

# [<sup>14</sup>C]- $\gamma$ -Hexachlorocyclohexane in a Flooded Soil with Green Manuring

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The fate of [<sup>14</sup>C]- $\gamma$ -hexachlorocyclohexane ( $\gamma$ -HCH) was studied in green manure amended and unamended flooded soils with a continuous-flow system permitting <sup>14</sup>C mass balance. There was a greater loss of radioactivity in the form of organic volatiles and <sup>14</sup>CO<sub>2</sub> in green manure amended than unamended soil. The organic volatile compound formed was identified as benzene. Green manure amendment considerably decreased the levels of extractable residues from soil. Bound residue formation was also less in green manure amended than unamended soil.

1,2,3,4,5,6-Hexachlorocyclohexane (HCH) commonly referred to as benzene hexachloride (BHC) is an insecticide widely used in India for agricultural and public health purposes. Technical-grade HCH consists of  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  isomers of which  $\gamma$ -HCH is insecticidal. The use of technical-grade HCH has been banned in many countries, but the use of  $\gamma$ -HCH or lindane is permitted (U.S. EPA, 1985).

Degradation of  $\gamma$ -HCH is rapid in flooded soils (Raghu and MacRae, 1966; MacRae et al., 1967; Yoshida and Castro, 1970), which is further enhanced by organic matter amendments like rice straw (Yoshida and Castro, 1970; Siddaramappa and Sethunathan, 1975) and green manure (Ferreira and Raghu, 1981; Zhang et al., 1982). Anaerobic bacteria viz. *Clostridium sp.* have been reported to be responsible for the rapid degradation of HCH in flooded soil (MacRae et al., 1969, 1984; Heritage and MacRae, 1979). The U.S. Environmental Protection Agency (1985) has stated that data on aerobic and anaerobic metabolism of lindane are required to fully assess its environmental fate. The purpose of this investigation was to examine the degradation of [<sup>14</sup>C]- $\gamma$ -HCH in flooded soil with and without green manure amendment under a continuous-flow system permitting a <sup>14</sup>C mass balance.

## EXPERIMENTAL SECTION

**Apparatus.** A manifold assembly system was used to study the degradation of  $\gamma$ -HCH in flooded soil. It consisted of three flasks: two containing 10 N KOH to remove CO<sub>2</sub> from the incoming air and the last containing water to maintain a saturated moist atmosphere. Volatile organic compounds were absorbed in 2-ethoxyethanol (Guth, 1981), and <sup>14</sup>CO<sub>2</sub> was trapped in 1 N NaOH. The polyurethane filter (Kearney and Konston, 1976) was used to trap any volatile compounds that may have escaped from 2-ethoxyethanol traps. The air flow was maintained at about 5 mL min<sup>-1</sup>.

**Soil.** Black clay soil (Vertisol) collected from Experimental Field Station, Trombay, Bombay, was used. The soil was air-dried and sieved through a 2-mm sieve. The soil had the following characteristics: pH 7.2; organic carbon, 1.26%; sand, 12.0%; silt, 15.0%; clay, 73.0%.

**Soil Treatment and Incubation.** Erlenmeyer flasks (250-mL capacity) containing 50 g of soil and flooded with 100 mL of distilled water were incubated at 30 °C in a water bath. After 7 days of flooding, green leaves of *Glyricidia sepium* (Jacq) Walp. Syn. *Glyricidia maculata* (H.B.K.) were mixed into the soil at the rate of 28 t/ha. Ten days after flooding the soil was treated with [<sup>14</sup>C]- $\gamma$ -HCH (sp act. 54 mCi/mmol obtained from Amersham International plc, Amersham, England) in acetone to a final concentration of 1  $\mu$ g g<sup>-1</sup>. Duplicate flasks were used for green leaf manure amended and unamended [<sup>14</sup>C]- $\gamma$ -HCH-treated soils. A control soil flask untreated with  $\gamma$ -HCH was also maintained.

**Analysis of Volatiles and Soil Samples.** Data on the formation of volatile organic compounds and <sup>14</sup>CO<sub>2</sub> evolution were obtained during the 21-day incubation period. A 0.5-mL aliquot from 2-ethoxyethanol traps and 0.2 mL from NaOH traps were analyzed by liquid scintillation (LS) counting. A 3-mL portion of Cab-O-Sil thixotropic gel powder (Packard Instrument Co. Inc.) was added to 10 mL of scintillation cocktail (PPO, 5 g; naphthalene, 100 g; dioxane, 1000 mL) for counting of the NaOH samples. The polyurethane filters were extracted with 100 mL of hexane in a Soxhlet apparatus, and <sup>14</sup>C was determined by LS counting.

