25 and 55 days showed considerable radioactivity, a decrease in total radioactivity was more rapid with rice straw amendment. Evidently, rice straw accelerated the degradation of not only endrin, but also its breakdown products.

Effect of Liming. It was noticed earlier (Table II) that of the two acid sulfate soils used, the degradation of endrin was slow in kari soil in contrast to its rapid breakdown in pokkali soil, perhaps due to the extremely low pH of the former soil even under flooded conditions. Whether an increase in soil pH by liming would accelerate the degradation of endrin in kari and alluvial soils was studied. The radioactivity counts recorded for endrin in limed and unlimed kari soils were 23.2 and 21.7 cpm $\times$ $10^4/20$ g of soil after 25 days whereas the corresponding values for alluvial soils were 14.7 and 12.9. Similarly, with regard to total radioactivity recovered in the solvent fraction, no appreciable difference was noticed between limed and unlimed soils. Liming was thus not very effective in increasing the degradation rates of endrin and/or its breakdown products in both soils in spite of a favorable pH in limed soils resulting in an increase in bacterial numbers (Rao et al., 1975). Recent studies in this laboratory showed that both γ and β isomers of benzene hexachloride persisted in this unique acid sulfate soil under flooded conditions even after liming (Siddaramappa, 1975). In contrast, Parr and Smith (1974) reported that liming a muck soil from pH 5.3 to 6.8 caused a rapid conversion of DDT to DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane) in a moist anaerobic environment.

CONCLUSION

The data presented in this report demonstrate the instability of endrin in most Indian soils tested under flooded conditions in contrast to the reported stability of endrin in three out of four Philippine rice soils. Degradation of endrin in flooded soils is mediated by both chemical and biological means; biological degradation is more extensive. Interestingly, metabolites formed showed resistance to further degradation chemically or biologically. Unless these metabolites are characterized with regard to their chemical nature and toxicity and unless means of inactivating these metabolites in a flooded soil environment are developed, the use of endrin in rice poses problems of environmental pollution despite its rapid breakdown to several metabolites under flooded conditions. The rapid degradation of endrin in two unique acid sulfate saline rice soils, particularly in pokkali soil of Kerala, is of applied significance since substantial portions of rice soils in coastal areas of India are highly saline and data regarding pesticide behavior in such environments are rather limited.

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Electrochemical Reduction and Anaerobic Degradation of Lindane

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The anaerobic reduction of lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) in sewage sludge and soil is compared with its electrochemical reduction in dimethyl sulfoxide (Me$_2$SO). Benzene was found to be an anaerobic reduction product along with the previously recognized γ-BTC (γ-3,4,5,6-tetrachlorocyclohexene). The electrochemical reduction of lindane at a mercury coated platinum electrode has a single wave at -1.520 V vs. SCE. γ-BTC was found to be an intermediate in the reduction to benzene at this potential. No other intermediate chlorinated compounds were detected in either type of reduction. This is another example of the modeling of anaerobic reduction of halogenated compounds by electrochemical reduction.

Organochlorine insecticides, which have been used extensively for the last 25 years, are generally considered to be persistent compounds. However, Hill and McCarty

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(1967) reported that under anaerobic conditions (such as in sewage sludge, in the sediment of river or lake bottoms, or in the soil of flooded fields) some of the pesticides have very short lifetimes. Lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane), for instance, was shown to have a half-life of approximately 1 day.

In our laboratory we have been studying the electrochemical reduction of organochlorine compounds, especially those of environmental concern (Farwell et al., 1973, 1975a,b). During the course of these investigations we

hypothesized that perhaps reductive electrochemistry could be used to predict the degradative pathway of organochlorine compounds under anaerobic conditions. For example, DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane) is the anaerobic degradation product of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) (Plimmer et al., 1968; Glass, 1972), and this same reaction pathway is observed electrochemically (Rosenthal and Lacoste, 1959; Farwell, 1973). Furthermore, under anaerobic flooded-soil conditions DTE (1,1,1,2-tetrachloro-2,2-bis-(p-chlorophenyl)ethane) loses vicinal (i.e., adjacent) chlorines to form DDE (1,1-dichloro-2,2-bis(p-chloro-phenyl)ethylene) (Beland et al., 1974), and again this is the reaction which occurs electrochemically (Rosenthal and Lacoste, 1959; Farwell, 1973).

