resonance fluorescence for I_R. We believe that the intensity of the resonance fluorescence would be quite weak.

No great effort was made to increase the capability of our instrument to the limit. With improved electronics it should be possible to determine Hg as low as 10^{-11} g/cm^3, corresponding to a sensitivity of about 1 ppb for a 10-mg sample.

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Riboflavin Photosensitized Oxidation of 2,4-Dichlorophenol: Assessment of Possible Chlorinated Dioxin Formation

Abstract. *Dimeric products are formed by riboflavin-sensitized photooxidation of 2,4-dichlorophenol. The products of this reaction were examined to determine whether chlorinated dibenzo-*p*-dioxins could be formed from chlorophenols in water by the action of light of wavelengths greater than 280 nanometers. Dimers are formed by union of phenoxy radicals through carbon-carbon or carbon-oxygen bonds. 4,6-Dichloro-2-(2,4-dichlorophenoxy)phenol was obtained in greater quantity than other dimers. Products were characterized by combined gas chromatography and mass spectrometry. Chlorinated dibenzo-*p*-dioxins which could result from ring closure of a 2-phenoxyphenol derivative were not detected in the products of photolysis. The failure to detect chlorinated dibenzo-*p*-dioxins may result from the rapid photolytic breakdown of the lower chlorinated dibenzo-*p*-dioxins. Under environmental conditions, dioxins are unlikely products of the lower chlorinated phenols or phenoxyalkanoic acids.*

Chlorinated dibenzo-*p*-dioxins are formed by pyrolysis of chlorophenols and their salts (*1*). The extremely high mammalian toxicity and teratogenic potential of the chlorinated dioxins are significant because of their possible occurrence as contaminants of commercial chlorophenols and their derivatives (*1*, *2*). A report that riboflavin sensitized the photodecomposition of 2,4-dichlorophenoxyacetic acid (*3*) prompted us to investigate the course of riboflavin-sensitized photooxidation of 2,4-dichlorophenol and especially the possibility that sensitized photolysis of 2,4-dichlorophenol may give a chlorinated dioxin by dimerization and loss of hydrochloric acid. Although Joschek and Miller did not detect dibenzo-*p*-dioxin during irradiation of phenol, carbon-carbon and carbon-oxygen dimers were formed (*4*). In the presence of photoexcited dyes similar reactions may occur through intermediate phenoxy radicals (*5*). Other photochemical reactions of chlorophenols have been reported. Under mercury arc irradiation in organic solvents, chlorinated phenols are reductively dehalogenated (*6*), and, in water, replacement of halogen by hydroxyl is possible (*7*).

Sunlight irradiation of the rice herbicide, pentachlorophenol, gives a number of products. Munakata and Kuwahara elucidated the structures of these and found that some dimeric ethers were formed (*8*). Later work indicates

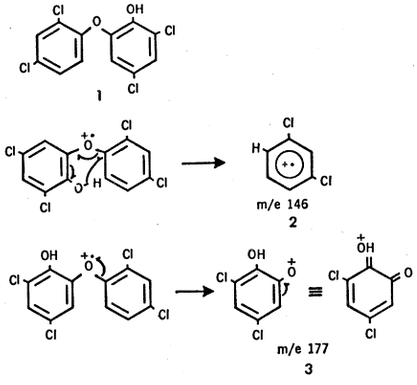

Fig. 1. Mass spectroscopic fragmentation of **1** to produce ions **2** and **3**.

that octachlorodibenzo-*p*-dioxin can be formed by photolysis of pentachlorophenol (*9*).

The herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) are manufactured by condensation of the corresponding phenol with sodium chloroacetate. Since the photolysis of these herbicides in water yields chlorophenols (*7*), it is important to establish the ultimate fate of chlorophenols in the environment.

We have now demonstrated that photosensitized reactions of 2,4-dichlorophenol irradiated in water (at a wavelength greater than 280 nm) yields principally dimeric products. Only trace quantities of dechlorinated products could be detected.

A saturated solution of 2,4-dichlorophenol in water saturated also with riboflavin was irradiated at room temperature by a 450-watt mercury lamp with a borosilicate glass filter for 1 hour to obtain maximum yield of dimer. Oxygen was bubbled through the solution and, since yields were much lower in the presence of nitrogen, we concluded that oxygen may function in regenerating riboflavin from its photoreduction product (*10*). Products were isolated and examined by combined vapor phase chromatography and mass spectrometry (*11*).

Mass spectrometry of the products and their methylated derivatives showed that tetrachlorinated phenoxyphenols and tetrachlorodihydroxybiphenyls were present. A trace quantity of a trichlorophenoxyphenol was also obtained. There was no evidence of ring closure to a substituted dibenzo-*p*-dioxin.