At the end of 21-day incubation period the soils were filtered without suction to separate the standing water, the radioactivity of which was determined by LS counting of 1-mL aliquots. The soils were extracted twice with 100 mL of acetone followed by two extractions with 100 mL of methanol. Extractable radioactivity was determined by LS counting of 1-mL aliquots. Soils (50-mg aliquots) remaining after acetone and methanol extractions were combusted in a Biological Material Oxidiser (J.B. Harvey Instruments) to determine the unextractable (bound) residues. The <sup>14</sup>CO<sub>2</sub> evolved was trapped in Oxysolve-C cocktail and quantitated by LS counting. All radioactive samples were counted in Packard Tricarb Model 3255 LS spectrometer and corrected for background and quenching.

2-Ethoxyethanol, standing water, and acetone extracts of soil were partitioned with hexane. The hexane extracts were analyzed on precoated TLC plates (silica gel 60F.254; E. Merck, Darmstadt, Germany) with hexane-methanol-acetone-diethylamine (100:1:1:1) as the developing solvent system. TLC plates were sprayed with a chromogenic agent (Kovacs, 1965) and exposed to UV (254 nm) for 20 min. Autoradiograms were obtained by exposing Indu X-ray films to another set of TLC plates to locate <sup>14</sup>C metabolites. The regions corresponding to  $\gamma$ -HCH as determined from standard  $\gamma$ -HCH spotted alongside were scraped, and radioactivity was quantitated by LS counting. The hexane extracts were also analyzed by GLC as described by Ferreira and Raghu (1981).

**Product Identification.** The formation of benzene from  $\gamma$ -HCH was established with nonradioactive  $\gamma$ -HCH. A 25-mL portion of a slurry of green manure amended flooded soil (incubated for 30 days) was treated with 1  $\mu$ g g<sup>-1</sup> of  $\gamma$ -HCH in a 50-mL volumetric flask and the flask stoppered with a rubber septum. Duplicate flasks were incubated for 3 days. The headspace gas was analyzed by GLC using a Shimadzu GC RIA with a flame ionizing detector and a microprocessor RPR-GI. The stainless steel column (3.05 m  $\times$  0.3175 cm) containing 5% Carbowax 400 on Chromosorb G (60-80 mesh) was maintained at 60 °C, and N<sub>2</sub> flow rate was 40 mL min<sup>-1</sup>. Under these conditions the retention time for benzene was 2.06 min.

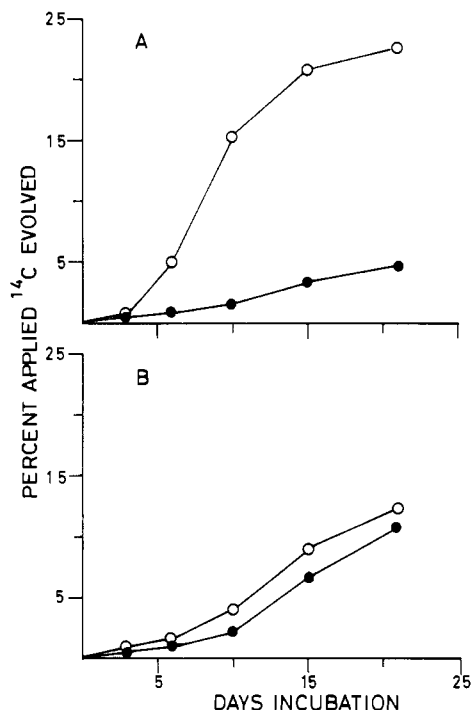
## RESULTS AND DISCUSSION

The loss of radioactivity from soil in the form of volatiles was greater in green manure amended soil than in unamended soil (Table I). The maximum production of

**Table I.** Distribution of <sup>14</sup>C in CO<sub>2</sub>, Volatiles, and Aquatic and Soil Phases Recovered from [<sup>14</sup>C]- $\gamma$ -HCH-Treated Flooded Soil

	% <sup>14</sup> C recovered as					
	<sup>14</sup> CO <sub>2</sub>	organic volatiles	soil			total
			flood-water	extractable	bound	
unamended	10.7	4.7	4.4	30.0 (28.9) <sup>a</sup>	27.4	77.2
green manure amended	12.3	22.6	1.6	4.7 (2.2) <sup>a</sup>	16.9	58.1

<sup>a</sup> Figures in parentheses indicate percent recovered as  $\gamma$ -HCH.



