Aldrin and Dieldrin: Loss under Sterile Conditions

Abstract. *After their application to sterile nutrient agar, both aldrin and dieldrin disappeared rather rapidly from the agar in glass-covered petri dishes. In most instances this disappearance was considerably retarded from agar inoculated with either fungi or bacteria. In the presence of microorganisms, aldrin was also epoxidized into dieldrin. Half of the applied aldrin volatilized from the agar during the first day of incubation; dieldrin volatilized more slowly and at a constant rate.*

Total disappearance of aldrin from sterile nutrient agar within 2 weeks of application was reported in 1963 (*1*); it was incorporated in Thornton's culture medium at a concentration of 10 parts per million (ppm); after incubation at 30°C for 6 or 12 days, 73 and 96 percent, respectively, had disappeared. Addition of Sesamex to the medium, or inoculation of the medium with a soil-water suspension, reduced the loss of aldrin; after 4 weeks of incubation, recoveries of aldrin amounted to 40 and 10 percent, respectively.

Subsequently it became evident that dieldrin also disappeared from sterile nutrient agar surfaces, but contamination of the surfaces with fungi retarded this disappearance to some extent. Moreover, almost 50 percent of dieldrin applied to sterile glass surfaces disappeared during a 2-week holding period at room temperature.

Aldrin labeled with ^{14}C was incorporated in Gochenauer's nutrient agar (*2*) at 1 ppm, or was deposited atop solidified sterile nutrient agar or on a sterile glass plate. Nutrient agar treated with aldrin at 1 ppm (*1*) was poured into each of eight sterile petri dishes (100-mm diameter). To determine the initial concentration of insecticide in the solidified nutrient agar, the contents of two petri dishes were extracted with a mixture of hexane and isopropanol (1:1) in a Potter homogenizer. The homogenate was then transferred quantitatively into a 250-ml erlenmeyer flask, along with solvent rinsings from the inner surfaces of the petri dishes. The homogenate was further extracted by refluxing for 15 minutes on a steam bath with use of a Vigreaux column. Finally, the isopropanol was removed with water within a separatory funnel; a hexane phase and a water-isopropanol phase resulted, both of which were analyzed by liquid-scintillation counting (*3*). The hexane phase was also analyzed by gas-liquid chromatography (*4*).

It had been found (*1*, *5*) that aldrin and dieldrin often persisted longer in or on nutrient agar contaminated with microorganisms. For this reason the nutrient agar in two of the six remaining dishes was inoculated with 1 ml of a soil suspension (dilution, 1:1000) prepared from a silt loam; the other four dishes were left sterile. All six plates were placed in a 12.5-cm-wide desiccator, containing water in the desiccant compartment, and incubated at 30°C.

After solidification of insecticide-free nutrient agar in eight more petri dishes, 1 ml of pentane containing 10 μg of ^{14}C-labeled aldrin was pipetted onto each agar surface. After the pentane had evaporated from the covered plates, the contents of two plates were similarly extracted and analyzed. The six remaining dishes were similarly incubated at 30°C in a second desiccator.

Radioactive aldrin was also deposited in the glass bottom of each of eight more sterile petri dishes by pipetting 1 ml of pentane containing 10 μg of aldrin; the pentane was then evaporated from the covered dishes. For determination of the initial insecticidal deposit, the inner surfaces of the bottoms and covers of two petri dishes were rinsed with hexane and then with a 1:1 mixture of water and isopropanol. The hexane fraction was analyzed by both gas-liquid chromatography and liquid-scintillation counting; the water-isopropanol fraction, by liquid-scintillation counting only. The six remaining dishes were similarly incubated at 30°C in a third desiccator.

The same experiment was conducted with ^{14}C-labeled dieldrin, so that three desiccators contained agar or glass plates treated with aldrin; three, with dieldrin.

Table 1. Volatilization of aldrin (A) and dieldrin (D) from sterile nutrient agar or glass surfaces.

