# Persistence and Biodegradation of Four Common Isomers of Benzene Hexachloride in Submerged Soils

I. C. MacRae,[1] K. Raghu,[2] and T. F. Castro

The persistence of the $\gamma$ isomer of benzene hexachloride (lindane), when added to submerged tropical soils at a rate approximately three times that recommended for the protection of rice from stem borer infestation and of the $\alpha$, $\beta$, and $\delta$ isomers of benzene hexachloride applied at similar rates was between 70 and 90 days. Losses of all four isomers from sterilized, flooded soil samples were much slower than from nonsterilized samples, indicating that the microflora of submerged soils is able to degrade benzene hexachloride. Microbial degradation of $\gamma$-BHC was demonstrated by the release of $C^{14}O_2$ from submerged soils treated with $C^{14}$-labeled $\gamma$-BHC. An application of $\gamma$-BHC at a rate approximately five times the usual field rate apparently inhibited $CO_2$ evolution from two tropical soils.

The persistence of a number of chlorinated hydrocarbon insecticides in soil and water for very lengthy periods has been reported and attributed either to their resistance to biodegradation or the fact that the insecticide forms a complex with some component of the environment which is largely resistant to microbial attack (Alexander, 1965). The gamma isomer of benzene hexachloride ($\gamma$-BHC) has been listed among these recalcitrant molecules (Alexander, 1965) because of the exceedingly long persistence recorded for this insecticide in nonflooded soils (Hetrick, 1957; Lichtenstein and Schultz, 1959; Lichtenstein and Polivka, 1959; Lichtenstein *et al.*, 1960).

However, previous studies (Raghu and MacRae, 1966) revealed that $\gamma$-BHC did not persist for extended periods in submerged tropical soils, for only a small percentage of the amount originally applied was detected in the soils 90 days after application. Information was also obtained which indicated that $\gamma$-BHC was degraded by the microflora of the submerged soils. These findings were encouraging, as $\gamma$-BHC has proved to be a most effective insecticide for protecting rice from stem borer [*Chilo suppressalis* (Walker)] infestation in the tropics (Pathak, 1967). For effective stem borer control, the insecticide is applied in two doses to give a total application of 5 kg. of $\gamma$-BHC per hectare (surface area basis) which is made directly into the standing water of the rice field (Pathak, 1967).

Commercial preparations of $\gamma$-BHC (6% $\gamma$ isomer) marketed for use in rice stem borer control contain considerable amounts of some of the other isomers of benzene hexachloride—viz., $\alpha$, $\beta$, and $\delta$ isomers. Although previous studies (Raghu and MacRae, 1966) showed that the $\gamma$ isomer did not persist for long periods in submerged soils and indicated that $\gamma$-BHC was biodegradable under the conditions prevailing in submerged soils, no information was available on the persistence and biodegradability of the $\alpha$, $\beta$, and $\delta$ isomers of benzene hexachloride. Because all four isomers enter the soil when commercial preparations for stem borer control are applied to the standing water of the rice field, the present investigations were aimed at determining the persistence of the $\alpha$, $\beta$, $\gamma$, and $\delta$ isomers of benzene hexachloride in submerged tropical rice soils. Also, attempts were made to evaluate the significance of biodegradation to persistence of these substances in submerged soils. The microbial degradation of $\gamma$-BHC in submerged soils also was determined by measuring the release of $C^{14}O_2$ from labeled $\gamma$-BHC added to soils.

## MATERIALS AND METHODS

**Persistence of BHC.** The persistence of the four common isomers of benzene hexachloride in two soils of Luzon (Philippines) was determined. Gas chromatographic analysis revealed that each isomer was free of the other isomers. The air-dried soils (Maahas clay, pH 6.6, organic matter 2.0%, total N 0.14% and a latosolic soil, Luisiana clay, pH 4.7, organic matter, 3.2%, total N 0.21%) were screened (2-mm. diameter), placed in large test tubes in 20-gram amounts, flooded to provide a column of standing water (7 cm.), and plugged with cotton. The tubes containing the soil samples were divided into two series so that two different treatments could be made. In the first treatment the soil samples received only one isomer of benzene hexachloride at the rate of 300 $\mu$g. per 20 grams of soil. The second series received all four isomers (300 $\mu$g. of each isomer per 20 grams of soil). Separately weighed samples of the isomers of benzene hexachloride were added as the dry crystalline material to the standing water in the tubes containing the soils.

