*-toluenesulfonate.

Because of the important role that male chemosterilants may play in the sterile-male technique of insect control, further investigation of the sulfonamides related to ENT 52766 should be directed toward the naphthyl substituent, particularly to its replacement with various substituted phenyl or other groups.

Supplementary Material Available. A listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N. W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-74-197.

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Photochemistry of Bioactive Compounds: Photoproducts and Kinetics of Polychlorinated Biphenyls

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Several symmetric tetrachlorobiphenyls were synthesized by modified Ullmann coupling of the corresponding dichloroiodobenzenes. Hexane and methanol solutions of these compounds were irradiated at 300 nm for different time periods and the photoproducts analyzed by gas chromatography and mass spectrometry. In hexane only, dechlorination products were observed, while in methanol solution additional methoxylated products were formed. Photoreaction rates have been measured and a marked difference in reactivity has been observed for tetrachlorinated biphenyls with chlorine substituents in different positions on the aromatic rings. Mechanistic pathways for this reaction consistent with our observations are discussed.

Ultraviolet radiation is known to induce chemical reactions in many chlorinated hydrocarbons under laboratory conditions (Mitchell, 1961). Some of these reactions have also been found to occur under field conditions (Crosby and Tutass, 1966). Identification of the resulting photoproducts and investigation of their chemical, toxicological, and pharmacological properties are necessary in order to have a correct evaluation of the merits involved in their continued use.

Polychlorinated biphenyls (PCB) are among those compounds which have received considerable attention in recent years. They have been found to occur together with chlorinated pesticides in human adipose tissue and milk (Acker and Schulte, 1970; Biros *et al.*, 1970). Several reviews have appeared describing their chemical and toxicological properties (Edwards, 1971; Vos, 1970). It is generally agreed that their presence in the environment and in foods is detrimental.

We have undertaken the study of PCB photochemistry in order to determine the structure of the resulting photoproducts, the effect of solvents on product formation and rates of reaction, and any correlations between PCB structure and their rate of photolysis.

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Table I. Retention Times of Polychlorinated Biphenyl Standards

PCB	Time, sec[a]
2,2′-Dichlorobiphenyl	70
2,6-Dichlorobiphenyl	74
2,5-Dichlorobiphenyl	84
2,4-Dichlorobiphenyl	89
2,3-Dichlorobiphenyl	96
3,5-Dichlorobiphenyl	114
3,3′-Dichlorobiphenyl	128
3,4-Dichlorobiphenyl	141
4,4′-Dichlorobiphenyl	146
2,2′,5-Trichlorobiphenyl	141
2,3′,5-Trichlorobiphenyl	168
2,4,4′-Trichlorobiphenyl	188

[a] Retention time determined at 180°, 6 ml/min nitrogen flow.

The tetrachlorobiphenyls selected for our work are among those found to have the greatest toxicity in biological systems (Fugita, 1971; Hoopingarner *et al.*, 1972). At the present time little is known about the photochemical properties of substituted biphenyls. Several studies have appeared (Safe and Hutzinger, 1971; Ruzo *et al.*, 1972) which have shown that the main reaction at environmentally significant wavelengths (>290 nm) is stepwise dechlorination. However, the actual dechlorination products have seldom been identified.

In the present study our aim has been the total identification of products arising from PCB photolysis in different solvents and the acquisition of kinetic data which will allow elucidation of mechanistic pathways involved.

EXPERIMENTAL SECTION

Synthetic Methods. *Tetrachlorobiphenyls.* Several synthetic methods have been used in PCB syntheses (Beaven, 1956; Taylor, 1970). 2,2′,5,5′-Tetrachlorobiphenyl (I), 2,2′,4,4′-tetrachlorobiphenyl (II), 2,2′,3,3′-tetrachlorobiphenyl (III), 3,3′,4,4′-tetrachlorobiphenyl (IV), and 3,3′,5,5′-tetrachlorobiphenyl (V) were prepared by treating the corresponding dichloroiodobenzene with magnesium metal in ether solution, and subsequent coupling of this Grignard reagent with cupric chloride yields the desired tetrachlorobiphenyl. This procedure is similar to that used by Tsutsui (1957). The yields obtained by this procedure were in the 20–80% range, with lower yields being characteristic of those PCB-containing chlorines in the ortho position, presumably due to increased steric hindrance to coupling. 2,2′,6,6′-Tetrachlorobiphenyl (VI) and all mono-, di-, and trichlorinated standards were obtained commercially (Analabs Inc., North Haven, Conn.) and purified by recrystallization from ethanol until gas chromatographic purity was achieved.