The tetrachlorophenoxyphenols were shown to be a mixture of two isomers separated by thin-layer chromatography on silica gel GF_{254} in benzene (Fig. 1). The major product was allocated structure (**1**), 4,6-dichloro-2-(2,4-dichlorophenoxy)phenol. The mass spectrum showed molecular ion at *m/e* (mass/charge) 322; and ratios of abundances of peaks at *m/e* 322, 324, 326, and so on confirmed the presence of four chlorine atoms in the molecule. A peak at *m/e* 146 (two Cl atoms) (**2**) is indicative of an *o*-phenoxyphenol, since the hydrogen transfer shown is typical of the fragmentation of an orthohydroxyphenoxy ether (*12*). A fragment, *m/e* 177 (two Cl) (**3**), indicates the presence of the phenoxyphenol system, as does also the formation of a monomethyl ether, *m/e* 336, on treatment

with diazomethane. An isomeric compound, *m/e* 322, present in lower yield, must also be a tetrachlorophenoxyphenol as it formed only a monomethyl ether with diazomethane. The absence of *m/e* 146 in the spectrum of the isomer leaves only the two alternative possibilities with an ether linkage meta to the free hydroxyl group. Two isomeric tetrachlorodihydroxybiphenyls (*m/e* 322) were also present in the reaction products. They formed dimethyl ethers (*m/e* 350) on treatment with ethereal diazomethane.

Studies of product composition were made at several intervals. Conversion to dimeric products was usually less than 5 percent, and no dibenzo-*p*-dioxins could be detected in the reaction mixture. In solution, by sunlight irradiation, the rate of photolysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and trichlorodibenzo-*p*-dioxins is relatively rapid (*13*). This probably holds for all tetrachlorodibenzo-*p*-dioxin isomers, so that the extremely rapid breakdown of any dioxin by light may account for our failure to detect dioxins as reaction products. Octachlorodibenzo-*p*-dioxin, in contrast, is broken down more slowly by light than are tetrachloro compounds, and may thus accumulate as a reaction product (*9, 13*). It seems unlikely, therefore, that in an aquatic environment dioxins will be formed from di- or trichlorophenol derivatives such as 2,4-D or 2,4,5-T. At the surfaces of soils or plants, dimerization of chlorophenols is probably limited by the ready availability of other molecular species for reaction with the radicals initially formed by photolysis.

The presence of both riboflavin and oxygen was necessary to obtain measurable yields of products in water. The reaction may proceed through the intermediacy of singlet oxygen which can function by abstraction of hydrogen from the phenol (*14*). Alternatively, riboflavin may participate in hydrogen abstraction reactions (*15*). Further experiments indicate that some reaction also occurs in methanol, but the addition of 2',7'-dichlorofluorescein as sensitizer does not change the quantity of dimer obtained. The former hypothesis, therefore, appears unlikely.

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Dopamine: Release from the Brain in vivo by Amantadine

Abstract. *After dopamine stores in the caudate nucleus of cats were labeled with [^3H]dopamine, the ventricular system was perfused with artificial cerebrospinal fluid. The addition of amantadine to the perfusing fluid caused a dose-related increase in the concentrations of [^3H]dopamine appearing in the perfusion effluent. Subthreshold concentrations of amantadine also enhanced the efflux of [^3H]dopamine induced by electrical stimulation of the caudate nucleus.*

Several clinical reports (*1*) have now confirmed the serendipitous discovery (*2*) that amantadine (*l*-adamantanamine hydrochloride, Symmetrel) ameliorates the symptoms of Parkinson's disease. Efforts are now under way to determine the mechanism of this action.

Reports that the brains of patients suffering from Parkinson's disease contained reduced amounts of dopamine (*3*) have stimulated research on the functional role of this amine in the central nervous system. This research has led to the identification of a dopaminergic nigrostriatal pathway (*4*) which probably has an inhibitory action on neurons in the basal ganglia (*5*). This basic research has culminated in the successful use of L-dopa in the treatment of Parkinson's disease (*6*).

It is reasonable, therefore, that attempts have been made to relate the pharmacological actions of amantadine to an interaction with dopaminergic neuronal systems. In studies in vitro with rat brain striatal slices amantadine increased the rate of dopamine synthesis and increased the release of this amine into the incubating medium (*7*). The pressor effects of amantadine in dogs are enhanced by prior treatment with dopamine, suggesting that amantadine releases dopamine from peripheral sympathetic nerve terminals (*8*). We now report that amantadine causes a selective and dose-related efflux of dopamine from brain structures that line the cerebroventricular system, most likely from the caudate nucleus. Furthermore, low doses of amantadine enhance the efflux of dopamine resulting from electrical stimulation of the caudate nucleus. These results suggest that the antiparkinsonian properties of amantadine may be related to the ability of this drug to increase the concentration of dopamine at postulated receptor sites in the caudate nucleus.

Cats weighing 2 to 3 kg were prepared for perfusion of the cerebroventricular system and for electrical stimulation of the caudate nucleus by modifications of described methods (*9*). The animals were anesthetized with methoxyflurane and placed in a stereotaxic apparatus. The spinal cord was sectioned at the level of the atlas and the animal was maintained on artificial respiration. All wound margins and pressure points were infiltrated with local anesthetic (hexylcaine). A screw-type stainless steel cannula was fixed in a lateral ventricle, and a polyethylene catheter was inserted through the cisterna, under the cerebellum and