through the bait and soil during the 6-month period and no trace of radioactivity could be detected in the leachate. Thin-layer chromatography and autoradiography of the extracts from weathered bait and soil showed all radioactivity to be in a single spot corresponding to mirex. There was no evidence of mirex degradation.

These field studies support the conclusion drawn in laboratory tests that mirex is not degraded by soil microorganisms. Under field conditions, mirex probably remains in unconsumed bait on the soil surface until the bait is completely decomposed. Decomposed bait and mirex are probably incorporated into the soil slowly. Because of the small amount of mirex applied to the soil and its low solubility in water, it is doubtful that significant amounts of mirex leach into ground water. Transportation of bait particles by surface runoff is a more likely source of stream and lake contamination.

ACKNOWLEDGMENT

This work is the result of a cooperative agreement between the National Institutes of Environmental Health Sciences and the U. S. Department of Agriculture Forest Service.

LITERATURE CITED

Gibson, J. R., Ivie, G. W., Dorough, H. W., *J. Agr. Food Chem.* **20**, 1246 (1972).
Martin, J. P., *Soil Sci.* **69**, 215 (1950).
Mehendale, H. M., Fishbein, L., Fields, M., Matthews, H. B., *Bull. Environ. Contam. Toxicol.* **8**, 200 (1972).
Stahl, E., "Thin-Layer Chromatography. A Laboratory Handbook," Springer-Verlag, Berlin, Heidelberg, and New York, 1969, p 896.
Thornton, H. G., *Ann. Appl. Biol.* **9**, 241 (1922).

Received for review November 26, 1973. Accepted January 28, 1974.

Kinetics of Azinphosmethyl Losses in the Soil Environment

Bruno Yaron,* Bruria Heuer,[1] and Yehudith Birk[1]

The kinetics of Azinphosmethyl losses in sterile and nonsterile soil as affected by temperature and moisture content was studied. The fate of Azinphosmethyl in soil involved two steps: one after application when the initial concentration remained constant and the second when the disappearance starts and the Azinphosmethyl losses follow first-order kinetics. It was found that the "lag period" before Azinphosmethyl losses begin is more than a biological effect. The water and temperature affect both the lag period and the rate of disappearance.

One of the pesticides commonly used in irrigated agriculture of arid and semiarid regions is the Azinphosmethyl (*O,O*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3(4*H*)-yl methyl] phosphorodithioate) also called Guthion or Gusathion. Studies on the persistence and degradation of Azinphosmethyl *in vitro* dealt with chemical decomposition of this pesticide in an organic solvent system (Harvey *et al.*, 1969) and in an aqueous solvent system (Liang and Lichtenstein, 1972). In soils the persistence of Azinphosmethyl was studied only with respect to the formulation and mode of application (Schultz *et al.*, 1970).

The aim of the present research was to isolate three environmental factors (biological, temperature, and moisture) important in the irrigated field and to establish their contribution to the disappearance of Azinphosmethyl in the soil.

EXPERIMENTAL SECTION

Soil. The soil used in the experiment was a silty loamy loessial sierozem (Haplargid) (from the Gilat Regional Experiment Station) with an organic matter content of less than 1%, pH 8.4, and a cation exchange capacity of 13.4 mequiv/100 g.

Sterile soil was obtained by irradiating subsequent samples of the initial soil in a JS-6000 irradiator provided with a ^{60}Co radiation source. The radiation dose was 3 Mrads.

Chemicals. Pure Azinphosmethyl has been synthesized in our laboratory from the commercially available methyl anthranilate in an overall yield of 35%. The intermediate and final products were identified by nmr spectroscopy. In the nmr spectrum (recorded with a Varian A-60 spectrometer in deuteriochloroform solution with tetramethylsilane as internal standard), the crystallized product displayed three sets of signals: doublet at τ 6.20 (J_{PCH3} = 15.5 Hz) for the methoxy group protons, doublet at τ 4.18 (J_{PCH} = 16 Hz) for the methylene protons, and multiplet at τ 1.9 for the aromatic protons.