All tetrachlorobiphenyls prepared were analyzed by infrared (ir), nuclear magnetic resonance (nmr), and mass spectrometry (ms) for chemical authenticity.

Trichloromethoxybiphenyls. 2,2′,4-Trichloro-4′-methoxy- and 2,4,4′-trichloro-2′-methoxybiphenyl were prepared by Ullmann coupling of the corresponding chloroiodoanisoles (Pfaltz & Bauer Chemicals) in dimethylformamide (DMF) (Kornblum, 1952). The resulting products, tetrachlorobiphenyl, trichloromethoxybiphenyl, and dichlorodimethoxybiphenyl, were separated by gas chromatography and identified by mass spectrometry.

Reagents. Hexane and methanol used in photochemical reactions were glass distilled and obtained from Burdick and Jackson Laboratories, Inc., Muskegon, Mich. At all other times, analytical grade solvents (Mallinckrodt Chemical Works, St. Louis, Mo.) were used.

Benzophenone, 2,5-dimethyl-2,4-hexadiene, and 1,3-cyclohexadiene were obtained from Aldrich Chemical Co. and purified; benzophenone was recrystallized twice from ethanol, 2,5-dimethyl-2,4-hexadiene was sublimed at low temperature, and 1,3-cyclohexadiene was obtained in gas chromatographic purity.

All chlorinated anilines and anisidines required for the syntheses of the tetrachloro- and trichloromethoxybiphenyls were obtained from Pfaltz & Bauer Chemicals, Flushing, N. Y.

Photochemical Equipment. All photolyses were carried out in a Rayonnette Photochemical Reactor (The Southern N.E. Ultraviolet Co.) fitted with RUL lamps having a peak energy output at 300 nm. During irradiation the samples were contained in borosilicate glass test tubes (13 × 125 mm, uv cutoff at 285 nm) fitted with Teflon stoppers or, if degassed, the tube was sealed under vacuum. Photolysis was carried out at temperatures ranging from 25 to 40°. A "merry-go-round" arrangement was employed to ensure equal exposure of all samples to uv radiation.

Analytical Equipment. Glc analyses were performed on a Varian Aerograph 1400 gas chromatograph equipped with a flame ionization detector and a 50-ft stainless steel SE-30 SCOT column (Perkin-Elmer, Norwalk, Conn.). The column oven was maintained isothermally in the 160–200° range, with a nitrogen carrier gas flow of 6 ml/min. Detector and inlet temperatures were 300 and 250°, respectively (see Table I).

Mass spectra were obtained from a DuPont 21-490 apparatus interfaced with a Beckman GC-65 gas chromatograph equipped with a 15% QF1, 10% DC 200, 6 ft × $\frac{1}{8}$ in. stainless steel column. The oven temperature was maintained at 220°, with a nitrogen carrier gas flow of 25 ml/min.

Infrared spectra were obtained with a Perkin-Elmer 337 spectrophotometer. Nuclear magnetic resonance spectra were obtained in carbon tetrachloride (spectrophotometric grade) using a Varian T-60 instrument. Ultraviolet spectra were taken in hexane and methanol with a Beckman DB-G grating spectrophotometer.

