system is produced at the crest, because active volcanoes exist on the flanks. However, it appears that very linear features, roughly parallel to the crest, generally are formed at the crest and preserved on the flanks. According to Table 1, the relief of the topography is proportional to the thickness of the second layer and inversely proportional to the spreading rate. Both relationships seem reasonable. The construction of a relatively thick second layer by lava flows and intrusions might be expected to produce a relatively high relief. The prolonged faulting of crustal blocks during slow spreading might produce more relief at the crest than the briefer faulting possible with fast spreading. In short, the relief can be correlated with the time of exposure to the relief-producing processes at the crest of a rise.

If the relationships and correlations above are correct they can be used to predict rates of spreading and the thickness of the second layer in regions where only the topography is known. One such prediction has been attempted and appears successful. The East Pacific Rise in the South Pacific typically has a relatively smooth crest, mountainous belts in the center of each flank, and smooth outer flanks (*7*). This information and Table 1 were sent to Heirtzler with the suggestion that the unpublished Lamont magnetic data in the region might provide a test (*8*). In this region, spreading at the crest is at a rate of about 4 cm/yr, on the mid-flanks it appears to be at about 1.5 cm/yr, and on the outer flanks at about 4 cm/yr (*9*). Although this is a relative time and spreading scale (*10*), it appears to confirm the relationships in Table 1. It should be obvious that the relationships apply only to the measured range of spreading rates and certainly not to zero spreading. The relationships cannot be extrapolated to the initiation or termination of motion, whether long continued or intermittent.

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Persistence of Chlorinated Hydrocarbon Insecticides in Soils

Abstract. *The percentages of technical aldrin, chlordane, endrin, heptachlor, Dilan, isodrin, BHC, and toxaphene remaining in Congaree sandy loam soil after 14, years were 40, 40, 41, 16, 23, 15, 10, and 45, respectively; those of purified aldrin and technical dieldrin after 15 years were 28 and 31, respectively; and the percentage of technical DDT in three soils after 17 years was 39. Treatments and maintenance of the soils were such that leaching, volatilization, photodecomposition, mechanical removal, and probably biological decomposition were at a minimum. These values may approach an upper limit of persistence of insecticides in soil.*

In 1949 and 1951 plots were established at the Plant Industry Station, Beltsville, Maryland, to study the long-term persistence and rates of disappearance of several chlorinated hydrocarbon insecticides in soil. These soils received from 0 to 448 kg of insecticide per hectare, mixed uniformly throughout their profile. We recently determined that 40, 40, 41, 16, 23, 15, 10, and 45 percent of the original applications of technical aldrin (determined as dieldrin degradation product), chlordane, endrin, heptachlor (plus heptachlor epoxide degradation product, 76 percent), Dilan (*1*), isodrin (plus endrin degradation product, 95 percent), BHC, and toxaphene, respectively, remained in soil after 14 years; that 28 and 31 percent of the original purified aldrin (determined as dieldrin) and technical dieldrin, respectively, remained after 15 years; and that 39 percent of the original technical DDT remained after 17 years (*2*).

Duplicate soil plots were established in 1951 on a Congaree sandy loam soil (*3*). Several technical chlorinated insecticides and purified aldrin, at rates of 0, 56 or 112, and 224 kg/ha [approximately 0, 25 or 50, and 100 parts per million (ppm)], were thoroughly mixed with the soil before it was placed in small plots to a depth of 38 cm. The plots were bounded by concrete block walls to a depth of 60 cm and provided with gravel and tile drainage.

In 1949 DDT was thoroughly mixed with Chester loam, Sassafras loam, and Evesboro loamy sand soils at rates of 0, 28, 112, and 448 kg/ha (approximately 0, 12.5, 50, and 200 ppm) before they were placed in plots similar to those for Congaree sandy loam (*3*). These soils were moved to a new location on the Plant Industry Station in June of 1962. Soil depth at the new location was 23 cm, whereas the original depth was 25 cm.

Soils were cropped at various times until 1962, in both plot series. Weeds were controlled by cutting, cultivation, or black plastic film. Since 1962, weeds were cut when necessary and allowed to decompose on the soil surface.