To evaluate the significance of biodegradation in losses of the four isomers of benzene hexachloride from submerged soils, sterilized samples of the two soils were prepared and treated in the same way as the nonsterilized soils. Shallow layers of the moistened soils were sterilized by autoclaving at 121° C. for 1 hour on each of three successive days. The tubes containing the treated soil samples were placed in pots containing flooded soil so that the soil and water surfaces in the tubes and pots coincided, and the pots were kept in the greenhouse for the duration of the experiment.

After 0, 15, 30, 50, 70, and 90 days' incubation in the greenhouse, three replicate tubes of each treatment were withdrawn, the benzene hexachloride was extracted from the soils, and the amount of each isomer remaining in the soils was determined quantitatively by gas chromatography.

Department of Soil Microbiology, International Rice Research Institute, Manila Hotel, Manila, Philippines.

[1] Present address, Department of Microbiology, University of Queensland, Brisbane, Australia.

[2] Present address, Indian Agricultural Research Institute, New Delhi, India.

The method used for the extraction of the isomers of benzene hexachloride from the soils gave recoveries of 93 to 99% from standard soil, water, and benzene hexachloride mixtures and was as follows: The soil and water were transferred to a 1-liter volumetric flask by repeated washings with acetone (total volume 200 ml.), 20 ml. of petroleum ether were added, and the volume was made up to 1 liter with 2% $Na_2SO_4$ solution. Following the addition of each organic solvent, the contents of the flask were shaken for 10 minutes and allowed to stand for 1 hour. After the addition of the 2% $Na_2SO_4$ solution, the flask was shaken for 5 minutes and allowed to stand at room temperature overnight to allow separation of the two phases. One milliliter of the petroleum ether layer was removed and suitably diluted with petroleum ether prior to injection into the gas chromatograph.

Identification of the isomers of benzene hexachloride was based upon $R_f$ values, and quantities were calculated by relating the peak heights to those of standards analyzed on the same day. The $R_f$ values obtained for the $\alpha$, $\gamma$, $\beta$, and $\delta$ isomers were 1.2, 1.5, 1.8, and 2.0 minutes, respectively. The gas chromatograph was an Aerograph Model 200 (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) fitted with dual columns and electron-capture detectors. The spiral glass columns were 5 foot × 1/8 inch and packed with 5% silicone (Fluoro) QF-1 on Chromosorb W (60- to 80-mesh). The operating conditions were as follows: column temperature 182.5° C., detector temperature 192.5° C., injection port temperature 220° C., column pressure 60 p.s.i., and detector cell voltage 90 volts d.c. The carrier gas was prepurified nitrogen maintained at a flow rate of 27 to 31 ml. per minute.

**Degradation of $C^{14}$-Labeled $\gamma$-BHC.** To measure the microbial degradation of $\gamma$-BHC in submerged soils, the evolution of $C^{14}O_2$ from three Philippine soils that had been treated with $C^{14}$-labeled $\gamma$-BHC was followed. The soils used were Maahas clay, Luisiana clay, and a clay loam from Pila. Luzon (pH 7.6, organic matter 1.5%, and total N 0.09%). The air-dried soils were screened (2 mm.) and placed in large test tubes in 20-gram amounts. The soils were flooded, treated with $C^{14}$-labeled $\gamma$-BHC (500 $\mu$g. of $\gamma$-BHC per 20 grams of soil, activity approximately 0.1 $\mu$c.) and placed in a water bath at 30° C. By suitable glass and Tygon tubing connections, the tubes were inserted in an aeration train that permitted carbon dioxide-free air to be drawn over the water surface of the soil-water columns, and any evolved carbon dioxide was trapped in 10 ml. of 1*N* NaOH. Samples of the untreated, nonsterilized soils and $\gamma$-BHC-treated, sterilized soils were included in the aeration trains as controls. Three replicates of each treatment were prepared.

At 15-day intervals up to 60 days after the addition of the insecticide, the total amount of carbon dioxide and the radioactivity of the evolved carbon dioxide were determined. At each sampling period, 2 ml. of the 1*N* NaOH used to trap the evolved carbon dioxide were diluted to 50 ml. with carbon dioxide-free distilled water, and the carbon dioxide was titrated with standard 0.2*N* HCl with phenolphthalein as indicator.

