STUDY IDENTIFICATION:


REVIEWED BY:

Richard J. Mahler, Hydrologist
Review Section I, EFGWB

APPROVED BY:

Paul J. Mastradone, Chief
Review Section I, EFGWB

CONCLUSIONS:

1. EFGWB concludes this study does not satisfy the data requirements for an anaerobic soil metabolism study and is considered supplemental.

2. An adequate material balance was not reported in this study. Absence of this data does not allow EFGWB to validate the conclusions presented in the study.

3. While the author speculated on the reasons for the incomplete material balance, the report does not mention if any attempt was made to determine where the loss occurred nor the identity of the metabolite.

4. EFGWB concludes that lindane will be stable in anaerobic conditions with a half-life of 37 days. Any dissipation would be by degradation to CO₂ and an unknown metabolite and adsorption as soil-bound residues. The resolution of the data deficiencies is not likely to alter these conclusions.

MATERIALS AND METHODS:

Lindane-UL-¹⁴C (Specific activity: 4.20 mCi/mM, radiopurity = 97.66%) was added (1.97 µg/g soil) to a fine sandy loam soil with the following characteristics: pH 6.4; CEC, 10.0 meq/100 g; sand, 70%; silt, 15%; clay 15%; organic matter, 2.8%; 1/3 bar moisture content, 14.15%.

Moisture content was maintained at 75.12% of field capacity for thirty days before conversion to anaerobic incubation conditions by the addition of corn starch, 90 ml of water and purging each flask with N gas for 15 minutes to remove the O₂ from the system.

The treated soil was incubated anaerobically at 24.5°C in the dark. Flasks containing nontreated flooded soil were incubated at room temperature on a laboratory bench. Both treated and nontreated soils were maintained under N gas.
Volatilized $^{14}$C was trapped in NaOH, polyurethane foam plugs, and XAD-2 and XAD-4 resins.

Soil samples were collected and analyzed at 0, 32, and 67 days after flooding. NaOH trapping solutions were sampled and changed periodically in order to determine the amount of $^{14}$CO$_2$ recovered.

The water above the soil was analyzed by LSC to determine the amount of radioactivity. The water was then extracted sequentially with hexane and 2X with ethyl acetate and aliquots removed for LSC.

After removing the flood water, the soil was extracted with methanol and 2X with acetone-methanol-toluene (AMT) to determine extractable compounds. Air-dried extracted soil samples were then combusted in a sample oxidizer to determine soil bound residues after extraction with AMT.

The AMT extracted soils were air dried and acid hydrolyzed with 2% HCl-methanol to determine soil bound residues not readily extractable with AMT. The 2% HCl-methanol solutions were further separated with NaCl and ethyl acetate. The acid hydrolyzed soils were air dried and combusted in a sample oxidizer to determine soil bound residues not acid hydrolyzable.

The amount of radioactivity in extracts, combusted samples and as volatilized products were determined using LSC. TLC analysis was used with two solvent systems to identify the products by comparing the results with known standards of 2,3,4,5,6-pentachlorocyclohexene (PCCH), alpha 1,2,3,4,5,6-hexachlorohexane (BHC) and lindane.

REPORTED RESULTS:

The author reported that material balance varied from 88.54 to 71.72% of the applied radioactivity (Table 15). The radioactivity in the soil extracts decreased with increasing incubation time (73.57 to 42.44% of applied radioactivity); while soil bound radioactivity increased with time (8.70 to 16.48% of applied radioactivity at days 32 and 67, respectively.

Volatilization of lindane was not a major mechanism of loss from the soil under anaerobic conditions since no more than 0.42% of the applied radioactivity was recovered in the resins or polyurethane plugs at either sampling time (Table 15). The author speculated that a portion of lindane was metabolized to volatile $^{14}$C-compounds which could not be trapped, thus accounting for the incomplete recovery rates or material balance.

Degradation products of lindane were identified as CO$_2$ and alpha BHC, accounting for 6.25 and 0.56%, respectively, of the applied radioactivity at day 67(Table 15 and 22). An unknown metabolite, representing no more than 0.40% of the applied radioactivity, appeared in the water fraction after 67 days.
TLC autoradiographic analysis of the AMT extracts showed that 42.05 and 22.46% of the applied radioactivity was recovered as parent lindane after, respectively, 32 and 67 days.

The overall percent recovery of lindane-UL-\( ^{14} \text{C} \) from the soil and water fraction, as reported by the author, decreased with time from 46.74 to 24.65% of the applied radioactivity (Table 22).

The extracted and volatile products were qualitatively identified by co-chromatography with known standards of lindane, PCCH and BHC using two solvent systems.

The author calculated the anaerobic half-life of lindane to be 37 days using linear regression on data from Tables 13 and 22.

**DISCUSSION:**

1. The anaerobic and aerobic soil metabolism studies were combined into one report making it difficult to follow either study. The two studies should have been sent back to the author to be written into two reports.

2. There was no verification that the incubation conditions were actually anaerobic.

3. The water used for flooding was not characterized.

4. While the author calculated the half-life of lindane under flooded conditions using linear regression, it was not stated what data was used for the calculation. In order to verify the half-life calculation, the reviewer spent considerable time determining which data was used in the calculation.

5. The author speculated on the reasons for the incomplete material balance; however the report does not mention if any attempt was made to determine where the loss occurred nor the identity of the metabolite. Subsequent telephone conversation with the author revealed that he speculated that methane was a major metabolite of lindane which could not be trapped under the conditions of the experiment. The work of MacRae, et al (I.C. MacRae, K. Raghu, and T.F. Castro. 1967. Persistence and biodegradation of four common isomers of benzene hexachloride in submerged soils. J. Agric. Food Chem. 15:911-14.) supports this conclusion. They pointed out in their study that since lindane degradation was carried out by anaerobic organisms, that methane should be expected to be a product of lindane decomposition.

6. EFGWB accepts this study as supplemental because of the deficiencies noted and believes that correction of these deficiencies may not affect the conclusions resulting from the study.