Photochemical Procedures. Fifty milliliters of 0.005 *M* tetrachlorobiphenyl (I–VI) in hexane or methanol solutions was irradiated for 10–15 hr. During the reaction, HCl evolution was observed. After irradiation the solutions were concentrated to 5 ml and 2-μl aliquots injected in the gc–ms for product identification. Nmr evidence for product structure was obtained by allowing the reaction to proceed until only dichlorobiphenyl remained as the main component in solution. The solvent was evaporated and the residue taken up in carbon tetrachloride. The spectra obtained matched those of dichlorinated biphenyl standards. Photoproducts consisted of trichlorobiphenyls with a parent peak (M) at *m/e* 256 and fragments at *m/e* 221 (M − Cl), *m/e* 186 (M − 2Cl) and *m/e* 151 (M − 3Cl) and dichlorobiphenyls with M at *m/e* 222 and fragments at *m/e* 187 (M − Cl) and *m/e* 152 (M − 2Cl). After 10 hr of irradiation monochlorobiphenyls constituted less than 1% of the total product formation. The products obtained for each PCB (I–VI) in hexane solution are listed in Table II.

The photoproducts obtained in methanol solutions (yields of 90–95% based upon reacted starting material) consisted of dechlorinated PCB; however, methanol substitution products were also observed. In all cases the amount of methoxylated products did not exceed 5% of the total amount of products formed, with trichloromethoxybiphenyls being the main components. Methanol substitution has been found to take place at the same site from which a chlorine is lost. In the case of 2,2′,4,4′-tetrachlorobiphenyl photolysis in methanol, the main methoxylated product was identified as 2,4,4′-trichloro-2′-methoxybiphenyl by comparison of gc retention time and mass spectra with those of authentic samples prepared in our laboratory. The parent peak for this compound appeared at *m/e* 286 with fragments at *m/e* 271 (M − Me), *m/e* 255 (M − OMe), *m/e* 251 (M − Cl), and other characteristic masses.

Table II. Photoproducts in Hexane and in Methanol

PCB	Dechlorinated products	Time, sec	Methoxylated products
I	2,3′,5-Trichlorobiphenyl	167	Trichloromethoxybiphenyl
	3,3′-Dichlorobiphenyl	128	Dichlorodimethoxybiphenyl[a]
	3-Chlorobiphenyl[a]		
II	2,4,4′-Trichlorobiphenyl	190	Trichloromethoxybiphenyl
	4,4′-Dichlorobiphenyl	148	Dichlorodimethoxybiphenyl[a]
	4-Chlorobiphenyl[a]		
III	2,3,3′-Trichlorobiphenyl	182	Trichloromethoxybiphenyl
	3,3′-Dichlorobiphenyl	129	Dichlorodimethoxybiphenyl[a]
IV[b]	3,4,4′-Trichlorobiphenyl	192	Trichloromethoxybiphenyl
	4,4′-Dichlorobiphenyl	147	
V	3,3′,5-Trichlorobiphenyl[a]	181	
VI	2,2′,6-Trichlorobiphenyl	158	Trichloromethoxybiphenyl[a]
	2,2′-Dichlorobiphenyl[a]	69	

[a] Compound represented less than 1% of total product formation. [b] 3,3′,4-Tetrachlorobiphenyl was observed in <2% after 50 hr irradiation.

Table III. Photolysis Rates of PCB

PCB	$k_h \times 10^8$ M sec^{-1} (degassed)[a]	$k_h \times 10^8$ M sec^{-1} (O_2)	$k_m \times 10^8$ M sec^{-1} (degassed)	$k_m \times 10^8$ M sec^{-1} (O_2)
II	15.0	2.8	18.0	4.2
III	2.4	1.0	4.0	1.5
I	2.2	0.5	4.8	0.7
IV	1.4	0.8	3.5	2.2
VI	0.4	0.2	0.5	0.2
V	<0.1		<0.1	

[a] Rate of reaction in hexane solution. [b] Rate of reaction in methanol solution.

As shown in Table II, each tetrachlorobiphenyl irradiated which contained chlorines in the ortho position yielded products arising from the loss of these. Similarly, those tetrachlorobiphenyls containing only meta- and para-chlorines upon irradiation lost the meta-chlorines preferentially. This behavior can also be observed in the formation of the secondary products, dichlorobiphenyls, which result from the loss of ortho- or meta- but not of para-chlorines. The tertiary products, monochlorobiphenyls, were only formed in <1% yield (I and III) or not at all, either because of insufficient absorption by dichlorobiphenyls at the wavelengths employed or by decreased reactivity of meta- and para-chlorines relative to those on the ortho position.