The remaining 8 ml. of 1*N* NaOH used to trap evolved carbon dioxide was extracted by shaking with 10 ml. of *n*-hexane (nanograde) for 10 minutes to remove any of the insecticide which may have been carried over in the air stream. After the sodium hydroxide layer had stood for 30 minutes, it was separated from the hexane. The carbon dioxide contained in the sodium hydroxide was liberated by acidifying the solution with 15 ml. of 1*N* HCl in a sealed apparatus. The acidified solution was swept with argon for 1 hour and the carbon dioxide trapped in 5 ml. of hydroxide of hyamine-10 X (Packard Instrument Co., La Grange, Ill.). An aliquot (3 ml.) of the hyamine solution was added to 10 ml. of scintillation solution (5 grams of PPO and 0.3 gram of POPOP per liter of toluene, Packard Instrument Co., La Grange, Ill.) and the radioactivity of the trapped carbon dioxide was determined in a Tri-Carb liquid scintillation counter, Model 314 EX (Packard Instrument Co., La Grange, Ill.).

## RESULTS AND DISCUSSION

**Persistence of BHC in Submerged Soils.** While the rates of disappearance of the isomers of benzene hexachloride from nonsterilized samples of Maahas clay were somewhat slower than for Luisiana clay, the over-all results were similar, and therefore only those obtained for Luisiana clay are used for illustrative purposes. Regardless of whether the four isomers were added singly or in combination to samples of Luisiana clay, only very small amounts were detected in the nonsterilized soil samples 70 days after application (Figures 1 to 4). To avoid confusion, the results obtained for soil samples which received all four isomers are represented by Figures 3 and 4. The same results were obtained with samples of Maahas clay. These results agree with those obtained earlier for the persistence of the $\gamma$ isomer in flooded soils (Raghu and MacRae, 1966). No evidence for prolonged persistence of any of the four isomers of benzene hexachloride was

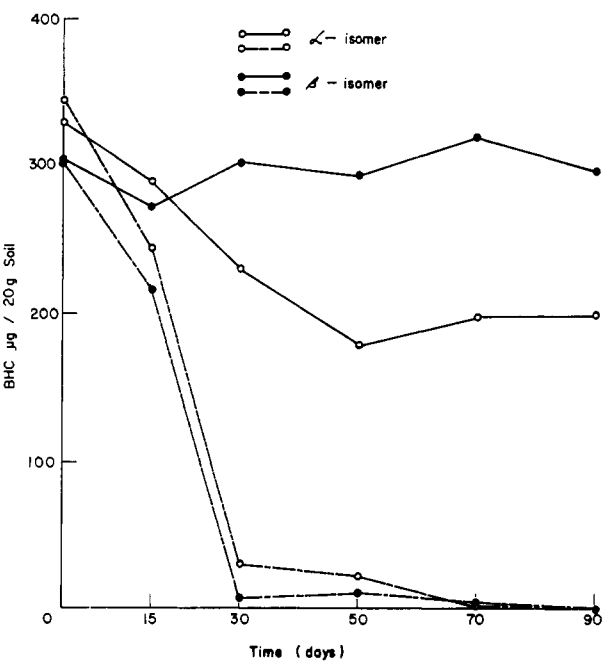


**Figure 1. Persistence of the $\alpha$ and $\beta$ isomers of benzene hexachloride, applied singly to samples of flooded Luisiana clay**

——— Sterilized
- - - - Unsterilized

found in the two soils examined. As the level of application used in the present study was approximately three times the level recommended for stem borer control in the field, no accumulation of BHC residues through applications of commercial preparations of γ-BHC to the two soils studied is expected.

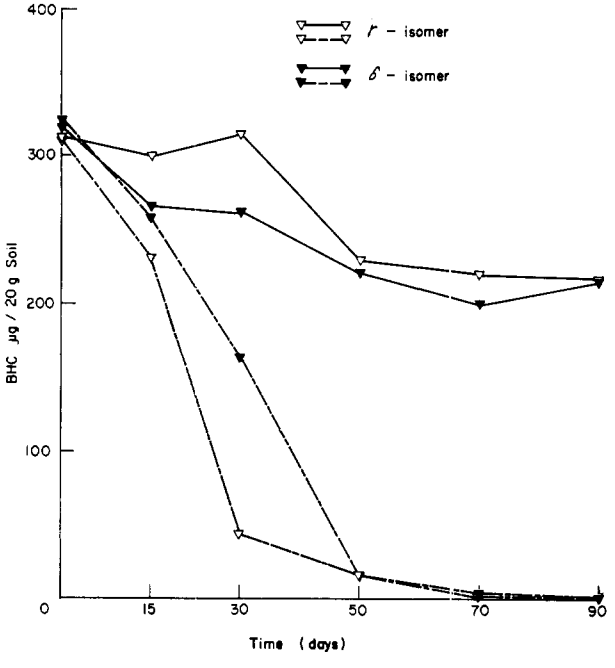


**Figure 2. Persistence of the γ and δ isomers of benzene hexachloride, applied singly to samples of flooded Luisiana clay**