Day	Recoveries (%) of applications					
	In agar		On agar		On glass	
	A	D	A	D	A	D
From paper traps around petri dishes						
1	49.0	4.5	51.0	4.2	48.0	7.0
2	17.0	6.3	14.5	5.5	14.0	8.0
3		5.7		6.0		8.2
From contents of petri dishes						
0	89.0	84.0	61.0	86.0	94.0	92.0
2	11.0		6.6		3.9	
3		56.0		55.0		51.0

After 8-day incubation the contents of duplicate petri dishes were similarly extracted and analyzed; four dishes of each series were left for further incubation and testing. Analyses of the hexane fractions showed that the levels of insecticide had diminished on the agar and glass surfaces. Since the data by gas-liquid chromatography and by liquid-scintillation counting were very similar, the indication was that the recovered residue was in the form of the originally applied aldrin or dieldrin. Aldrin, being more volatile than dieldrin, disappeared faster. After 8 days of incubation only 2 to 3 percent of the applied aldrin and 63 percent of the applied dieldrin were recovered from agar in which they had been incorporated at 1 ppm. The fact that less than 1 percent of the total radioactivity recovered was found in the water-isopropanol phase indicated the absence of breakdown into hydrophylic substances.

Nutrient agar containing aldrin or dieldrin and also inoculated with a soil suspension was covered with a dense layer of bacterial colonies; 8 days after application of aldrin and inoculation, the insecticide was still present at a concentration of 0.59 ppm (59 percent of the application). Dieldrin had been formed from aldrin and was present at a concentration of 0.17 ppm, so that the total recovery of insecticides amounted to 76 percent of the applications. After 1 more week of incubation, the second inoculated agar plate contained aldrin at 0.50 ppm and dieldrin at 0.13 ppm; 63 percent of the applications were recovered. Not only was aldrin more persistent under these conditions; part of it had been epoxidized into dieldrin because of the presence of microorganisms.

The persistence of dieldrin in agar was not significantly affected by the presence of microorganisms. Sixty-three percent of the insecticides applied was recovered after the 8-day incubation period from the sterile nutrient media; 69 percent, from the inoculated media. Results were similar after 15-day incubation.

Considerable amounts of the insecticides had been lost from the surface-treated agar or glass plates also: 18 percent of the applied aldrin and 55 percent of the applied dieldrin were recovered from the agar; 11 and 40 percent, respectively, from the glass surfaces. Thus the losses were, respec-

tively, 82 or 89 percent of aldrin and 45 or 60 percent of dieldrin during the 8-day incubation period. To test the hypothesis that aldrin and dieldrin (to a lesser extent) had volatilized, the following experiment was conducted 9 days after the applications.

Strips of filter paper were placed around and atop the remaining petri dishes within each desiccator in order to collect any insecticidal vapors that might escape from the covered dishes; the paper had been immersed in a 0.25-percent solution of corn oil in hexane before placement in the desiccator. After 3 more days of incubation (days 9 to 11), the filter papers were removed and rinsed with hexane. The solvent was then adjusted to 50 ml and analyzed by gas-liquid chromatography (*4*). The hexane was then further concentrated in a flash evaporator and finally placed in a counting planchet, evaporated, and tested for radioactivity with a Geiger-Mueller counter. The residue in the planchet was then redissolved in hexane and tested by thin-layer chromatography (*4*).

Their presence in the filter paper showed that aldrin and dieldrin had volatilized from the covered dishes. The amounts detected by Geiger-Mueller counting and gas-liquid chromatography were nearly identical; only aldrin or dieldrin was detected by thin-layer chromatography. Seven micrograms of aldrin were recovered from the papers kept in a desiccator with four plates of agar originally treated with a total of 82 μg of aldrin.