Lindane can also be reduced electrochemically. This reduction is a six-electron process whereby lindane is quantitatively transformed into benzene as shown in the following reaction (Fukami et al., 1953; Schwabe and Frind, 1951).

$$C_6H_6Cl_6 + 6e^- \rightarrow C_6H_6 + 6Cl^-$$
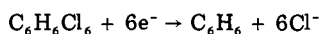
Thus, since the electrochemical reduction obtained in the laboratory correctly predicts the anaerobic environmental degradation products of DDT and DTE, we questioned whether or not the same type of correlation would hold true for lindane. However, γ-BTC (γ-3,4,5,-6-tetrachlorocyclohexene) is a known anaerobic degradation product of lindane (Tsukano and Kobayashi, 1972). In this paper we report that γ-BTC is an intermediate in the electrochemical reduction of lindane and that it is further reduced to benzene. We also show that lindane is further reduced beyond γ-BTC to benzene under anaerobic conditions in a manner analogous to the electrochemical reduction.

EXPERIMENTAL SECTION

The cell, electrode system, instrumentation, and experimental electrochemical procedures employed were the same as previously described (Farwell et al., 1975a). Reagent grade dimethyl sulfoxide (Me$_2$SO) was the solvent with 0.1 M tetraethylammonium bromide (Et$_4$NBr) as the supporting electrolyte. The concentration of the electroactive species was 5×10^{-4} M for all the electrochemical experiments.

Lindane was anaerobically incubated in two different systems, sewage sludge and Bowdoin clay loam soil (air-dried, pH 7.9, 1.44% organic carbon, 0.8% sand, 30.6% silt, 68.6% clay). The incubation and metabolite isolation procedures for this soil system have previously been described (Beland et al., 1974).

Lindane was incubated in sewage sludge as follows. The sludge was obtained from the 35 °C anaerobic digestor at the Bozeman, Mont. sewage treatment plant. The physical characteristics of this sludge were: pH 7.0, and 1.7% total solids of which 60% were volatile solids. Two 2-l. samples of sludge were collected at weekly intervals in stoppered 2-l. Erlenmeyer flasks. One flask was treated with 400 mg of lindane in about 10 ml of acetone. The other flask, used as the control, was treated with 10 ml of acetone. Each sample was stoppered and continually mixed with a magnetic stirrer while being incubated at 37 °C under a nitrogen atmosphere to ensure anaerobic conditions. In each case an exit tube passed through the cork stopper and led to a dry ice–acetone cold trap to collect volatile metabolites. The system was allowed to incubate for 24 h; then nitrogen was bubbled through the sewage for 12 h to help purge any volatile degradation products that had been formed. The material collected in the cold trap was an-

alyzed directly by gas chromatography.

Two different gas chromatographs (GC) were used for monitoring the reactions and for product identification. They were a Varian Aerograph Series 1200 equipped with an electron-capture detector and 10 ft × 1/8 in. silanized Pyrex columns, and an F and M Model 400 equipped with both thermal conductivity (for preparatory work) and flame ionization detectors, and 6 ft × 0.25 in. silanized Pyrex columns. Lindane and γ-BTC were separated on two different columns, one packed with 5% QF-1 on 80/100 mesh Chromosorb W, the other with 3% OV-101 on 80/100 mesh Chromosorb W. The presence of benzene was established by using 10% Carbowax 20M on 60/80 mesh Chromosorb W and a mixed 5% Bentone 34/10% OV-101 on 100/200 mesh Supelcoport.