**Figure 1.** Cumulative organic volatiles (A) and <sup>14</sup>CO<sub>2</sub> (B) evolved from unamended (●) and green manure amended (○) flooded soils treated with [<sup>14</sup>C]- $\gamma$ -HCH.

organic volatiles was observed at the 10-day sampling period in green manure amended soil and at the 15-day sampling period of unamended soil (Figure 1). LS counting of the hexane extracts of the polyurethane filters showed the absence of any radioactivity indicating efficient trapping of volatile organic compounds by the 2-ethoxyethanol traps. TLC analyses of the hexane extracts of 2-ethoxyethanol traps showed that all the radioactivity that was spotted had disappeared from the plate. Thus, it appears that the compound(s) trapped in 2-ethoxyethanol was extremely volatile and was not any of the chlorobenzenes or  $\gamma$ -pentachlorocyclohexene ( $\gamma$ -PCCH) or  $\gamma$ -tetrachlorocyclohexene ( $\gamma$ -TCCH), which can be analyzed by the TLC method used (Tu, 1976). GLC analysis of hexane extracts did not show the presence of  $\gamma$ -HCH or any other peaks. A separate experiment carried out to determine the formation of benzene did indeed show the presence of benzene in headspace gas of soils treated with  $\gamma$ -HCH and the absence of benzene in soils untreated with  $\gamma$ -HCH. After 68-h incubation 3.45 ng of benzene mL<sup>-1</sup> of headspace gas was formed while after 72 h the level of benzene had risen to 15.9 ng mL<sup>-1</sup> of headspace gas, indicating the formation of benzene from  $\gamma$ -HCH with an increase in incubation period. Benzene has been reported to be a product of lindane metabolism by *Escherichia coli* and *Clostridium sporogenes* (Allan, 1955).

Sewage sludge treated with lindane and incubated anaerobically showed the formation of benzene along with  $\gamma$ -TCCH (Beland et al., 1976). In our experiment it is likely that  $\gamma$ -TCCH formed as an intermediate in the degradation of  $\gamma$ -HCH to benzene, but due to its rapid conversion to benzene we have been unable to detect it.

Maximum production of <sup>14</sup>CO<sub>2</sub> was observed at 15 days incubation (Figure 1). A total of 12.3 and 10.7% of the applied radioactivity was found to be evolved as <sup>14</sup>CO<sub>2</sub> in green manure amended and unamended soils, respectively (Table I). The presence of <sup>14</sup>CO<sub>2</sub> in NaOH traps was confirmed by hexane extraction of NaOH traps by precipitation as BaCO<sub>3</sub> and LS counting of the <sup>14</sup>CO<sub>2</sub> released on acidification. The levels of <sup>14</sup>CO<sub>2</sub> evolved are higher than those reported earlier from soil. Brahmprakash et al. (1985) showed the evolution of CO<sub>2</sub> from flooded soils planted and unplanted with rice to be negligible and amounted to only 1–2% of the originally applied <sup>14</sup>C activity at 30 days. Kohnen et al. (1975) reported that 6% of the applied <sup>14</sup>C activity of lindane was evolved as <sup>14</sup>CO<sub>2</sub> after 71 days and 17.8% as <sup>14</sup>CO<sub>2</sub> after 140 days from submerged soil. Only 3% of the applied [<sup>14</sup>C]lindane was mineralized to <sup>14</sup>CO<sub>2</sub> at the end of 42 days in an anaerobic suspended soil (Scheunert et al., 1987). The high level of evolution of benzene from green manure amended soil suggests the greater enhancement of reductive degradation of  $\gamma$ -HCH to benzene compared to the ring cleavage of benzene to CO<sub>2</sub>. Green manure amendment is known to reduce the soil, bringing about a more rapid fall of redox potential (Motomura, 1962), thus explaining the enhancement of the reductive degradation of  $\gamma$ -HCH to benzene.