To obtain more-quantitative data, these experiments were repeated by similar preparation of six plates for each treatment. The contents of two plates of each set were used for initial analyses; the remaining four were placed in a desiccator; oil-impregnated filter papers were placed in each desiccator. After 1 day of incubation at 30°C, the filter papers were replaced by freshly oil-impregnated papers which were removed after the 2nd day of incubation. With dieldrin, similar papers were used for a 3rd day in the desiccators. Immediately after removal of the papers from the desiccators, they were extracted and analyzed.

The contents of dishes treated with aldrin were extracted after 2 days of incubation; those treated with dieldrin, after 3 days. The hexane phases were then analyzed by gas-liquid chromatography, liquid-scintillation counting, and thin-layer chromatography; the water-isopropanol phases, by liquid-scintillation counting only.

The fact that the data from gas-liquid chromatography and liquid-scintillation counting were similar indicated that the total radioactivity in the hexane phases was due to the presence of the original aldrin or dieldrin. Moreover, none or less than 1 percent of the total radioactivity recovered was present in the water-isopropanol phase; the absence of hydrophylic insecticidal metabolites was indicated. Thin-layer chromatography detected only aldrin or dieldrin. Results by gas-liquid chromatography are summarized in Table 1. Forty-eight to 51 percent of the applied aldrin and 4.2 to 7.0 percent of the applied dieldrin had volatilized during the first day of incubation and been trapped in the filter paper outside the dishes; 15 percent of the aldrin and 7 percent of the dieldrin volatilized during the 2nd day of incubation; 15 percent of the dieldrin during the 3rd day of incubation. While half of the applied aldrin volatilized immediately after application, dieldrin was lost at a constant rate during the 3-day incubation period—4.2 to 8 percent daily of the application. At the end of the incubation periods only 3.9 to 11 percent of the applied aldrin and 51 to 56 percent of the applied dieldrin were left on the glass surfaces or on the agar.

In the laboratory, the fate of pesticides in the absence of microorganisms should be determined by control experiments. Volatile compounds low in water solubility may be lost under sterile conditions; a cover of microorganisms to some extent prevents such volatilization. Thus it is more difficult to interpret data regarding the effects of microorganisms on pesticides under laboratory conditions.

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References and Notes

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Autocorrelation Functions of Noisy Electron Micrographs of Stained Polynucleotide Chains

Abstract. *Polyuridylic acid was deposited on carbon films in the presence of the thallous salt of mercury-*p*-dihydroquinone-*O,O*-diacetic acid under conditions leading to attachment of one of these molecules to each nucleotide. In electron micrographs of the stained polynucleotide chain, detail was obscured by the noise resulting from the substrate. Optically obtained autocorrelation functions of the distribution of density along the strands revealed peaks suggesting a structural regularity of 8 angstroms; this was interpreted as the internucleotide spacing.*

In electron-microscopic study of the sequence of bases of nucleic acids it is important to know the internucleotide distance; Highton and Beer (*1*) have inferred this from the lengths of tobacco mosaic virus RNA molecules; their values of approximately 6 Å, however, may be somewhat low since end-to-end distances rather than contour lengths were used. This spacing is now reexamined in an electron-microscopic study of extended polynucleotide chains stained with the dithallous salt of mercury-*p*-dihydroquinone-*O,O*-diacetic acid (Tl_2HgHDA). The chemistry of the staining reaction has been described (*2*).

Electron microscopy of such fine molecular detail falters not so much because of the capabilities of instru-

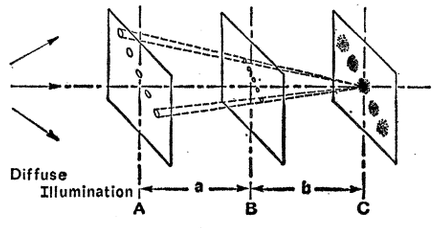

Fig. 1. Optical arrangement for obtaining autocorrelograms of an electron micrograph (*A*). *B* is a copy of *A* at $b/(a+b)$ times the magnification, and oriented parallel to it. *C* is an unexposed photographic emulsion. The system is illuminated from the left with a diffuse source. All rays through corresponding points of *A* and *B* converge on the optical axis at *C*.