Lindane was Baker grade from the J. T. Baker Company. γ-BTC was synthesized by a zinc reduction of lindane. Specifically, 5 g of lindane and 1.2 g of zinc dust were added to 20 ml of 95% ethanol and the mixture was refluxed for 1 h. Upon cooling, 20 ml of water was added and the solution extracted three times with 50-ml aliquots of hexane. Gas chromatographic product analysis revealed the presence of three major components: an unidentified early eluting fraction followed by γ-BTC, and then by unreacted lindane. The γ-BTC was isolated by preparatory GC and had a melting point of 88–89 °C (Fisher-Johns apparatus) and an infrared spectrum (Beckman IR-5) in agreement with the values reported by Orloff et al. (1953)

Benzene formation was confirmed by synthesizing the 1,3-dinitro derivative with the following procedure. First, 15 ml of concentrated sulfuric acid was added to 5 ml of concentrated nitric acid and the mixture was cooled with ice to about 5 °C. Then the solution containing benzene was slowly added to this acid mixture while cooling with ice. Following the addition, the mixture was warmed to approximately 50 °C for 10 min and then poured onto about 100 ml of ice and extracted three times with 50-ml portions of hexane. The combined hexane extracts were concentrated to about 1 ml on a rotary evaporator and analyzed by GC.

RESULTS AND DISCUSSION

Electrochemical. It is well known that lindane is readily reduced electrochemically. One pH-independent wave is observed in the reduction (Fukami et al., 1953; Schwabe and Frind, 1951). In our Me$_2$SO solvent system this reduction occurs at an E_{2d} of −1.521 V (vs. SCE) and represents the six-electron reduction of lindane to benzene. (E_{2d} is the interrupt potential determined by the zero crossing of the second derivative curve (Farwell et al., 1975a).) This reduction mechanism was confirmed by performing controlled-potential electrolysis and subsequently analyzing the electrolysis product(s) by gas chromatography. The only compound detected *after* the electrolysis current returned to its initial residual level was benzene.

After confirming that the reduction of lindane does indeed form benzene as a final product, we considered what potentially detectable intermediates might be involved in its overall reduction mechanism. For example, when there are adjacent chlorines on a saturated system, the two chlorines are generally lost in a one-step reduction with simultaneous formation of a double bond (Mann and Barnes, 1970). In the case of lindane this would present the possibility of finding tetrachlorocyclohexenes (after two electrons have been transferred) and dichlorocyclohexadienes (after four electrons have been transferred). In order to investigate these potential intermediates in the
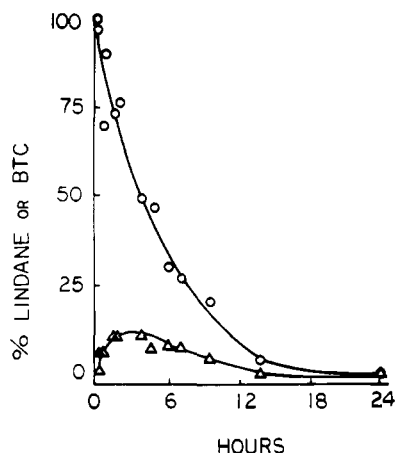
Figure 1. Controlled potential electrolysis of 5 × 10⁻⁴ M lindane in Me₂SO-0.1 M Et₄NBr: voltage, -1.520 V vs. SCE; (o) lindane; (△) γ-BTC.

Figure 2. Anaerobic formation of γ-BTC from lindane: (o) lindane; (△) γ-BTC.

Figure 3. Electrochemical reduction pathway for lindane. This same sequence is proposed for the anaerobic degradation of lindane.

electrochemical reduction, the electrolysis reaction course was followed as a function of time by GC analysis. We confirmed that the intermediate formed in the electrolysis of lindane was indeed γ-BTC by comparison of known GC retention times for the γ-BTC standard with the corresponding chromatographic peaks for the electrolysis reaction mixture. Further confirmation was obtained by simultaneous injection of the reaction mixture and a γ-BTC standard. In addition, a small amount of the compound responsible for the chromatographic peak in the electrolysis mixture was isolated by preparatory GC, and its melting point and infrared spectra were identical when compared with the same data obtained for the γ-BTC standard. As illustrated in Figure 1, the γ-BTC intermediate reached a maximum of 15% of the lindane concentration before slowly decreasing.