Samples of soil from the plots treated with DDT in 1949 were taken at the time of their establishment, and the concentration of DDT was determined. The first assay of soil plots treated in 1951 was made 1 year later. Soil samples of both series of plots were taken in the fall of 1952, 1953, 1954, 1955, 1958, 1962, and in June 1966. Several soil cores were taken; they were composited, thoroughly mixed, screened, and then subsampled for analysis. Through 1962, assay of insecticide was based on the total chlorine content of treated soil less the total

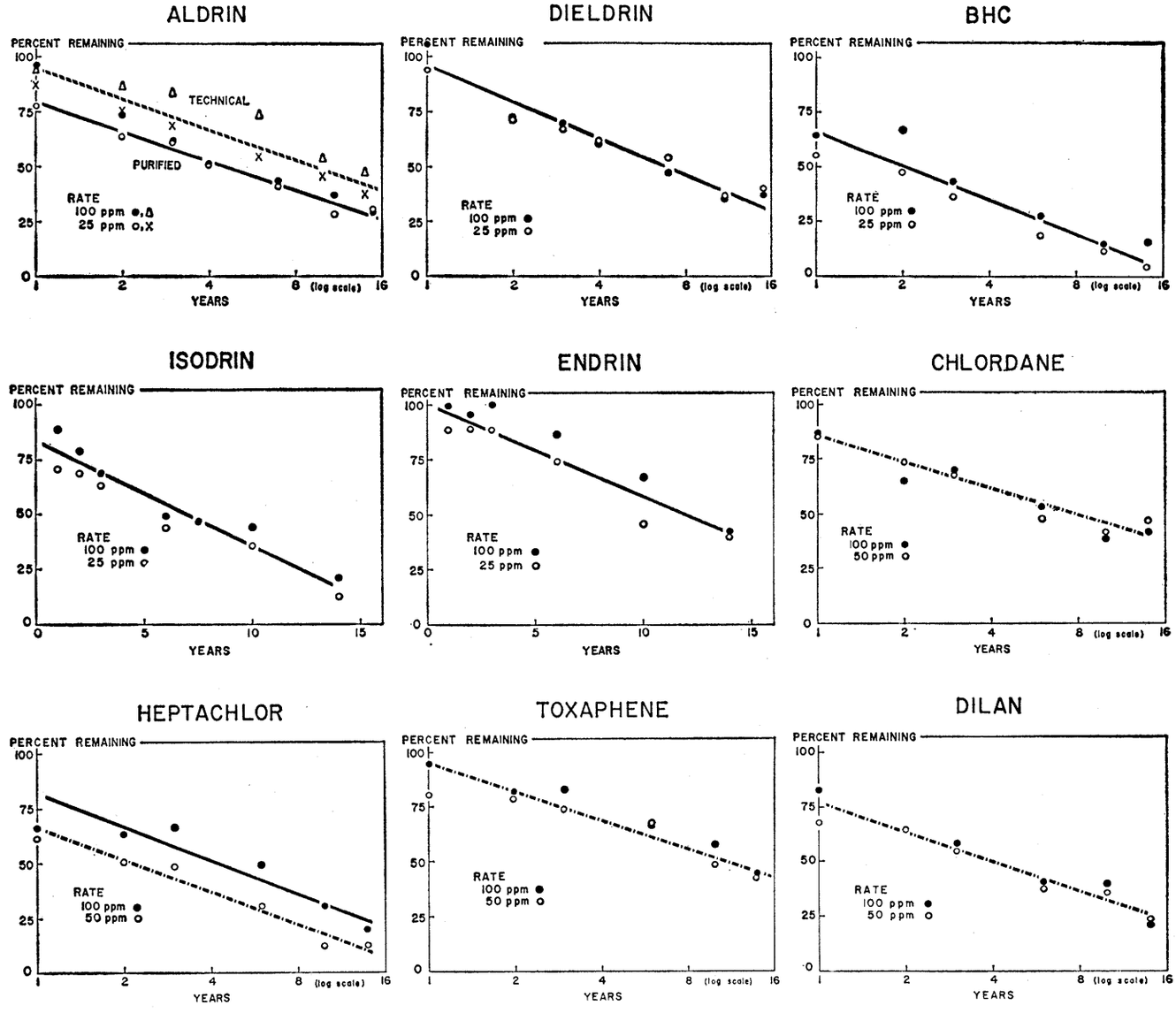

Fig. 1. Persistence of nine chlorinated hydrocarbon insecticides in Congaree sandy loam soil. Aldrin was determined as dieldrin; Dilan as Prolan and Bulan; and BHC as α-, β-, and γ-isomers. Heptachlor includes heptachlor epoxide (76 percent) and isodrin includes endrin (95 percent).

chlorine content of nontreated soil (*4*). Concentration of insecticide in soil was calculated to an air-dry basis.

