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Photochemical Transformation of the Fungicide Chlorothalonil by Ultra Violet Radiation

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Among the chlorinated aromatics, nitriles represent an increasingly important group of pesticides of which chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile, **I**) is a broad spectrum fungicide used against many plant pathogens affecting a large number of agricultural crops. Several studies on the photochemical transformation of **I** in different organic solvents, viz., benzene (Khan and Akhtar 1983 and references cited therein), dichloromethane (Dureja and Walia 1993), ethanol (Giumanini *et al.* 1989), methanol (Binkley *et al.* 1977) have been reported, but no attempt has been made to evaluate the toxicity of the transformed products. The present study has, therefore, been intended to re-investigate the transformation of **I** in alcohols (ethanol and methanol) under ultra violet irradiation for evaluation of the relative toxicity of the transformed products formed under the process.

MATERIALS AND METHODS

Chlorothalonil was purified from commercial 'Kaboch' formulation (75% wettable powder) by dissolving it in chloroform and filtered. The concentrated filtrate was further purified by coloumn chromatography over silica gel and crystallised from petroleum ether-chloroform. Its purity (98.0%) was determined by TLC and HPLC (mp 250-251°C) and its authenticity was confirmed by IR,MS and NMR spectral data. Solvents used in the study were HPLC grade (E Merck , India) and all inorganic reagents were laboratory grade. Melting points were determined by an electrically heated mp apparatus and are uncorrected. Infrared spectra (IR) were determined on potassium bromide discs using a Perkin Elmer model 1310 spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 100 MHz using a JEOL model JNM-FX 100 engine using tetramethylsilane (TMS) as the internal standard. Samples were dissolved in deuterochloroform. The mass spectra (MS) were obtained by direct sample probe on a JEOL Model JMS-DX 300 instrument at 70 ev.

High pressure liquid chromatograph (HPLC) of Hewlett Packard Model 1050 equipped with a UV variable detector Model 1050 coupled with a HP 3392A integrator was used for the analysis of chlorothalonil and its metabolites. The reverse phase column (length 25 cm; id 4.6 cm; od 0.635 cm) was packed with Hypersil

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(ODS) of Shadon HPLC, UK. The solvent system used was acetonitrile : water (7:3) at the flow rate of 1.0 mL min^{-1}. Chlorothalonil [retention time (Rt) 5.0 min] was detected at its maximum UV absorption (236 nm). Thin layer chromatography (TLC) was performed on 20 x 20 cm glass plates coated with 0.5 mm silica gel G (Qualigen, India, TLC grade) using different combinations of solvents , viz. , petroleum ether - benzene and benzene - ethyl acetate as developing solvents and iodine as chromogenic reagent for visualising the spots. Column chromatography was conducted using Silica gel (60-120 mesh , Qualigen, India) slurry prepared in petroleum distillate (60-80°C) in glass column (65 x 2.5 cm id). The column was successively eluted with petroleum distillate and various mixtures of benzene (9+1,7+3,1+1 by volume) followed by benzene and the mixtures of benzene + ethyl acetate (9+1,7+3,1+1 by volume).

Chlorothalonil , **I** (2.5 mg) was dissolved in 250 mL of ethanol and irradiated with a 16-watt UV lamp (Applied Photophysics, London). The reactor was equipped with a water cooled quartz immersion well to maintain a constant solution temperature (22°C) during the photolysis and with a magnetic needle inside the container for constant stirring of the solution. Samples were withdrawn initially and at appropriate time intervals for analysis. The experiment was also conducted dissolving **I** (2.5 mg) in 250 mL of methanol. Control sets were kept in complete darkness at the same time and analysed in both the experiments. For the isolation of photoproducts 250 mg of **I** was dissolved in ethanol (250 mL) and irradiated for 40 hr. The solution was concentrated in a rotary vacuum evaporator at 45°C and chromatographed over silica gel in glass column. Similar procedure was followed for 250 mg of **I** dissolved in methanol (250 ml).