——— Sterilized
- - - - Unsterilized

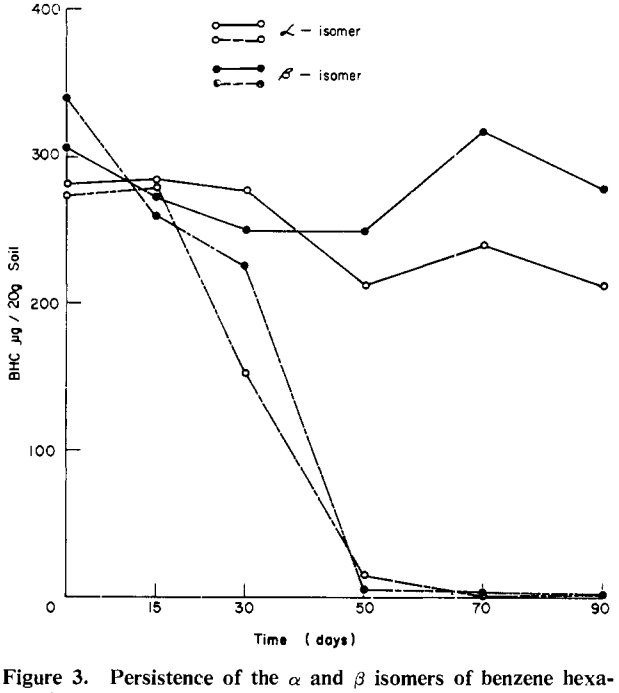


**Figure 3. Persistence of the α and β isomers of benzene hexachloride following application of all four isomers in combination to samples of flooded Luisiana clay**

——— Sterilized
- - - - Unsterilized

Losses of the four isomers of benzene hexachloride from sterilized samples of both soils were far less than from nonsterilized soil samples (Figures 1 to 4). These results indicate that all four isomers were degraded biologically, and that biodegradation is one of the most important factors governing the persistence of benzene hexachloride in submerged soils. Losses of the isomers of benzene hexachloride from the sterilized soil samples can be attributed to volatilization, and the magnitude of these losses followed the pattern which might be predicted from the vapor pressures of the four isomers—i.e., $\beta < \gamma < \delta < \alpha$. Losses due to volatilization were greater for Maahas clay than for Luisiana clay.

Of the four isomers examined in the present study, the γ isomer is the only one which possesses significant insecticidal activity (Metcalf, 1955). Therefore, because of the differences among the isomers in their biological activity as insecticides, differences in their susceptibility to microbial degradation might be expected. However, no conclusive evidence was obtained in the present study which would indicate differences among the isomers in their susceptibility to microbial attack.

In view of the exceedingly long persistence of benzene hexachloride reported for nonflooded soils (Hetrick, 1957; Lichtenstein and Schultz, 1959; Lichtenstein and Polivka, 1959; Lichtenstein *et al.*, 1960), the contrasting results reported here for flooded soils suggest that the anaerobic species of the soil microflora are more active than aerobic species in the degradation of benzene hexachloride. With the exception of a few millimeters or so of surface soil, the bulk of the profile of a submerged soil becomes anaerobic soon after flooding, and anaerobic species of the soil microflora become dominant. The results also suggest that,

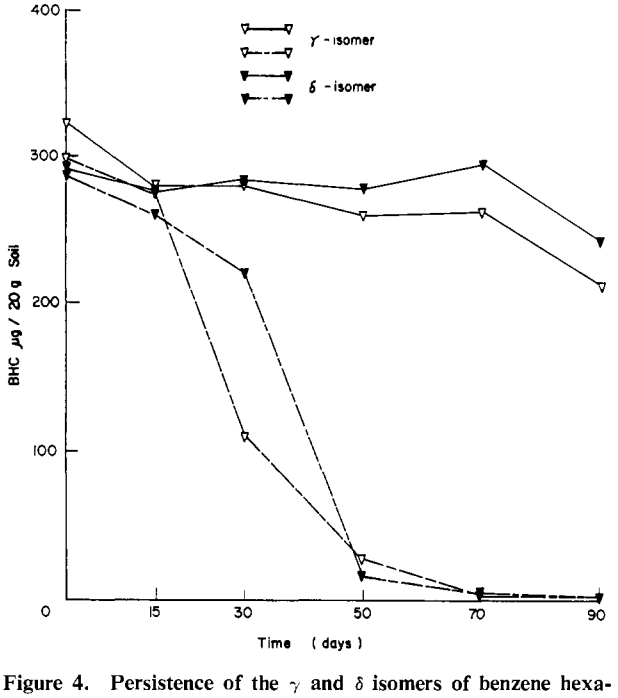


**Figure 4. Persistence of the γ and δ isomers of benzene hexachloride following application of all four isomers in combination to samples of flooded Luisiana clay**