The 1966 soil samples were assayed by gas chromatography. About 20 g of moist soil was extracted three times by shaking with a mixture of hexane and isopropanol (3 : 1, by volume) totaling 100 ml. Moisture contents of soil were taken at the time of sample extraction, and the results were based upon oven-dry soil (104°C for 24 hours). The following compounds were quantified by peak height with the use of electron-capture gas-liquid chromatography: aldrin (determined as dieldrin), dieldrin, DDT (as *o,p'*- and *p,p'*-DDT; *p,p'*-TDE; and *p,p'*-DDE), heptachlor plus heptachlor epoxide, endrin, and isodrin plus endrin. BHC (determined as α-, β-, and γ-isomers), chlordane, Dilan (determined as Bulan and Prolan), and toxaphene were quantified by total peak area. BHC, endrin, and isodrin were passed through a 1 : 1 mixture of 10 percent DC-200 and 15 percent QF-1 on Chromosorb W (acid-washed, dimethyldichlorosilane) column at 210°C. The remaining compounds were passed through a 5 percent SE-30 on Chromosorb W column at 210°C.

Identification of the compounds was made by comparison of relative retention times of all compounds on both columns. Further identification was accomplished by infrared spectra analysis of peaks trapped (10 to 20 μg of each insecticide) from the gas chromatograph. The compounds identified were: aldrin (determined as dieldrin), dieldrin, DDT (determined as *o,p'*- and *p,p'*-DDT; *p,p'*-TDE; and *p,p'*-DDE), endrin, heptachlor, and heptachlor epoxide.

Recovery efficiencies of purified insecticides were obtained by treating portions of the samples of moist control soil. The samples were thoroughly mixed and extracted after 36 to 48 hours. Recovery efficiencies from soil in the percentage of that applied were: dieldrin, 97; DDT, 85 to 97; endrin, 99; chlordane, 100; and heptachlor, 99. No recovery corrections were made, except for DDT in which a 90-percent mean recovery was used.

The decline of aldrin, dieldrin, chlordane, endrin, heptachlor, Dilan, isodrin, BHC, and toxaphene in Congaree sandy loam is given in Fig. 1, and the decline of DDT in Chester loam, Sassafras loam, and Evesboro