In order to evaluate the antifungal activity of the photoproducts, two plant pathogenic fungi, viz. *Rhizoctonia solani* and *Sclerotium rolfsii* were taken as the test organisms taking pure chlorothalonil **(I)** as the reference standard. The mycelial growth at different concentrations (0.1-10.0 µg mL^{-1}) of the products (**I-IV)** in potato dextrose agar (PDA) media was measured diametrically after 72 hr for *R.solani* and 96 hr for *S.rolfsii*. Seven petriplates formed one set for each concentration for an organism and each set was replicated thrice. The corrected per cent inhibitions were calculated over the control sets and the data were then subjected to Probit analysis (Finney 1971) to get the ED_{50} values for the products.

RESULTS AND DISCUSSION

The results of the photolysis of chlorothalonil **(I)** solutions (10µg mL^{-1}) in alcohols (ethanol and methanol separately) with increasing time of exposure to UV irradiation are shown in **Figure 1** which reveals that **I** was degraded to about 50% in 22 hr in ethanol while in methanol it took about 30 hr. At the end of 40 hr of exposure the concentrations of **I** remaining in solutions were about 34% and 48% respectively. No significant change was observed in the control set in methanol kept in dark while in ethanol slight decrease (2%) could be measured after 30 hr of

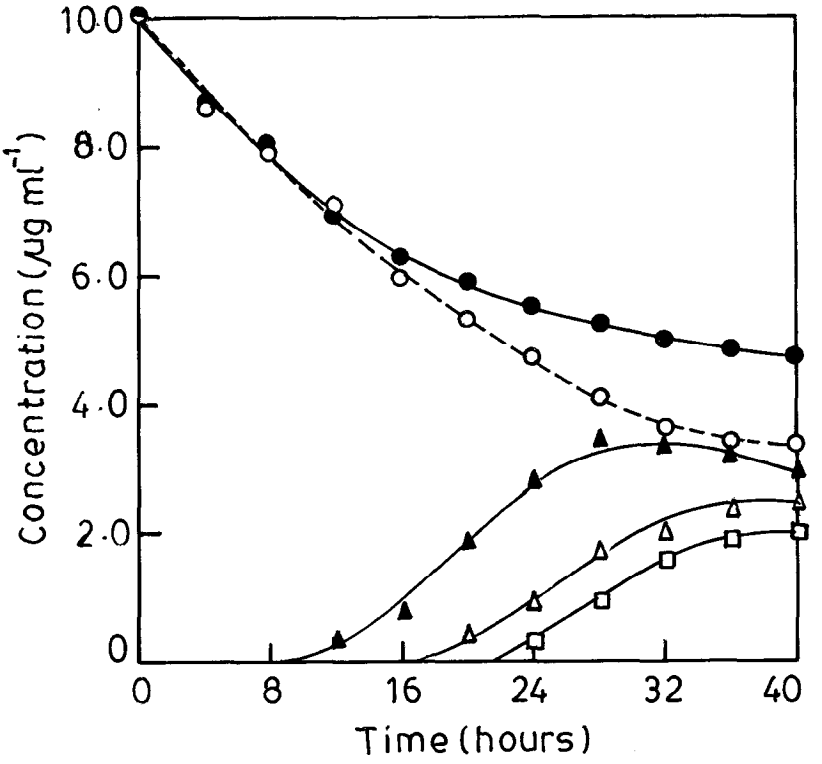

Figure 1 . Photolysis of chlorothalonil (**I**) in ethanol (O), and methanol (●) with the formation of photoproducts **II** (▲), **III** (□) & **IV** (Δ).

observation. The major products were found to be **II** and **III** in ethanol detected after 10 and 22 hr of exposure. The maximum concentration of **II** (3.4 µg mL^{-1}) was observed after 28 hr and started to decline thereafter. The concentration of **III** was found to increase, the maximum value (1.9µg mL^{-1}) obtained after 40 hr. In methanol, the only product (**IV**) was detected after 18 hr and reached the maximum (2.4mu g mL^{-1}) after 40 hr of exposure (**Figure 1**) .

