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In Vitro Photoconversion of Gamma-Hexachlorocyclohexane in the Presence of Chlorophyll

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The gamma-isomer of hexachlorocyclohexane (gamma-HCH) commonly used in agriculture and its slow biotransformation in the environment is a matter of great concern. Physical processes such as volatilization and photodegradation form a major dissipation route of pesticides in environment in addition to biodegradation (Spencer 1983). Many organic chemicals introduced into the environment absorb sunlight and undergo transformation to new molecular species (Gab et al. 1977). Parlar (1990) reported that the photodecomposition of pesticides in the adsorbed state on active surfaces was found to be more rapid than unadsorbed state. In this case, the active surfaces act as a catalyst and accelerate electron transfer causing abiotic conversion of pesticides in the environment.

Photochemical decomposition can be oxygen dependent or independent (Pathak and Joshi 1984). In the former case, UV induced generation of active oxygen species are considered responsible. Being a good singlet oxygen sensitizer, chlorophyll could be likely targets for excitation leading to generation of active oxygen in natural system (Loach and Hales 1976). In situations such as pesticide spray settling on leaves or in soil in the presence of leaf litter, hydrophobic interaction between chlorophyll and organic xenobiotics could be possible. If photochemically sensitized chlorophyll could initiate abiotic degradation of the pesticides, it would influence the persistence and ecotoxicological potential. Information on total photodegradation or conversion into non-toxic products could find applications in environmental detoxification and removal of toxic residues. In order to test these possibilities, the effect of UV radiation on gamma-HCH adsorbed on surface of acid washed sand alone and in the presence of carotene free chlorophyll was studied in vitro.

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MATERIALS AND METHODS

Gamma-HCH, commercial grade (99% purity) was purchased from Sigma Chemical Company. USA. Pesticides reference standards were from Riedel de Haen, Germany. Other chemicals used in the experiment were from British Drug House, England and E. Merck, Germany. Sephadex LH-20 gel was obtained from Pharmacia, Sweden. Pure acid washed, calcinated sea sand with particle size (0.1-0.3 mm) was used for sediment. The sand samples were washed with n-Hexane exhaustively and then with acetone, dried at 110 deg. C and cooled in dessicator till use.

For extraction of total chlorophyll free from carotenes which are known quenchers of active oxygen species, spinach leaves (27 g) were taken and crushed using an all glass pestle and mortar. The crushed leaves were then extracted with 95% chilled acetone (110 mL). In order to separate the chlorophyll a and chlorophyll b, the volume was reduced in vacuum to 50.0 mL and 2.0 mL of this was applied to the sephadex LH-20 gel filtration column of length 60 cm and diameter 2.5 cm using 80% methanol as mobile phase with flow rate 20 mL/hr. The column was wrapped with black paper to avoid any effect of light on chlorophyll. Both chlorophyll a and chlorophyll b (50 mL) were collected in amber colored conical flasks and the volume reduced to 25 mL.

A 5.0 - mL sample containing 2.5 mL chlorophyll a in acetone and 2.5 mL gamma-HCH (1 mg/L) in n-Hexane was applied on the LH-20 gel filtration column and after 70.0 mL, 20 fractions of 2.0 mL were collected and read at Spectronic 1001 spectrophotometer (Bausch and Lomb, Milton Roy Company, USA) at 645 nm and 663 nm for chlorophyll. The same experiment was repeated with chlorophyll b. Control in both the cases were devoid of gamma-HCH. The chlorophyll a and b concentrations were calculated by the formula of Arnon (1949) in terms of mg/mL.

The 4.0 - mL assay system contained chlorophyll a or b in acetone and gamma-HCH in n-Hexane in various concentrations. Controls were devoid of Gamma-HCH. Measurement was performed at excitation 600 nm and emission 662.4 nm on a RF-5000, Ratio fluorophotometer (Shimadzu Corp., Japan). To calculate the extent of quenching, Stern Volmer relationship described by Lee et al. (1991) was used.

$$KSV = \frac{Fa - F}{F \, . \, Qs}$$

where, Fa = Fluorescence Intensity in the absence of gamma-HCH; F = Fluorescence intensity in the presence of gamma-HCH; KVS = Stern volmer constant: Qs = Concentration of gamma-HCH in system.

