Sample Variability and the Measurement of Dieldrin Content of a Soil in the Field

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Dieldrin was incorporated to 7.5-cm depth in two 0.6-ha field plots. Dieldrin found in samples from five subsections of each plot varied widely and no loss could be statistically demonstrated after 530 days. The dieldrin contents of 108 individual cores taken from a 36-m² grid showed a 50-fold variation ranging from 16 to 797 mg/m² about a mean of 207. The variation could be attributed to differences in spray coverage coupled with irregularities due to poor mixing during cultivation. Under these typical agricultural conditions coefficients of variation of soil dieldrin analyses cannot be reduced below 20% at practical sampling rates, limiting the precision of measurements of degradation rates of pesticides in short-term field experiments. If two insecticides are applied in a mixed formulation, the relative rates of degradation can be found with much greater precision than that of either one.

The primary objective of the field experiment from which the data in this paper were taken was to measure the amounts of dieldrin lost by different pathways from field plots under conventional agricultural management. The pathways studied included losses in runoff water, by soil erosion, crop uptake, and volatilization to the air. Measurements of the amount of dieldrin remaining in the soil at various times after its application at the start of a 4-year maize-wheat-meadow rotation were an essential part of the data required. Since the confidence with which changes in the dieldrin content of the soil can be measured depends upon the reproducibility with which samples can be taken and analyzed, the original sampling program was designed to give an estimate of the uncertainty inherent in the results. To this end each experimental plot was each sampled in five sections. Comparison of the amounts of dieldrin in the five samples then permitted an estimate of the variance among them.

The smaller more intensive study described in this paper was performed to identify the source of the variability that was found, and to provide a basis for estimating how much improvement could be expected from improvement in the sampling procedures.

Experimental Treatments. The field plots from which samples were taken were two experimental watersheds at the North Appalachian Experimental Watershed at Coshocton, Ohio. The areas of the two watersheds were 0.63 and 0.68 ha. The soil on both was Muskingum silt loam. This soil contains 1.64% organic matter, has a pH of 5.8, and contains 51% by volume of water at saturation. The mineral fraction contains 28% sand, 55% silt, and 17% clay.

On May 2, 1968, the 0.63-ha watershed received a dieldrin application as a spray of an aqueous emulsion at a rate of 5.6 kg/ha of active HEOD (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene). The second watershed received a similar application of a mixture of dieldrin and heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) on May 1, 1969. Both insecticides were applied in the same spray at the rate of 5.6 kg/ha of each active material.

Immediately after application the insecticides were disced into the soil to a depth of 7.5 cm, and the watersheds were seeded to maize on the same day. The only subsequent tillage on either plot was a light discing of the surface after corn harvest in October to prepare a seed bed for winter wheat. In April, a mixture of red clover, alsike clover, and timothy was seeded in the ankle-high wheat without cultivation.

Analytical Methods. All samples were transported from the field plots to the laboratory in hermetically sealed cans to prevent any moisture loss or pesticide evaporation. On arrival the moist bulk samples were sieved through a 4-mesh screen. The fraction retained by the screen was measured and included in the total dry weight used in calculating the results. Earlier studies had demonstrated that the amount of dieldrin retained by the coarse fraction was negligible. After screening, a 10- to 20-g subsample was taken and the moisture content was adjusted to between 1 and 10 bars of tension, with a pressure membrane before extraction for 3 hr in a Soxhlet apparatus with a 3:1 hexane and 2-propanol mixture. The extracts were then passed through a short column of powdered alumina to break any emulsion and the column eluate was washed with water to remove the 2-propanol. The residual hexane was diluted to 250 ml and a 2- to 8-μl aliquot was injected into a gas chromatograph.

In the experiment where individual cores were analyzed (see below) these were not sieved. The entire core was extracted by shaking with 500 ml of 1:1 hexane:2-propanol mixture in the 1-qt Mason jar used to transport the sample from the field. No adjustment of the moisture content was required. After decantation, an aliquot of the extract was washed with water to remove the 2-propanol and an aliquot of the remaining hexane was injected into the gas chromatograph without any further volume adjustment.