The physical and spectral data of the isolated products **II, III** and **IV** have been presented in **Table 1.** Compound **II** obtained from petroleum ether - benzene (1: 1) eluates of the crude irradiated mixture of **I** under UV rays in ethanol was purified by repeated crystallisation from petroleum ether-benzene (mp 158-160°C). The IR spectrum of **II** exhibited a sharp peak at 2255 cm^{-1} due to the presence of cyano group and also at 1785 cm^{-1} for lactonic carbonyl group. The chemical ionisation mass spectra of II confirmed the molecular ion peak (M+) at m/z 275 (M + H = 276, $M + NH_4 = 293$, $M + N_3H_{10} = 327$) corresponding to the molecular composition of $C_{10}H_4NO_2Cl_3$. In addition, the mass spectrum exhibited the strong peak at 260 ($M^+ - CH_3$) with a distinct isotopic pattern for three chlorine atoms at 262, 264 and 266 in the usual ratio of intensities (Kemp 1991). The presence of the lactonic carbonyl group was also evidenced by the appearance of the mass fragment ions at

Table 1. IR, MS and NMR spectral data of the photoproducts (**II-IV**) of chlorothalonil (**I**)

Compounds	Spectral data
II : IR (cm^{-1})	3000, 2955, 2255, 1785, 1600, 1569, 1460, 1420, 1390, 1335, 1285, 1265, 1205, 1130, 1108, 1075, 1052, 990, 966, 946, 800, 788, 745, 722, 700, 645, 612.
MS(m/z)	279, 277, 275(M^+),266, 264, 262, 260, 236, 234, 232, 208, 206, 204, 171, 169, 134, 133, 99, 98, 86, 84, 43.
NMR-^{1}H(δ)	1.78d, (3H), J=7Hz; 5.62q, (1H), J=7Hz
^{13}C(δ)	163.6, 154.2, 142.5, 135.9, 127.3, 123.4, 118.0, 111.9, 75.7, 18.0.
III : IR (cm^{-1})	2995, 2940, 2860, 1795, 1765, 1615, 1472, 1455, 1385, 1360, 1300, 1225, 1150, 1125, 1100, 1092, 1070, 1030, 972, 955, 910, 810, 785, 720, 685, 675, 635, 618.
MS(m/z)	290, 288, 286(M^+), 275, 273, 271, 247, 245, 243, 231, 229, 203, 201, 173, 171, 145, 143, 109, 108, 99, 98, 87, 86, 75, 74, 73, 43.
NMR-^{1}H(δ)	1.79d, (6H), J=7Hz; 5.62q, (2H), J=6.5Hz.
IV: IR (cm^{-1})	3000, 2950, 2250, 1775, 1590, 1420, 1385, 1355, 1285, 1270, 1200, 1105, 1025, 1000, 980, 950, 920, 835, 780, 745, 720, 705, 672, 610.
MS(m/z)	265, 263, 261(M^+), 236, 234, 232, 206, 204, 133, 99, 98.
NMR-^{1}H(δ)	5.32s, (2H).

232 (M^+-CH_3CO) and 204 (M^+-CH_3CO-CO). Therefore, the compound (**II**) appears to be a product of **I** formed by the replacement of one chlorine atom with a lactonic carbonyl functionality. Earlier studies (Binkley et al. 1977) revealed that 4-chlorine atom is most susceptible to such replacement. The ^{1}H NMR spectrum of **II** revealed the presence of three methyl protons at δ 1.78(d) and one methine (CH) proton at δ 5.62(q). Based on this spectral evidence the structure of compound **II** has been assigned as 4,5,7 -trichloro - 6 - cyano - 3 - methyl - 1 (3H) - isobenzofuranone. The ^{13}C - NMR spectrum of **II** (**Table 1**) was also in well accordance with the proposed structure. However , the difference in mp and IR absorption values of **II** and that reported earlier by Giumanini *et al* (1989) may be due to different stereoisomeric forms arising out of the asymetric C - 3 carbon atom in **II** .

Continuation of the same column with benzene yielded compound **III,** mp 264-266°C, isolated by repeated crystllisation from hot - cold benzene. The IR spectrum of **III** did not show the characteristic absorption for cyano group but exhibited strong absorptions at 1765 and 1795 cm^{-1} for lactonic carbonyl