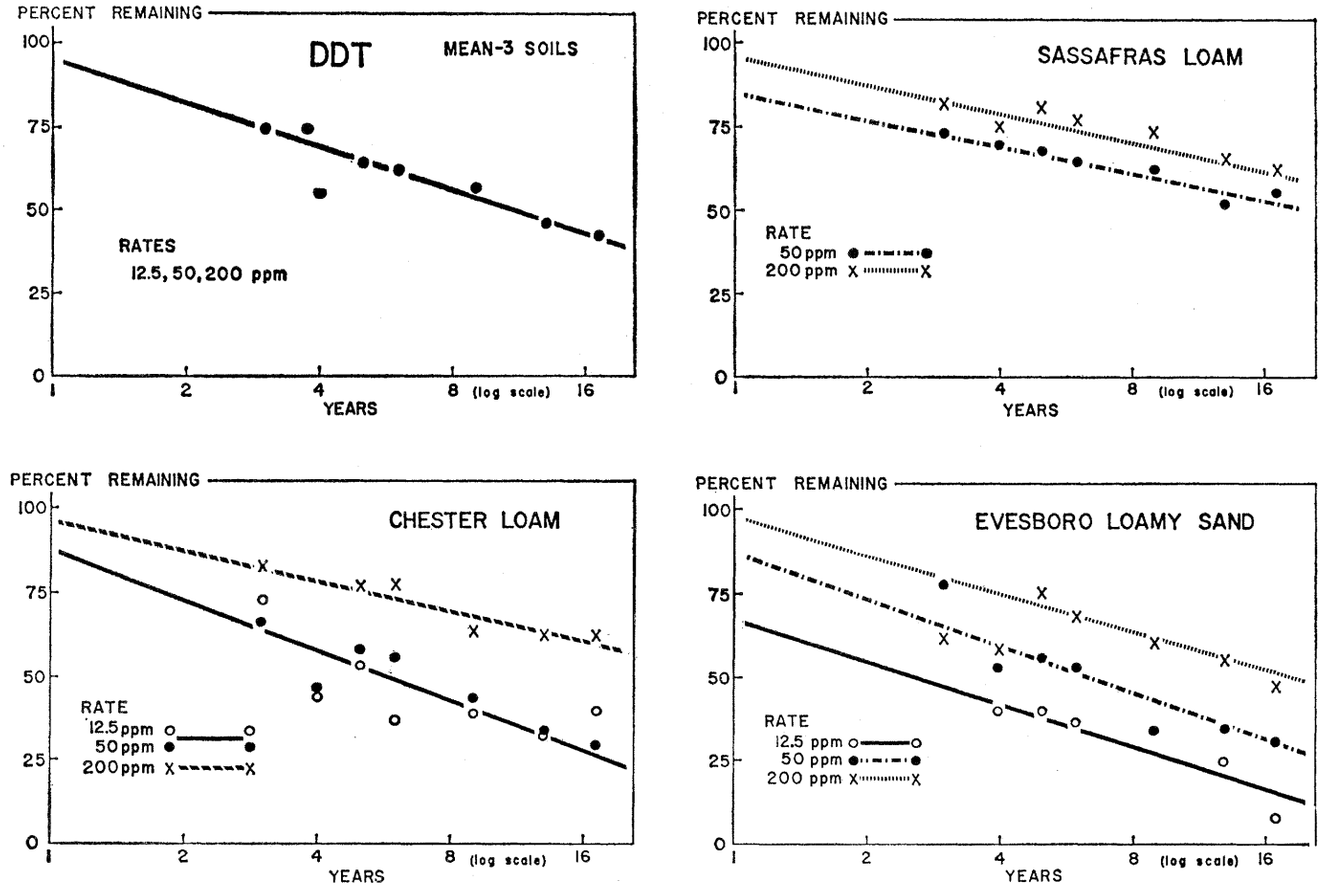

Fig. 2. Persistence of DDT in three soils. DDT was determined as *o,p'*-DDT, *p,p'*-DDT, *p,p'*-TDE, and *p,p'*-DDE.

loamy sand soils is given in Fig. 2. The time for half of the original amount of each insecticide to disappear from soil was determined from Figs. 1 and 2. They were found to be (in years): purified aldrin, 5; technical aldrin, 9; dieldrin, 7; chlordane, 8; heptachlor, 2 to 4; Dilan, 4; BHC, 2; toxaphene, 11; and DDT, 10.5 (2.5 to 35). Disappearance of endrin and isodrin was an arithmetic, rather than a geometric, linear function of time.

The percentages of DDT and heptachlor remaining in soil were greater after higher rates of application than after lower. The percentage of DDT remaining after 17 years, applied at the rate of 200 ppm, was about twice that at the rates of 12.5 and 50 ppm on Chester and Evesboro soils. Lichtenstein *et al.* (*5*) found that after 4.5 years 28 and 54 percent of DDT were recovered from soil treated with 11 and 112 kg/ha, respectively.

The establishment and maintenance of these soil plots have been such that an upper-limit persistence of soil insecticides would be expected. Thorough mixing of the insecticides throughout the soil profile, extremely high rates of application, and minimum tillage have all been reported to contribute to persistence of these insecticides in soil (*6*). DDT may be more persistent in acid soils (*7*); acidity of DDT-treated soils ranged from *p*H 4.7 for Evesboro to *p*H 5.2 for Sassafras soil. In addition, leaching of insecticides was minimized by the gravel layer beneath the soil profile.

Losses of insecticides may occur by processes of volatilization, chemical decomposition, photodecomposition, biological metabolism, and mechanical removal through crop absorption. Chemical decomposition per se in these soils should differ little from that in soils under normal field conditions. Volatilization and photodecomposition would be almost at a minimum in these largely undisturbed soils. Photodecomposition would occur only near the surface of the soil, and volatilization occurs most rapidly on the surface, but some of the insecticide could escape from soil by diffusion upward or possibly by codistillation with water as reported by Acree *et al.* (*8*) for DDT. The extremely high rates of application of insecticides may have eliminated much of the soils' zoological population, thereby reducing both micro tillage and biological decomposition. The complete pathway of biological decomposition of the chlorinated insecticides in soils is unknown, though BHC (technical) is known to be metabolized, aldrin and heptachlor are metabolized to their epoxides, and DDT is metabolized to DDD (TDE) (*9*). Mechanical removal through absorption by crops, and subsequent removal of these crops, was also near a minimum, because these plots were not cropped as often as normal agricultural soils are, and the crops were not always removed from the plots. Recent results indicate that quantities of less than 1 ppm of these insecticides were found in several plant species grown in soils contaminated with the chlorinated insecticides (*10*).