Analytical Methods. Azinphosmethyl in soil was extracted by mechanically shaking with a 1:2 mixture of chloroform–methanol (9:1) and water in a soil:solvent ratio of 1.1. The soil was separated from the solvents by centrifugation (4000 rpm) and the aqueous phase was separated from the organic solvent in a separatory funnel. Preliminary tests showed that by this procedure a 100% recovery is obtained. A Packard Model 873 gas chromatograph with a flame ionization detector was used. The conditions for glc were: glass column, 180 cm × 3 mm i.d. filled with 10% SE-30 on Gas Chromosorb Q, 80–100 mesh; inlet and column temperature, 245°; detector temperature, 200°; nitrogen carrier gas flow, 60–70 ml/min. Azinphosmethyl solution (2 μl) at a concentration of 50 ppm in chloroform was injected as a standard after each two injections of sample. Peak height was used for quantitation.

Procedure. Two grams of soil (air dried and passed through a 60-mesh sieve), sterile and natural, respectively, were shaken with 2 cm^3 of an Azinphosmethyl solution of 50 ppm in chloroform for 30 min. Preliminary tests showed that the soil population was not affected by the treatment with chloroform. The solvent was then evaporated and 1 cm^3 of water (50% moisture content equivalent to the soil-saturated paste value) was added to half of the samples. Samples were incubated at three temperatures, 6, 25, and 40°, and analyzed at different intervals for up to 2 months. The entire experiment was conducted in duplicate. The bottles were sealed with paraffin in

Institute of Soils and Water, Agricultural Research Organization, Bet Dagan, Israel.
[1] Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel.

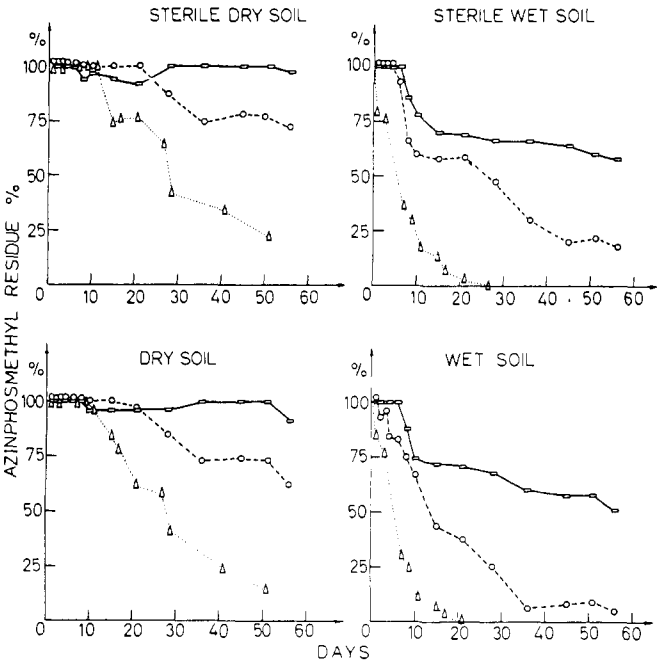

Figure 1. Disappearance of Azinphosmethyl in a sterile and natural Gilat soil as affected by moisture and temperature: (□-□) 6°; (O- - -O) 25°; (Δ···Δ) 40°.

Table I. Half-Life ($T_{1/2}$ in Days) of Azinphosmethyl in Natural and Sterile Soils as Affected by Soil Moisture and Temperature

Temp, °C	Sterile soil		Natural soil	
	Dry	Wet	Dry	Wet
6	484	88	484	64
25	135	29	88	13
40	36	6	32	5

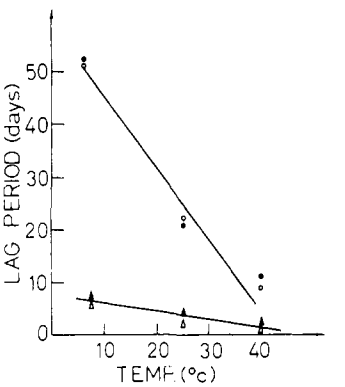

Figure 2. The effect of the temperature on the "lag period" during the incubation of Azinphosmethyl in a sterile and natural Gilat soil: (●) sterile dry soil; (O) dry soil; (▲) sterile wet soil; (Δ) wet soil.

order to avoid water evaporation. The samples were extracted as described and the chloroform phase was injected and tested by gas chromatography.