Reaction Rates (Table III). Solutions of I-VI were prepared so that approximately 30% of the light was absorbed. Greater absorption could not be achieved due to PCB's low solubility in methanol. The reaction was found to be zero order.

The rates of photolysis (k) of I-VI (1-6 × 10^{-3} M) were measured by integration of their gc peaks obtained after irradiation intervals of 0.5-20 hr with photoconversions being carried out to less than 10% in order to avoid absorption by the products and any possible sensitization or quenching of the reaction. Docosane was used as an internal standard to normalize all injections.

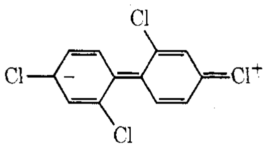
A marked increase in rate was observed when solutions were degassed prior to irradiation. Oxygen is known to act as a triplet quencher by accepting excess electronic energy from excited molecules before any chemical change occurs.

An increase in the rate of reaction was also observed when methanol was used as a solvent. The increased solvent polarity lowers the energy of the π-π^* transitions (Braunton, 1972).

Ultraviolet Spectroscopy. The spectra of I-VI showed maxima at 220-230 nm (A) corresponding to the π-π^* transition of benzene. Additional maxima between 240-260 (B) and 275-285 (C) nm were also present. Presumably these correspond to the biphenyl system as a whole.

The position of chlorine substituents affects bands B and C; when ortho-chlorines are present (I, III, VI) the molar extinction coefficient (ϵ) decreases and, conversely, when para-chlorines are present it increases (II, IV). Meta substitution apparently has no effect on band intensity and the spectrum of V closely resembles that of biphenyl, yielding the following values for PCB and ϵ (290 nm), respectively: I, 120; II, 140; III, 116; IV, 6740; V, 1410; and VI, 30.

Reaction Mechanism. The geometry of biphenyl has been shown to be planar in the excited state (Wagner, 1967), involving a high degree of conjugation between the two phenyl rings, and reactions of several monochlorobiphenyls are believed to proceed through triplet excited states (Wagner, private communication). In the excited state the charge distribution could be represented as either 1 or 2. The ultraviolet spectra obtained support this assumption since PCB with ortho-chlorines would be less likely to achieve this planar structure due to steric interactions, resulting in a decreased ϵ for the absorption band from this transition. Para substitution would bring about increased electron donation into the ring system. The ortho substitution probably contributes to the resonance effect on the same order of magnitude as the para substitution; however, the destabilizing steric effect appears to be more important. Tetrachlorobiphenyls containing chlorines in the meta position do not show either a stabilizing resonance effect or destabilizing steric interactions.



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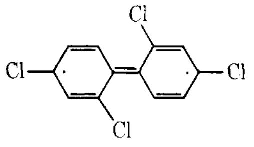

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The rate of dechlorination can then be considered to depend upon the extent to which the particular chlorine substituents enhance or decrease the likelihood of a particular excited state geometry, both physical and electronic.

Table II shows that whenever present, it is the ortho-chlorines that cleave. In the absence of chlorines in the ortho position, the meta-chlorines react. Para-chlorines have not been observed to react to any significant extent for any of the compounds studied.

It is possible that for 2,2′,4,4′-tetrachlorobiphenyl the rate is enhanced by a greater intersystem crossing rate coupled with the increased reactivity of the excited state caused by the steric interactions brought about by the chlorines in the ortho position. Studies are now underway in our laboratories to determine the triplet lifetimes, isc rates, phosphorescence, and quantum yields of I-VI.