The radioactivity levels in the standing water of the soil were 4.4 and 1.6% in unamended and amended soils, respectively (Table I). Such a low level of <sup>14</sup>C radioactivity had been reported in water phase of anaerobic soil at the end of 42 days (Scheunert et al., 1987). TLC of hexane extract from standing water of unamended soil revealed only one spot corresponding to  $\gamma$ -HCH, while no radioactivity could be extracted into hexane fraction from standing water of green manure amended soil, indicating the absence of  $\gamma$ -HCH or its metabolites. The acetone extracts were reextracted a second time with hexane after adjusting to pH 1. This was done to recover any chlorophenols that might have resulted from lindane degradation. No radioactivity could be recovered in a second hexane extraction, indicating the absence of chlorophenols. The radioactivity in standing water of amended soil probably represents those compounds formed in the degradation of benzene.

The organic solvent extractable radioactivity levels (acetone and methanol) from soil were 30.0 and 4.7% in unamended and amended soils, respectively (Table I). Of the radioactivity 93.1% could be recovered into hexane from acetone extracts of unamended soil while only 75% could be recovered from amended soil. The acetone extracts were reextracted a second time with hexane after adjusting to pH 1. No radioactivity could be extracted in a second hexane extraction, which indicated the absence of chlorophenols. The hexane extracts were spotted on TLC plates and scraped without development, and radioactivity was counted. Losses of radioactivity were 12 and 15.4% in the unamended and amended soils, respectively. Standard [<sup>14</sup>C]- $\gamma$ -HCH gave nearly 100% recovery after spotting and scraping. Hence, the loss of radioactivity could be attributed to the presence of benzene in the soil extracts. TLC analyses of the hexane extracts showed the presence of only one radioactive spot as  $\gamma$ -

HCH while, with the amended soil, most of the radioactivity remained at the origin. GLC analysis confirmed the presence of  $\gamma$ -HCH in soil extracts. Cliath and Spencer (1972) found that two-thirds to three-fourths of the volatilized material was lost as  $\gamma$ -PCCH from an unflooded calcareous soil treated with lindane. Mathur and Saha (1975) reported that lindane degraded slowly (90% recovered) in a sandy loam soil incubated for 6 weeks under flooded conditions, with the formation of  $\gamma$ -TCCH (>5%) and  $\gamma$ -PCCH (>2%) as major degradation products. However, in the present investigation, it is likely that  $\gamma$ -HCH metabolites were not accumulated due to rapid degradation in flooded soil. Hence, no intermediate metabolites from  $\gamma$ -HCH to benzene could be detected.

Soil-bound residues of  $\gamma$ -HCH constituted 27.4 and 16.9% of the applied radioactivity in unamended and amended soils, respectively (Table I). Other studies have also shown a decrease in bound residues of  $\gamma$ -HCH with green manure amendment (Raghu and Ferreira, 1984). Thus, although organic matter is implicated in the formation of bound residues, green manure did not increase but instead decreased the extent of bound residue formation from [ $^{14}\text{C}$ ]- $\gamma$ -HCH. This probably occurred due to an extremely fast rate of degradation of  $\gamma$ -HCH in green manure amended flooded soil. Benzene had been reported to be volatilized and totally degraded to  $\text{CO}_2$  so fast that only low levels of bound residues are formed (Scheunert et al., 1985). From Table I, it can be seen that 77.2 and 58.1% of the applied radioactivity could be accounted for in unamended and amended flooded soils, respectively. The unaccounted radioactivity could have been lost in the form of volatilized benzene during the process of soil filtration.

The results obtained in this investigation indicate that benzene and  $\text{CO}_2$  are the main metabolites in the degradation of  $\gamma$ -HCH only under flooded conditions. Green manure amendment increased the formation of both metabolites and decreased the levels of extractable and bound residues of  $\gamma$ -HCH in flooded soil. The usefulness of flooding and green manure amendment to soil had been pointed out for decontamination of HCH isomers (Ferreira and Raghu, 1981) and toxaphene (Mirsatari et al., 1987). The faster degradation of  $\gamma$ -HCH in green manure treated flooded soil observed in this investigation strengthens the recommendation that green manure is suitable for the decontamination of HCH-polluted soils.

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**Registry No.**  $\gamma$ -HCH, 58-89-9;  $\text{CO}_2$ , 124-38-9; benzene, 71-43-2.

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