The glc column used was a 165-cm × 4-mm (i.d.) glass column packed with a 1:1 mixture of 10% DC-200 on Gas Chrom Q and 15% QF-1 on Gas Chrom Q. The temperature was 200° C and the flow rate of the 95:5 argon:methane carrier gas was 70 ml per min. A Ni^{63} electron capture detector was used and quantitation was made in terms of peak area using an electronic digital integrator. The retention time of dieldrin was about 5 min. Calibration of the method with fortified soils demonstrated that the recovery was very close to 100%.

SAMPLING PROGRAM AND RESULTS

To measure the changes in the amount of insecticides in the soil, the watersheds were divided into five sections of approxi-

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mately equal area (Figure 1). A single bulk sample obtained by combining a number of individual spade or core samples was taken from each section of the 1968 watershed on the dates listed in Table I. The first samples were taken on the day after application.

In 1968, 12 randomly spaced holes were dug in each section and a vertical slice of soil was cut from the side of each with the spade. Each slice was then cut horizontally to retain the slice to 17.7 cm (7-in.) depth. These samples were then composited to give the bulk sample for each section of the watershed. This procedure is described as Method A in Table I. In 1969, 75 cores, each 21-mm in diameter and 17.7-cm in length, were taken from each section and composited to make the bulk sample. This procedure, described as Method B in Table I, also was used for all the samples taken from watershed treated in 1969.

The results obtained from the 1968 watershed are presented in Table I. Mean values for the whole watershed on each date, with the coefficients of variation, are included. The results are clearly erratic. Comparison of the results from the same sections on different sampling dates does not reveal any consistent tendency for high (or low) values to be found in the same sections of the watershed.

Following an analysis of variance, an examination of the data using Tukey's method (Snedecor, 1965) showed that the means of the data on the 1st and 532nd days are not significantly different at the 5% level. The data of days 159 and 341 are significantly different from the 1st but not from the 532nd day. These differences, however, cannot have any physical significance. The variations are so great that no decrease in the amount of insecticide in the soil can be demonstrated over the 532-day period.

Analytical Variations. Application of the two insecticides in the same spray in the 1969 experiment presented an opportunity to evaluate the amount of variation due to the chemical analyses. The results of the sampling on the first day after application are presented in Table II. These data are calculated directly in terms of the amount of insecticide per unit area of watershed surface. This is a convenient method of presentation since the total insecticide in each bulk sample represents that beneath the area corresponding to 75 cores, each 21 mm in diameter.

The formulation used also contained a significant amount of γ-chlordane associated with the heptachlor. All three compounds were measured in the same extracts. The coefficients of variation in the amounts of each insecticide in the five bulk samples are very similar and lie within the range of those found in the 1968 experiment. Comparison of the relative amounts of the three organochlorines in each sample, presented in Table II as ratios, shows much smaller variations; high (or low) levels of heptachlor are always associated with high (or low) levels of both dieldrin and chlordane. The coefficients of variation of the ratios are close to the values expected from the variations in extractability indicated by the fortified test samples. The effect of chemical errors is clearly a small fraction of the overall variability which must, therefore, reflect genuine differences in the insecticide content of the bulk samples from different parts of the watershed.