——— Sterilized
- - - - Unsterilized

Table I. Release of $C^{14}O_2$ from $C^{14}$-Labeled γ-BHC Applied to Submerged Soils

| Soil | Radioactivity in Evolved $CO_2$, C.P.M.[a] | | | |
|---|---|---|---|---|
| | 15 days | 30 days | 45 days | 60 days |
| Luisiana clay | 9,780 | 51,570 | 18,510 | 6,580 |
| Maahas clay | 10,440 | 11,880 | 10,260 | 7,260 |
| Clay loam (Pila) | 7,000 | 9,510 | 12,010 | 7,630 |

[a] All figures adjusted for background and for activity obtained for sterile controls. Range for sterile controls was 80 to 450 c.p.m.

Table II. Effect of γ-BHC upon $CO_2$ Evolution from Submerged Soils

| Soil | Evolved $CO_2$ after 60 Days, Ml.[a] | |
|---|---|---|
| | Treated | Untreated |
| Luisiana clay | 63.5 | 107.7 |
| Maahas clay | 73.4 | 77.9 |
| Clay loam (Pila) | 44.8 | 104.0 |

[a] All figures represent total carbon dioxide evolved during 60-day incubation.

where feasible, flood-fallowing may be effective in reducing levels of benzene hexachloride in soils which through repeated applications have developed levels of the insecticide which produce toxicity symptoms or off-flavors in certain crops.

**Degradation of $C^{14}$-Labeled γ-BHC.** By determining the radioactivity of carbon dioxide evolved from flooded, sterilized, and nonsterilized samples of three soils which had been treated with $C^{14}$-labeled γ-BHC, further evidence was obtained that the insecticide undergoes microbial degradation in submerged soils. The results of this study are given in Table I. More rapid release of $C^{14}O_2$ was found for Luisiana clay than for Maahas clay, and this result is consistent with the earlier finding (Raghu and MacRae, 1966) that γ-BHC disappeared more rapidly from Luisiana clay.

The results demonstrate microbial degradation of γ-BHC. Evidently, the six-carbon ring structure was broken, and some of the carbon was oxidized to carbon dioxide. As the degradation of γ-BHC seems to be carried out chiefly by anaerobic species of the microflora, methane also may be expected to be a mineralization product of the degradation of γ-BHC.

Carbon dioxide evolution from Luisiana clay and the clay loam was retarded by an application of γ-BHC equivalent to five times the recommended field rate (Table II). Very little effect was noted in the case of Maahas clay. These results indicate that γ-BHC may have had an inhibitory effect upon the mineralization of native organic matter. This finding is surprising, in view of the results obtained earlier which showed that applications of γ-BHC at 10 times the recommended rate had no inhibitory effect upon the mineralization of soil nitrogen (Raghu and MacRae, 1967). The method employed in the present study only accounted for carbon dioxide actually released from the water surface. No measure of dissolved carbon dioxide or methane (an important product of carbon mineralization in submerged soils) was made. Because of the numerous factors influencing the retention of carbon dioxide in submerged soils—e.g., pH, iron, and manganese content—it seems more likely that the γ-BHC influenced one of these factors rather than inhibiting the mineralization of native soil carbon.

LITERATURE CITED


Alexander, M., *Advan. Appl. Microbiol.* **7,** 35 (1965).
Hetrick, L. A., *J. Econ. Entomol.* **50,** 316 (1957).
Lichtenstein, E. P., de Pew, L. J., Eshbought, E. L., Sleesman, J. P., *J. Econ. Entomol.* **53,** 136 (1960).
Lichtenstein, E. P., Polivka, J. B., *J. Econ. Entomol.* **52,** 289 (1959).
Lichtenstein, E. P., Schultz, K. R., *J. Econ. Entomol.* **52,** 124 (1959).
Metcalf, R. L., "Organic Insecticides, Their Chemistry and Mode of Action," Interscience, New York, 1955.
Pathak, M. D., "Proceedings of Symposium on Major Insect Pests of Rice," Johns Hopkins Press, Baltimore, Md., in press, 1967.
Raghu, K., MacRae, I. C., *Can. J. Microbiol.*, in press, 1967.
Raghu, K., MacRae, I. C., *Science* **154,** 263 (1966).



*Received for review February 23, 1967. Accepted May 29, 1967. Study supported in part by a grant from the National Science Development Board of the Philippines.*