It is not surprising that γ-BTC is formed at the first step during the reduction. The conformation of the chlorines in lindane is eeeaaa (e = equatorial, a = axial) while γ-BTC has the conformation eeea or aaae, the former being the preferred conformation (Orloff et al., 1953). Thus, there are four equivalent positions (allowing for ring inversion) on lindane by which the loss of adjacent chlorines will generate γ-BTC. The only other tetrachlorocyclohexene that could be formed is the α isomer (eeaa), through the loss of adjacent ae chlorines. However, we did not detect the formation of this isomer. This result supports the observation of Zavada et al. (1963) that compounds will reduce more easily when the dihedral angle of the halogens is either 180 or 0°. They also found that the greatest $E^{1}/_{2}$ occurred when the angle was 90°. The angle between the chlorines whose loss would form γ-BTC is 180°, while the angle between the chlorines whose loss would form α-BTC is close to 60°. Consequently, the electrochemical reduction mechanism seems to agree with Zavada's prediction.

Subsequent reduction of γ-BTC should yield a dichlorocyclohexadiene before benzene is generated, this again being due to the simultaneous loss of adjacent chlorines with concomitant double bond formation. However, by controlled potential electrolysis we were unable to detect the formation of any species other than γ-BTC or benzene, even when performing the electrolysis on γ-BTC at an applied voltage of -1.520 V vs. SCE.

Anaerobic Degradation. When lindane was incubated in the experimental anaerobic soil system, γ-BTC was found in the amounts shown in Figure 2. The γ-BTC product reached a maximum concentration at the end of 2 weeks and then steadily declined for the duration of the
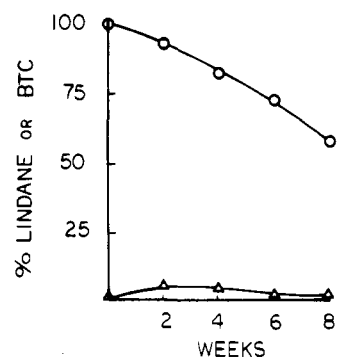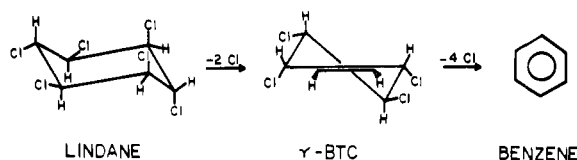
experiment. The maximum amount of γ-BTC obtained was just 5% of the initial lindane concentration, which agrees with previous observations. For example, Tsukano and Kobayashi (1972) observed γ-BTC to reach a maximum concentration of 2% after γ-BTC was incubated for 2 weeks in flooded rice field soil, whereas Benezet and Matsumura (1973) found 19% formation of γ-BTC after a 1 month anaerobic incubation period.

The low recovery of γ-BTC could be due to either irreversible binding to the soil system or to further degradation, or both. However, further degradation seems to be a more tenable explanation. γ-BTC is formed as an intermediate during the single potential electrochemical reduction of lindane to benzene. Since γ-BTC is also detected during the anaerobic degradation of lindane, it seems plausible that any γ-BTC formed could be in turn reduced (Figure 3). To test this hypothesis, lindane was incubated in anaerobic sewage sludge where it has a reported half-life of approximately 24 h (Hill and McCarty, 1967).