Intensive Sampling Experiment. On May 25, 1970, a special sampling experiment was performed on a part of the watershed treated in 1968. The object of this experiment was to examine the variations in the dieldrin content of individual cores taken in a systematic pattern from a limited area. A square grid pattern of 6 m on each side was marked out on the B_2 section of the watershed, with one side of the grid (the abscissa) oriented along the slope contour. In the application of the insecticide the spray boom was moved directly up the slope, so that the ordinate of the grid was parallel to the path of the spray boom and perpendicular to the orientation of the boom itself. Three cores were taken to a depth of 17.7 cm within each of the 36 m^2 of the grid. Core diameters were 21, 29, and 44 mm, respectively, giving cross-sectional areas with an approximate ratio of 1:2:4. The three samples were

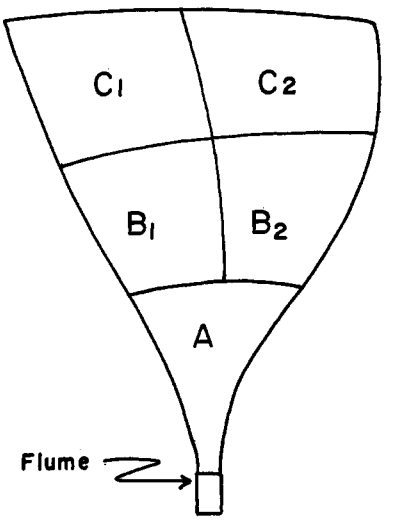

Figure 1. Location of sections used in the sampling of the soil on watersheds treated with insecticides

Table I. Dieldrin Content of Samples of Soil Taken to 17.7-cm Depth from Sections of a Treated Watershed

	Dieldrin content (ppm dry soil)			
Date	**5/3/68**	**10/8/68**	**4/8/69**	**10/17/69**
Sampling method	**A[a]**	**A**	**B**	**B**
Days after application	**1**	**159**	**341**	**532**
Section A	3.04	1.45	1.29	1.83
Section B_1	2.59	0.54	1.31	1.32
Section B_2	3.84	1.51	1.47	2.36
Section C_1	3.48	2.34	1.46	2.39
Section C_2	1.72	1.40	2.02	3.24
Mean	2.93	1.45	1.51	2.23
Standard deviation	0.82	0.63	0.30	0.71
Coefficient of variation (%)	28	44	20	32

[a] In Method A, each sample comprised 12 individuals; in Method B, each sample comprised 75 individuals.

Table II. Dieldrin, Heptachlor, and γ-Chlordane Contents of Samples of Soil (17.7-cm Depth) Taken 24 Hr after Application to the Field

	Pesticide content (mg/m^2)[a]			Ratios	
Subsection	**Dieldrin**	**Heptachlor**	**Chlordane**	**Heptachlor/dieldrin**	**Heptachlor/chlordane**
A	248	251	47.9	1.012	5.24
B_1	265	262	39.2	0.989	6.68
B_2	372	369	60.2	0.992	6.13
C_1	179	189	32.0	1.059	5.91
C_2	219	232	42.8	1.059	5.42
Mean	257	261	44.4	1.022	5.88
Coefficient of variation (%)	28	25	24	3.4	9.8

[a] All compounds were applied in the same spray emulsion.

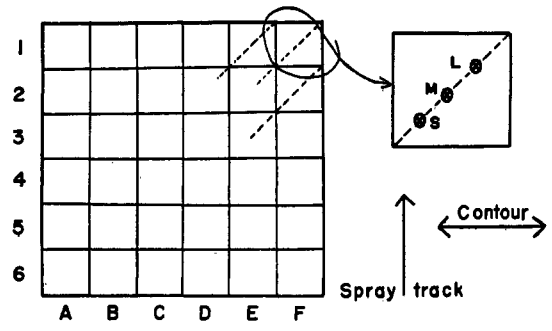

Figure 2. Sampling grid (6 × 6 m) and location of individual cores within each grid square. (Large core, L; medium core, M; small core, S)

always taken from the same relative position in each square, equally spaced along the diagonal with the smallest closest to and the largest farthest from the origin, as indicated in Figure 2.

At the time of sampling the vegetative cover in the watershed was grass-legume meadow, about 30 cm high. The grass was removed from each sampling point before the corer was inserted in the soil. All the cores were placed in individually sealed 1-qt Mason jars immediately after they were drawn and stored in these jars until analyzed.