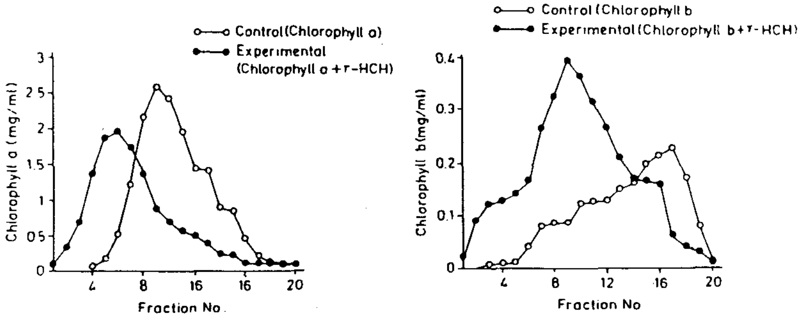
A layer of 5.8 g of sand was evenly spread in each of seven petri dishes (diameter 9.0 cm) to a height of 1.5 cm. A 80.0 - mL solution of 20 mg gamma-HCH was prepared in n-Hexane. Ten (10.0) mL of this solution was sprayed with the help of chromatography sprayer on the sand in each petri dish. It was dried at room temperature. In two of the petri dishes, 10.0 mL of chlorophyll a and in another two, 10.0 mL of chlorophyll b was sprayed. The remaining three petri dishes were devoid of chlorophyll. All the petri dishes except the one that served as a control were irradiated with UV (Sun lamp) light at room temperature UP to 30 min or 1 hr. The irridance of the measured light was measured by the method described by Kumar and Joshi (1992). The dose of UV radiation was 4 and 8 Joules for 30 and 60 min, respectively. After exposure to UV light,the gamma-HCH from each petri dish was extracted from sand with three portions of 50.0 mL AR grade n-Hexane in a separatory funnel. All the extracts were pooled, dried over anhydrous sodium sulphate, and filtered through glass wool. The filtered extract was evaporated on flash evaporator to get 3.0-4.0 mL. Finally, the volume was made up to 5.0 mL.

Gamma-HCH was measured in all the fractions by Gas Liquid Chromatography (GLC). The extracts were diluted accordingly and subjected to gas chromatotographic analysis on Varian vistas 6000 model with column size: length 6 ft and diameter 4.0 mm: detector: electron capture; filling material: 1.5% ov-17 and 1.95% ov-210: column temperature: 180 deg C; inject ion and detector temperature : 250 deg C ; and gas flow : 60 mL/min.

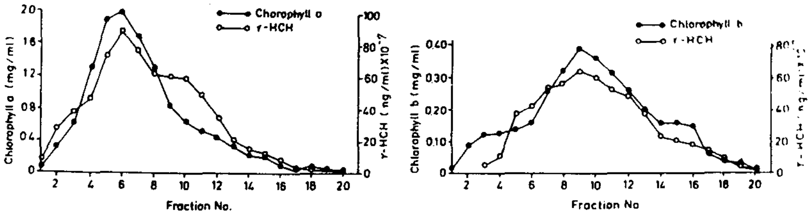
RESULTS AND DISCUSSION

In the Chlorophyll-HCH interaction study, chlorophyll a and b alone showed peaks at fraction no. 10 and 17 respectively. On treatment with gamma-HCH, the above peaks were shifted to fraction no. 6 and 9 respectively (Fig. 1 and 2).

Shifting of the chlorophyll peak towards an earlier fraction on treatment with gamma-HCH indicated an increase in molecular weight and thereby binding of gamma-HCH. Further, presence of a significant amount of gamma-HCH in fraction no, 6 in chlorophyll a and fraction no. 9 in b both confirmed the binding (Fig. 3 and 4).



Figures. 1 and 2. Molecular sieving profile of chlorophyll a and b (O—O) and chlorophyll a gamma-HCH (●—●) on sephadex LH-20 column.



Figures. 3 and 4. Graph showing the amounts of chlorophyll a and b in mg/mL (●—●) and gamma-HCH in ng/mL x 10^{-7} (O—O) in different fractions.

To see the stoichiometric combination between chlorophyll (a & b) and gamma-HCH, the number of molecules involved in the reaction were calculated by dividing the amount of chlorophyll (a & b) and gamma-HCH by molecular weight and multiplied by Avogadro's number (6.023×10^{23} molecules/mole).

Fluorescence spectra of chlorophyll a and b after addition of various concentrations of gamma-HCH showed quenching. However, the quenching was not noteworthy . Stern volmer fluorescence quenching constant (KSV) of chl a and b calculated on the basis of fluorescence intensity in control and experimental cases also showed fluorescence quenching (Table 1).

Effect of UV light on sand sprayed with gamma-HCH showed a 81% decrease of gamma-HCH content at 60 min as compared with the control. Further, when sand

Table 1. Fluorescence intensity and stern volume fluorescence quenching constant (KSV) of chlorophyll a and b after addition of various concentrations of gamma-HCH in the system (listed in the table)

Conc. gamma-HCH ug/mL	Fluorescence Intensity				Fluorescence quenching constant (KSV)	
	Chl. a		Chl. b		Chl. a	Chl. b
	Cont.	Exp.	Cont.	Exp.		
0.95	1.4	1.4	0.1	0.1	0	0
0.90	5.2	4.5	2.0	1.3	0.172	0.518
0.85	8.3	7.4	3.9	3.0	0.143	0.235
0.80	10.7	10.5	5.0	4.5	0.023	0.138
0.75	13.7	13.0	7.6	6.6	0.071	0.202
0.70	17.0	16.0	10.0	8.4	0.089	0.272
0.65	20.0	19.0	11.3	10.8	0.080	0.071
0.60	23.0	21.0	13.4	12.3	0.158	0.149
0.55	25.3	23.2	15.9	13.4	0.165	0.373
0.50	28.0	25.0	18.9	16.5	0.240	0.290

Values are represented as arithmetic mean of three replicates.