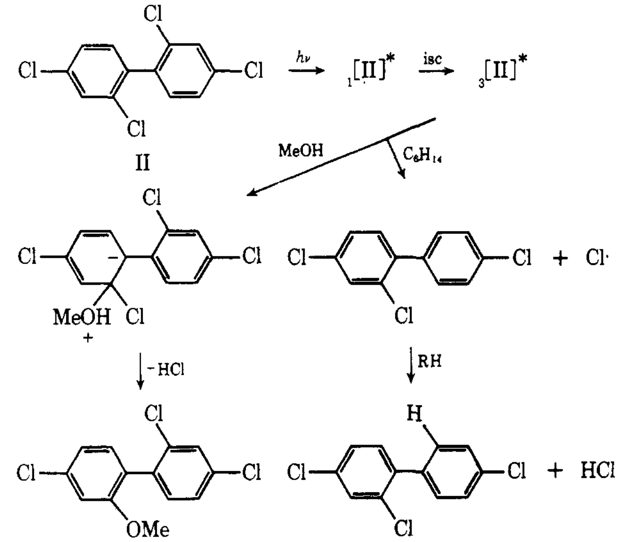
Quenching studies conducted with 2,2′,4,4′-tetrachlorobiphenyl using 1,3-cyclohexadiene indicate that the excited state reacting is indeed a triplet. Degassed methanol solutions of this PCB irradiated with varying concentrations (Q) of quencher showed decreased k values:

$k \times 10^7 M\,sec^{-1}$	Q, $10^{-3} M$
1.80	0
1.52	2.69
1.21	5.58
1.04	8.07
0.62	13.45

The fact that oxygen, also a triplet quencher, reduces the rate suggests the same conclusion.

The intermediate involved in this reaction appears to be similar to that observed by Kharasch (1968) in the photoreaction of iodobiphenyl in benzene. The main product observed was terphenyl, arising from free radical attack on the solvent. In other solvents biphenyl is formed by hydrogen abstraction. In both cases a free radical type of intermediate has been invoked, although no iodine scavenging was observed. It is probable that PCB's yield similar "free" radical intermediates when photolyzed in hexane or methanol solutions. This would explain the occurrence of dechlorination products. Methoxylated products must form through a different mechanism, probably involving nucleophilic attack by methanol and subsequent elimination of chlorine and/or hydrochloric acid. This mechanism would explain the hydroxylated products obtained by Hutzinger and coworkers (1971) in the photolysis of PCB's in aqueous dioxane solutions (see Scheme I).

Scheme I



CONCLUSIONS

The photolysis of polychlorinated biphenyls at wavelengths greater than 290 nm indicates the environmental significance of such nonbiological degradations. The determination of photoproduct structure and reaction rates is intimately related to the mechanism of the reaction. The presence of methanol substitution products suggests the possibility of degradative "handles" which can be introduced photochemically to compounds which are otherwise stable to most biological metabolic pathways. The effects of different chlorine substitution patterns upon reaction rates and the resulting products are of ecological significance.

At present we are investigating other photochemical properties of these compounds.

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Solubility and Molecular Structure of 4-Amino-3,5,6-trichloropicolinic Acid in Relation to pH and Temperature

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The solubility of 4-amino-3,5,6-trichloropicolinic acid (picloram) was investigated at pH 0.20, 1.10, 2.0, 2.8, 4.2, and 4.7, and at 10, 20, 30, and 40°. The pK_a values at these temperatures and the molar heats of solubility, $\Delta H°_{sol}$, of picloram at these pH's are reported. Explanation is offered for the variation of solubility of picloram with equilibrium pH and temperature. Dominant structural species and equilibria involved at various pH ranges are suggested and elucidated. Infrared studies of picloram at different pH's show that the intensities of the absorption bands at 2600 and 2650 cm^{-1}, the stretching vibration of $=N^+H$, increase inversely with pH. Existence of a number of solid structures of picloram was suggested. The possible formation of the zwitterion and cationic species at low pH is speculated. These findings are discussed as applied to picloram absorption on soil and its potential as an environmental contaminant. Also discussed is application of the values of $\Delta H°_{sol}$ as a correction of solubility-temperature effect on the standard enthalpy of pesticide adsorption processes.

Picloram (4-amino-3,5,6-trichloropicolinic acid) has been introduced as a herbicide for controlling weeds in cereal grains including wheat, oats, rice, and corn (Gantz and Warren, 1966; Haagsma and Wiffen, 1966). Broadcast application of a mixture of picloram and 2,4,5-T has been very effective in controlling undesirable woody species, broadleaf weeds, and brushes infesting over 88 million

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