The results presented in Table III are again calculated on an area basis, each value representing the amount of dieldrin found per unit of ground area removed by each core. The data from the three corers of different sizes are thus normalized for direct comparison. Core sizes are identified as small (S), medium (M), and large (L). For convenience, the squares of the grid are identified as rows 1–6 and columns A–F (see also Figure 2).

The data show a 50-fold variation, ranging from 16 (in 6D) to 797 mg/m² (in 6B); both these extreme results were obtained from the large cores. The overall mean of all the cores was 207 mg/m², with a standard deviation of 166 about the mean. The median value of the data was 166 mg/m². The difference between this and the mean indicated that the population distribution contained a larger number of lower values, the higher mean reflecting the presence of a small number of high values or "hot spots" in the area sampled.

The means and standard deviations of the results from the large, medium, and small cores were 196 ± 133, 209 ± 178, and 217 ± 186 mg/m², respectively. An analysis of variance applying Fisher's "F" test shows that there is no significant difference among these at the 5% level despite the slight but consistent increase in the mean with decreasing core size. The three sizes may therefore be regarded as equivalent and the whole group of observations can be treated as a single sample.

An analysis of variance of all the samples in each row of grid squares (1A-F, 2A-F, etc.) shows that there were no significant differences among the means of the rows, but a similar analysis of the data in the columns (A1-6, B1-6, etc.) reveals significant differences at the 5% level. A more detailed statistical examination using Tukey's test reveals three levels of significant difference at the 5% level among the six means (Snedecor, 1965). These can be classified as high, medium, and low, as indicated in Table III. The most striking of these differences is evident in the original data, which show a preponderance of low values in the E column. Although the original experimental records of the spraying operation do not state the precise path of the spray boom, the distribution of low numbers coincides closely with the direction in which the boom was moved and suggests that this part of the grid lay between two adjacent passes so that the coverage was less complete. The distribution of the high values in the B and C columns suggests that the pesticide was applied at a higher than average rate along this strip. The exact cause of the differences between columns cannot be identified except to say that they probably reflect irregularities inherent in the spraying operation. This irregularity is evident over distances of about 2 m and has an amplitude between about 50 and 350 mg/m². Inspection of the individual data in Table III reveals a further irregularity superimposed on that shown by the means, with up to sixfold variations between adjacent samples. This superimposed variation appears to be random in direction, the variations within individual grid squares being similar to those between neighboring squares. The cause of the random short-distance variation may be

Table III. Dieldrin Contents (mg/m²) of Individual Soil Cores Taken from a Rectangular 6-m² Grid of Treated Field Soil

Row	Core	Column A	B	C	D	E	F	Mean	Standard deviation
	L	218	353	406	359	50	294		
1	M	51	139	623	127	47	483	250	165
	S	167	315	307	350	34	262		
	L	217	396	215	54	86	89		
2	M	442	456	200	55	39	65	194	141
	S	332	287	244	181	29	106		
	L	263	507	79	22	29	234		
3	M	126	380	110	57	42	222	175	155
	S	145	107	529	117	38	122		
	L	108	152	190	71	48	102		
4	M	143	376	566	79	39	232	174	143
	S	195	251	379	68	39	93		
	L	622	283	125	21	93	276		
5	M	299	385	170	25	59	218	200	155
	S	287	237	175	34	30	335		
	L	115	797	325	16	64	528		
6	M	195	626	212	20	126	160	246	225
	S	153	465	201	28	64	339		
Mean		223	362	281	94	54	231	207	
Standard deviation		135	172	160	105	25	130	166	
Group		m	h	hm	l	l	m		

attributed to the incomplete mixing of the soil during the discing operation, when the insecticide lying on the uncultivated surface is turned under into the 7.5-cm depth disturbed by the disc cultivator (Read *et al.*, 1968).