Five 2-l. samples of sewage sludge were incubated, each containing 400 mg of lindane. At the end of 36 h, gas chromatographic analysis indicated the formation of a new compound that had an identical retention time to benzene on two different columns (10% Carbowax 20M and 5% Bentone 34/10% OV-101). The average amount of benzene detected in the five runs was 11.1 mg with a range from 4.0 to 18.3 mg. Five control incubations were run, four of which revealed no trace of benzene. (The minimum amount of benzene that could be detected under the operating conditions, based upon a set of applied controls, was equivalent to 0.4 mg of benzene). One control sample did have a peak corresponding to benzene with a concentration of 1.4 mg. However, the average amount of benzene found in the five experimental samples was eight times the amount found in this one control, so it is clear that benzene is being formed anaerobically and presumably from lindane.

To confirm the formation of benzene, the m-dinitro derivative of benzene was made from each of the experimental samples. A peak was found that had an identical GC retention time to m-dinitrobenzene. This peak was not detected in any of the controls, with the exception of the one previously discussed. The amount of benzene

detected is only approximately 10 mol % of the amount of lindane applied, assuming complete conversion. There are two apparent reasons that could cause this low recovery. First of all, there may not have been efficient transfer of all the benzene to the cold trap. This could have been due to an inadequate flow of nitrogen through the sewage vessel. Secondly, and perhaps a more plausible reason, is that the anaerobic organisms in the sewage sludge were utilizing the benzene as a carbon source. For instance, labeled carbon dioxide has been found after the anaerobic incubation of [^{14}C]lindane (MacRae et al., 1967). This implies that lindane, or more probably one of its degradation products (i.e., benzene), is being used as a carbon source.

CONCLUSION

Thus, we have another example of the utility of electrochemical reduction data to predict anaerobic degradation products of organohalogen compounds in the environment. In this instance electrochemistry indicated that the γ-BTC formed during anaerobic degradation should be a transient intermediate and that benzene should be the final reduction product. This was established to be the case by incubating lindane in sewage sludge.

This concept may be carried further. If the anaerobic degradation of compounds is purely a chemical phenomenon, as has been recently suggested by Zoro et al. (1974), one might expect to be able to predict the final degradation products from just a knowledge of the electrochemical reduction potentials. For example, DDT can be anaerobically reduced because its E_{2d} is only -1.240 V (Farwell, 1973). However, its reduction product, DDD, which has an E_{2d} of -2.068 V (Farwell, 1973), cannot be anaerobically reduced because the redox potential associated with the anaerobic conditions is not sufficiently low to promote this reduction. DTE has a reduction potential more anodic than DDT's; thus it is anaerobically reduced, whereas DDE, the reduction product of DTE, is not because its first reduction potential of -1.757 is too cathodic. These data imply that compounds with an E_{2d} more positive than -1.521 V vs. SCE in Me$_2$SO will be anaerobically degraded and compounds with an E_{2d} more negative than -1.757 V will not be anaerobically reduced.

The validity of this generalization will have to be tested by investigating the reduction of more compounds of this type.

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In Vitro Inhibition of Lactic Acid Dehydrogenase by Insecticidal Polychlorinated Hydrocarbons. 2. Inhibition by Dieldrin and Related Compounds

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Dieldrin and related compounds, including aldrin, endrin, and chlordane, inhibit lactate dehydrogenase (EC 1.1.1.27, crystalline from rabbit muscle). Inhibition is competitive with respect to pyruvate and to NADH; apparent inhibition constants range from 0.02 to 0.06 mM (11 ppm for dieldrin). Levels at which this inhibition occurs are well below the usual lethal in vivo dosages and are comparable to accumulation concentrations. Lactate dehydrogenase occupies a key position in the anaerobic glycolytic pathway of skeletal muscle and in other tissues; impairment of this enzyme's function could result in possible harmful effects on enzyme function.

In previous papers we have described the inhibition of crystalline rabbit muscle lactic acid dehydrogenase by the

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insecticide Mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene) (Hendrickson and Bowden, 1973, 1975a) and DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (Hendrickson and Bowden, 1975a, 1976b), and have speculated upon the mode and mechanism of the in vitro inhibition of lactic acid dehydrogenase by these neuroactive high molecular weight polychlorinated hy-