functionality. The mass spectrum revealed the molecular ion peak (M^+) at m/z 286 (with a distinct isotopic pattern for two chlorine atoms at 288, 290) which corresponds to the molecular composition of $C_{12}H_8O_4Cl_2$. The other significant ion peaks at m/z 271, 243, 229, 201, 171 and at 145 revealed the exclusion of two lactonic carbonyl groups from the molecular structure of **III.** The ^{1}H-NMR spectrum of **III** appeared to be similar to that obtained for compound **II** but the intensity of the proton signals were just double in **III** (1.79 d, 6H and 5.62 q, 2H). Based on this spectral evidence the structure of **III** has been assigned either as **III**a or **III**b. A close scrutiny of the PMR spectrum of **III** with those reported for **III**a and **III**b (Giumanini et al. 1989) revealed the similarities between **III** and **III**b. Therefore, the structure of **III** has been assigned as 4,8- dichloro-3,5- dimethyl - 3H,5H - benzo - difuran - 1,7 - dione **(III** b). However, the significant variations in melting point and in IR absorption values of **III** and those reported earlier for **III**b (Giumanini *et al.* 1989) could only be explained due to different stereoisomeric forms arising out of the asymetric carbons at C - 3 and C-5. Paucity of material precluded us to carry out further study for identification of the exact isomeric form.

The UV irradiated product of **I** in methanol was also subjected to column chromatography. The petroleum ether - benzene (9:1) eluates of the column yielded compound **IV** which was crystallised repeatedly from petroleum ether - chloroform (mp 231-233°C). The IR spectrum of **IV** indicated the characteristic cyano absorption at 2250 cm^{-1} and lactonic carbonyl functionality at 1775 cm^{-1} .The mass spectrum of the compound revealed the molecular ion peak [M^+] at m/z 261 [with the distinct isotopic pattern for three chlorine atoms at 263 and 265 in the usual ratio of intensities (Kemp 1991), but the peak at 267 could not be encountered possibly due to low intensity] corresponding to the molecular composition of $C_9H_2NO_2Cl_3$.The other significant ions appeared at 232 (M-CHO), 204(M^+-CHO-CO), 133 (M^+-CHO-CO-HCl-Cl), 99, and 98 (m/z 133 - Cl). Therefore, the compound (**IV**) appears to be formed by the replacement of one chlorine atom of chlorothalonil (**I**) with a lactonic carbonyl functionality. The ^{1}H NMR spectrum exhibited a sharp singlet for two protons at 6 5.32. All the above data could only be fitted with the structure of 4,5,7 - trichloro - 6- cyano - 3, 3 - dihydro - l-isobenzotiranone previously reported by Binkley *et al.* (1977).

The results of the antifungal assay of chlorothalonil **(I)** and its different transformation products **(II-IV)** on the growth inhibition of two plant pathogenic fungi, viz. *Rhizoctonia solani* and *Sclerotium rolfsii* at different concentrations have been presented in **Table 2.** Significant lower inhibitions were observed for the products in comparison to **I** against both the fungi. The ED_{50} values calculated from probit analysis were found to be 0.74, 2.93 and 1.62 mg L^{-1} for **II, III** and **IV** respectively much higher than that obtained for **I** (0.37 mg L^{-1}) in case of *R.solani,* whereas the corresponding values were 1.39, 5.22 and 3.43 mg L^{-1} much higher than **I** (0.80 mg L^{-1}) in case of *S.rolfsii* (**Table 3**) .

The products formed from chlorothalonil **(I)** by photolysis using ultra violet rays appeared to be the results of several reactions in complex sequences **(Figure 2).**

Table 2. Radial growth inhibition of *Rhizoctonia solani* and *Sclerotium rolfsii* by different concentrations of chlorothalonil **(I)** and its photometabolites **(II-IV)**

Compounds	Concentration of compounds in PDA medium (mg L^{-1})	Per cent inhibition of radial growth[a] of	
		R . solani	*S . rolfsii*
I	0.10	25	14
	0.25	38	18
	0.50	51	23
	1.00	81	69
	2.00	85	79
	5.00	88	83
II	0.25	35	17
	0.50	39	25
	1.00	60	35
	2.00	66	66
	5.00	76	78
III	0.50	12	8
	1.00	21	14
	2.00	33	18
	7.20	78	63
IV	0.25	17	8
	0.50	27	15
	1.00	36	20
	2.00	53	25
	4.84	79	67
	9.68	81	74

[a]Average of three sets each containing seven petriplates.