DISCUSSION

The data in Table III can be used to estimate the effect of the sampling density, defined as the number of cores taken per unit area of ground, on the variability of the insecticide content of the bulk sample made by combining them. The amount of pesticide that would be present in a bulk sample made from any set of cores drawn from the 6-m grid can be calculated by taking the mean of the appropriate set of data from Table III. The variability expected among different bulked samples of the same size can then be found by calculating the total amounts in different sets containing the same number of cores. The effect of increased bulk sample size, or sampling density, on variability may then be found when the same calculation is done for sets containing 2, 3, 4, 6, 9, and 12 numbers. The results of such a calculation are presented in Figure 3, in which the coefficients of variation of the means of 54 pairs, 36 sets of 3, 27 sets of 4, 18 sets of 6, 12 sets of 9, and 9 sets of 12 numbers taken from Table III with the aid of a table of random numbers are plotted against the sampling density. The curve shows a continuous decrease in the expected variability over the whole range of sampling densities examined, although the improvement becomes progressively less as the number of cores per set is increased.

The sampling rate of 75 cores per section used in 1968 corresponds to a sample density of about one core per 17 m^2. Comparison of the coefficients of variation in Tables I and II with Figure 3 shows that the variability of the bulk samples from the sections is somewhat less than that predicted in Figure 3. In view of the limited number of bulked samples available, and the inherent assumption in the calculation that the variability of the data in Table III is representative of the whole watershed, no critical comparison is possible. It is clear, however, that the variability of the insecticide content of the bulk samples and the consequent uncertainty in the results can be attributed to the wide variations in the insecticide content of the cores, which reflects the irregular distribution of the insecticide in the soil itself. This irregularity is due to both the spraying operation and to the incomplete mixing of the treated surface soil into the lower layers during discing, which results in large differences in insecticide content over short distances. These differences are persistent, remaining prominent for at least 2 years after the original treatment.

This result has important implications in the measurement of the rate of decay or loss of pesticides in field conditions. If the rate of decay or loss is slow, considerable errors can be encountered if the measurements are confined to short periods of time and no estimate of sample variability is made. As an example, the mean values for the whole watershed in the first three sets of data in Table I would lead to an erroneous result for the rate of dieldrin loss if they were not examined critically in the light of the uncertainties inherent in them. In planning any experimental or monitoring program to study a persistent pesticide, it is essential to design the sampling program in the light of the expected rate of pesticide loss and the anticipated variability of the results. As an example, the calculation summarized in Figure 3 suggests that the coefficients of variation of the bulk samples would be approximately halved if the number of cores were increased from 8 to 30 per 100 m^2. Whether such an improvement can be achieved in practice depends upon practical considerations such as the size of the area to be characterized, the size of the soil sample that can be handled, and the number of analytical determinations to be made. Since such considerations may well be the main factors that control the accuracy of the final result, it is clearly desirable that some experimental determination of the sampling error should be included in studies of this kind. Details of the way such measurements should be made will depend upon the character of the experiment, because this will influence the type of statistical analysis of the data. Although the experiment summarized in Table III clearly indicates the nature and magnitude of the problem, it is not suitable as a basis for statistical predictions of variations

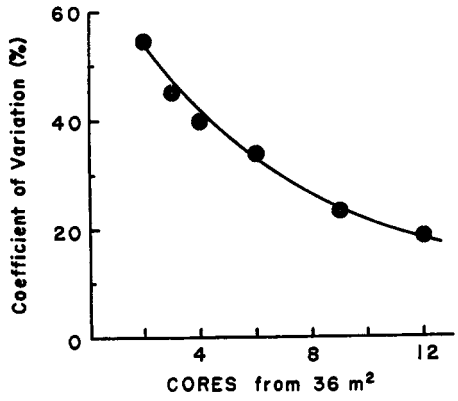

Figure 3. Predicted changes in the coefficient of variation of dieldrin content of bulk samples containing differing numbers of individual cores drawn from a 36 m^2 sampling area