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References and Notes

1. Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.
2. Abbreviations: DDT: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDD: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene TDE: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; BHC: 1,2,3,4,5,6-hexachlorocyclohexane; DC-200: silicone, Dow-Corning 200; SE-30: silicone gum rubber (methyl); and QF-1: silicone(fluoro) (FS1265).
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Collagen Proline Hydroxylase in Wound Healing, Granuloma Formation, Scurvy, and Growth

Abstract. *Compared to homolgous tissues from adult animals, rapidly growing embryonic and fetal tissues contain large amounts of collagen proline hydroxylase. Lung and skin from scorbutic guinea pigs contain one-third as much enzyme as normal tissues do. A rapid increase in proline hydroxylase occurs on the 2nd day of formation of granuloma after injection of carrageenan and on the 4th day of wound healing. The increase in enzyme activity is associated with the onset of collagen biosynthesis and deposition.*

Healing of wounds, formation of granulomas, growth, and differentiation are all characterized by distinctive rates of synthesis of connective tissue, and collagen is the major component of connective tissue in animals. The conversion of prolyl to hydroxyprolyl residues is an enzymatic step uniquely important to collagen biosynthesis, since collagen is the only animal protein that contains hydroxyproline. In a study of the factors that govern the rate of collagen synthesis in intact animals, we determined the amount and specific activity of collagen proline hydroxylase (*1–3*) in different organs and tissues under various experimental conditions. Our data suggest that changes in the amount of this enzyme may be of decisive importance in regulating the rate of formation and deposition of collagen.

Tissues were excised, blotted, weighed, and cut into small pieces with scissors. Ten milliliters of cold 0.25*M* sucrose were added per gram of tissue, and the mixture was homogenized for 1 minute at high speed in a Servall Omni-Mixer. After centrifugation at 15,000*g*, supernatant fractions were collected. Rehomogenization and reextraction of the 15,000*g* pellets yielded approximately 20 percent as much enzyme as the initial extraction. Concentrations of protein were estimated by the method of Lowry (*4*). Collagen proline hydroxylase was assayed in a 2.0-ml volume of incubation mixture by measuring the release of tritium from specially prepared protein substrate labeled with 3,4-^{3}H-proline, as described previously (*2*). Ascorbic acid, α-ketoglutarate, and ferrous ion, cofactors of the enzyme (*2*), were present in optimum amounts. Specific activities of homogenates are reported as counts per minute of hydroxyproline formed per milligram of protein per hour. The standard error of the mean (S.E.) is reported in many cases. The amount of hydroxyproline formed per 30-minute incubation was linearly related to the amount of homogenate added to the assay from 0 to 1000 μg of protein. A requirement for α-ketoglutarate is characteristic of partially purified preparations of peptidyl proline hydroxylase obtained from chick embryo (*2*, *3*), granuloma of guinea pig (*2*), and skin of fetal rat (see *2*).

To rule out the possibility of nonenzymatic release of tritium, crude homogenates were assayed for peptidyl proline hydroxylase activity before and after dialysis, in both the presence and absence of added α-ketoglutarate. Dialyzed extracts were always inactive in the absence of added α-ketoglutarate, whereas the activity of undialyzed extracts was stimulated two- to threefold by its addition. No evidence of nonenzymatic hydroxylation was obtained.

Peptidyl proline hydroxylase activity of homogenates prepared from organs of 2- to 3-month-old Sprague-Dawley rats, weighing 300 g, are shown in Table 1. The enzyme is present in many tissues but is most highly concentrated in extracts of lung, heart, and skin. No activity was detected in whole blood or its components. In general we found that rapidly growing embryonic and fetal tissues contain more proline hydroxylase per unit weight and per milligram of extractable protein than comparable tissues from adult animals do. As shown in Fig. 1, activity in rat tissues was very high in 3-day-old animals, but by 5 to 10 days after birth the specific activity of homogenates decreased to levels comparable to those of adult animals. A characteristic amount of protein was extracted per gram of tissue for each organ (Table 1), and this amount did not vary significantly with changes in the age of the animal.

Chick embryo is convenient for studying variations in the level of peptidyl proline hydroxylase during development. No enzyme activity was detected in either the yolk or the white of the egg; this activity was first detected in 4-day-old embryos weighing 50 to 60 mg. Prior to day 4 it is difficult to obtain sufficient tissue to assay; after day 4 both specific activity and total amount of enzyme in the embryo increased at a rapid rate. On day 6 the average specific activity of homogenates was 500 count/min of hydroxyproline per milligram of protein per hour, whereas on day 14 the average specific activity was 2000. Other investigators (*5*) determined the amount