The primary photochemical reaction is the homolytic cleavage of the 4 - Cl - C bond followed by alkylation by the α - radicals ($R\text{-}\dot{C}HOH$) where R may be H or CH_3) derived by photoirradiation of alcohols with the concomitant formation of HCl in the solution. Although the nitrile group cannot be easily substituted and does not undergo changes but in this case the stereochemically ideal proximity of the nucleophile resulted in an accelarated irreversible condensation with the neighbouring alcohol group to yield the hydrolytically sensitive imidines to produce the product **II** in case of ethanol and **IV** in case of methanol. When more than one Cl is substituted both equivalent Cl of **I** are assumed to be replaced. So cleavage of the 4 & 6 - Cl - C bond following the same mechanism resulted in the formation of **III (Figure 2).**

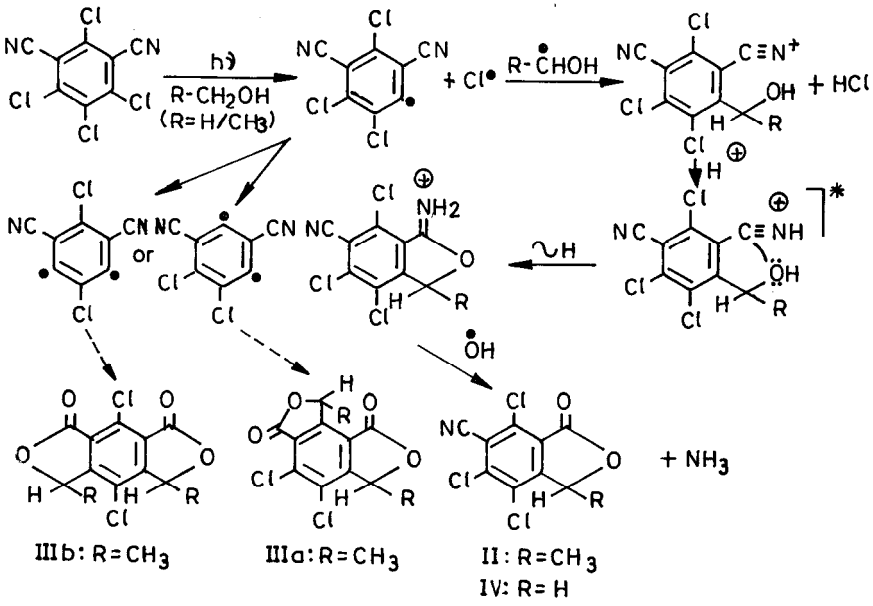

Figure 2. Mechanism of phototransformation of Chlorothalonil (**I**) in alcohols

The mechanism of fungicidal action of **I** is attributed to the inhibition of thiol dependent enzymes through chlorine substitution resulting in strong inhibition of glucose oxidation (Vincent and Sisler 1968; Tillman *et al.* 1973). Thus, the products (**II,III &IV**), formed by chlorine replacement of **I,** exhibited lower toxic action against both the fungi. The decrease in fungicidal activity of **I** has been observed to vary with the type of substituent and the number of chlorine atoms

Table 3. Results of probit analysis of growth inhibition percents of *R.solani* and *S.rolfsii* by chlorothalonil **(I)** and its photometabolites **(II-IV)**

Organism	Compounds	Regression equation	ED_{50} Value * (m g L^{-1})	Decrease in activity in terms of **I**
R. solani	**I**	Y=3.05+1.25X	0.37	-
	II	Y=3.37+0.87X	0.74	2.0
	III	Y=0.76+1.72X	2.93	7.9
	IV	Y=2.28 +1.23X	1.62	4.4
S. rolfsii	**I**	Y=2.20+1.47X	0.80	-
	II	Y=1.88+1.46X	1.39	1.7
	III	Y=0.76+1.56X	5.22	6.5
	IV	Y=1.48+1.39X	3.43	4.3

* Standard Error of ± 0.01 was calculated for each ED_{50} value.

replaced. When one chlorine atom was replaced by methyl group (**IV**) followed by rearrangement , the activity was reduced by more than 50 % than that observed for ethyl substituent (**II**) (**Table 3**). In case of two chlorine substituted product (**III**) the activity was reduced by about 75% in comparison to one chlorine replacement product (**II**). Therefore, the antifungal activity of chlorothalonil and the photo-products may be presented in the descending order as : **I** > **II** > **IV** > **III** , which indicates the possibility of utilising UV rays as a means for detoxification of the fungicide (**I**) residues.

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