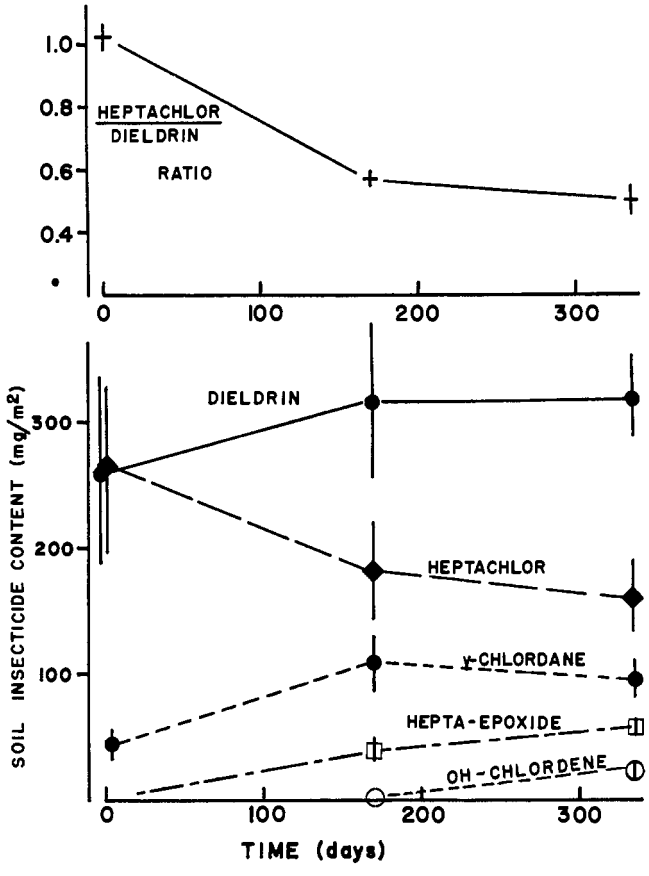

Figure 4. Measured changes in concentration in field soil of dieldrin, heptachlor, γ-chlordane, heptachlor epoxide, and hydroxychlordene for 335 days after application, together with changes in the ratio of heptachlor/dieldrin content of soil samples over the same period. Height of bars indicates standard deviations about the means

to be expected in samples taken over much larger areas. The exact significance of such predications will depend upon the assumptions implicit in the different ways the problem can be analyzed, and experiments to make more critical analyses of sampling errors in other circumstances must be designed with these statistical problems in mind.

Where less persistent pesticides are being examined, the confounding effect of the variability is much less critical because the changes to be measured will be much more rapid. This difference is exemplified in the comparison of the dieldrin and heptachlor data obtained from the 1969 watershed. The results obtained over the first year are presented in Figure 4 in two ways. When the mean insecticide content of the five bulk samples obtained from the five sections of the watershed are plotted against sampling time, it is evident that, as with the 1968 data, no loss of dieldrin can be demonstrated over the 335-day period. The decrease of the heptachlor content of the soil is clearly apparent, with the average dieldrin and heptachlor contents on the 170th day being separated by considerably more than the sum of their standard deviations. The decay of the heptachlor is also confirmed by the appearance of the heptachlor epoxide and hydroxychlordene degradation products, which could not be detected on the first day, and by the increased amounts of γ-chlordane found in the later samplings. Since both dieldrin and heptachlor were measured in the same samples, the change in the amount of heptachlor relative to that of dieldrin can be calculated in terms of the heptachlor/dieldrin ratios similar to those presented in Table II. The means of these ratios for the five bulk samples, together with the standard deviations about the means, are also plotted in Figure 4. When the magnitude of the change in the ratio is compared to the standard deviation on each sampling date, the validity of the measurements of the difference in the behavior of the two compounds is evident, and is not confounded by the variability of the amounts of both insecticides from sample to sample. This demonstration that the differences between the behavior of two compounds in the field can be measured with much more confidence than the individual behavior of either one suggests the possibility of using stable nondegrading materials as reference blanks in experimental situations where sample variability is likely to present serious difficulties.

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