Table 2. Effect of UV radiation on sand sprayed with gamma-HCH and chlorophyll

Systems	Length of exposure to UV light in (min)	HCH in mg/mL		Dose of UV radiation in Joules
		alpha	gamma	
Sand+gamma-HCH-UV	-	93.61	1130.24	-
Sand+gamma-HCH+UV	30	94.04	1114.57	4J
Sand+gamma-HCH+UV	60	97.41	216.14	8J
Sand+gamma-HCH+Chl a+UV	30	91.03	556.93	4J
Sand+gamma-HCH+Chl a+UV	60	118.98	395.30	8J
Sand+gamma-HCH+Chl b+UV	30	92.76	172.20	4J
Sand+gamma-HCH+Chl b+UV	60	100.65	148.68	8J

Values are represented as arithmetic mean of three replicates.

sprayed with gamma-HCH and chlorophyll b was irradiated with UV light for 30 min and 60 min, the level of gamma-HCH decreased by 65% and 67% respectively.

Similarly sand sprayed with gamma-HCH and chlorophyl1 a after irradiation with UV light showed a 51% decrease of gamma-HCH in 30 min and a 65% decrease in 60 min. Apart from this, the isomeric conversion of gamma-HCH to alpha-HCH (27%) was noticed with the Gamma-HCH and chlorophyll system, exposed to UV radiation for 1 hr, but with gamma-HCH alone, the conversion was not noteworthy (Table 2).

Lipophilicity is the most important physico-chemical property influencing movement of unionised pesticides through soil (Nicholls 1988). The importance of interaction between lipophilic xenobiotics with hydrophobic sits of biomolecules and structures and their toxicity is being increasingly understood in recent years as such Processes are likely to be factors influencing environmental persistence. intercompartmental distribution kinetics, bioavailability, biomagnification and target species and tissue effects (Bruggeman 1982). The results of the present study conducted with the purpose of understanding any role of chlorophyll on the photodegradation of HCH, showed indication of both hydrophobic interaction and photochemical detoxification. The observed shifting of peaks of chlorophyll towards earlier fractions and presence of gamma-HCH in these fractions along with fluorescence quenching of chlorophyll (though not significant) after addition of gamma-HCH clearly indicated the binding between these two molecules. However, from the present data it is very difficult to decide the target sites and nature of binding. The ratio of number of molecules of chlorophyll and HCH involved in the binding on the basis of Avogadro’s no. showed that the binding is non-stoichiometric and non-specific. Thus on the basis of these observations, the possibility of cluster formation as a result of aggregation and formation of charge transfer complexes between fatty acid chain of chlorophyll a and b and gamma-HCH cannot be ruled out.

Organochlorine pesticides are known to volatilize despite their low vapor pressure. Suntio et al. (1988) studied the vapor pressure of HCH and found that the vapor pressure increases in the order of gamma-HCH (0.025) < delta-HCH (0.031) <beta-HCH (0.033) < alpha-HCH (0.073). The primary degradation product of lindane or gamma-hexachlorocyclohexane is pentachlorocyclohexene (PCCH). Cliath and Spencer (1972) on the basis of field measurements reported that two - third to three - fourths of the volatilized material is lost as PCCH from a calcareous soil.

The decrease of gamma-HCH in sand sprayed with gamma-HCH after exposure to UV light indicated the volatilization. Further, significant decrease of gamma-HCH in the presence of chlorophyll b and UV light indicated the fast photoconversion of gamma-HCH to PCCH which could be due to the dechlorination of lindane molecule, which may be explained on the basis of stereochemical arrangement of chlorine atoms in lindane (containing two pairs of Vicinal, antiparallel-oriented chlorine atoms), which makes dechlorination easier.

Earlier studies by Misra et al. (1992) reported that linear alkyl benzene sulfonate (LAS) facilitates the conversion of gamma-HCH into alpha-isomer from gamma-HCH-loaded sediment in an aquatic system. Benezet and Matsumara (1973) demonstrated the isomerization of gamma-HCH in alpha-HCH in aouatic sediment in the presence of microorganisms. Some alpha-HCH in the atmosphere may also be formed as a result of isomerization of lindane by UV radiation (Malaiyandi and Shah 1984). Similar to these findings our present data also showed the isomeric conversion of gamma-HCH to alpha-isomer (27%) in the presence of chlorophyll and UV light. The data further indicate that such photochemical conversion of gamma-HCH in presence of chlorophyll could be significant in relation to the environmental dynamics of the pesticide in sprayed plants, soil surface in presence of leaf litter or aquatic ecosystem in presence of algae and macrophytes.

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