RESULTS AND DISCUSSION

Figure 1 shows that there are two definite steps in the kinetics of Azinphosmethyl persistence in soil. The first one is immediately after its application when the initial concentration (C) remains constant during a period of time (t_0); the second period begins when the disappearance process starts and when the rate of reaction at any time (t) is a function of the concentration of the pesticide. In this second period, when plotting the logarithm of the Azinphosmethyl concentration in soil *vs.* disappearance time, a linear relationship was observed. This confirms the fact that the Azinphosmethyl losses follow first-order kinetics

$$\log ([A]_0 - x) = [-(k_1/2.303)]t + \log [A]_0 \quad (1)$$

where $[A]_0$ is the initial concentration of Azinphosmethyl, x is the amount of A decomposed per unit volume at any time t, and k_1 is the rate constant for the reaction or proportionality constant. By plotting $\log ([A]_0 - x)$ *vs.* t, the y intercept is $\log [A]_0$ and k_1 is found from the slope of the line by $k_1 = -2.303(\text{slope})$.

In order to compare the influence of different environmental factors in Azinphosmethyl in the soil, it is useful to determine its half-life ($T_{1/2}$)

$$T_{1/2} = t_0 + t_{1/2} \quad (2)$$

where t_0 is the lag period between the application of pesticide and the beginning of the disappearance process and $t_{1/2}$ is the time required for one-half of the initial Azinphosmethyl to be lost during the disappearance process. t_0 is determined experimentally; $t_{1/2}$ may be calculated from eq 1 ($t_{1/2} = 0.693k_1$).

It is often observed that upon applying pesticides to the soil there is a "lag period" before losses begin. This lag was considered as the time required for the development in the soil of an effective population of pesticide-degrading organisms (Kaufman, 1970; Hamaker, 1970). Figure 1 shows, however, that with Azinphosmethyl a lag phase occurs in both sterile and natural soil and, consequently, the lag period is not only of a biological origin.

Water and Temperature Effect. Table I gives the half-life ($T_{1/2}$) of the Azinphosmethyl in the sterile dry and wet Gilat loessial loamy sierozem at three temperatures as calculated from eq 2. It is pointed out that an "air-dry soil" with about 2-3% moisture content is a normal status for the upper layer of a nonirrigated soil in the arid and semiarid zones. The effect of both factors, water and temperature, is significant. Water and temperature affect differently the two components of the $T_{1/2}$. Figure 2 shows the influence of the temperature on the lag period (t_0) in dry and wet Gilat soil. The delay in the beginning of the loss processes is affected by the temperatures. However, the temperature effect is much greater in a dry soil than in the soil with 50% moisture. A 10° increase in the temperature causes the disappearance process to start 11 days earlier in a dry soil and only 1 day earlier in the wet soil. The mechanism which affects the delay in the chemical decomposition of Azinphosmethyl in soil will be clarified by further research. The Azinphosmethyl residues at varying dates during the incubation period as affected by water and temperature are presented in Figure 1. This process may be defined by the changes in the loss rates (k_1). For the same temperature, *e.g.*, 25°, the presence of water in soil causes an increase of loss rate (k_1 from 6.1×10^{-3} to 2.8×10^{-2}). At the same water content (wet soil), the rate of disappearance is 8.5×10^{-3} at a soil temperature of 6° and 1.3×10^{-1} at 40°. The contribution of soil water to the loss of Azinphosmethyl at different temperatures was calculated from the differences between the losses in wet and dry soil (Figure 3). It should be noted that data for Figure 3 were taken from experiments done in sterile soil and under sterile conditions, and therefore microbiological degradation was avoided.

Biological Effect. The biological effect was defined from the differences between the disappearance of Azinphosmethyl on wet sterile and nonsterile soils. In Figure 1, there are no large differences in the lag periods for sterile and natural soils, and therefore this constant was not affected by the biological factor only. The effects of the bio-

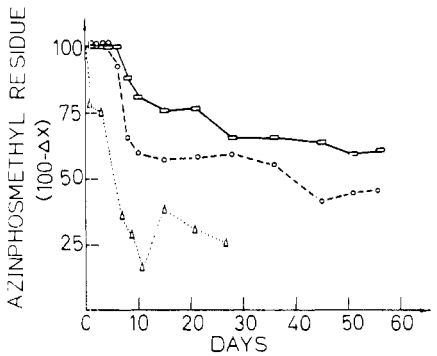

Figure 3. Calculated effect of soil moisture on the disappearance of Azinphosmethyl in soil. Δx is the difference of per cent recovery between dry and wet sterile soils: (□–□) 6°; (O- - -O) 25°; (Δ···Δ) 40°.

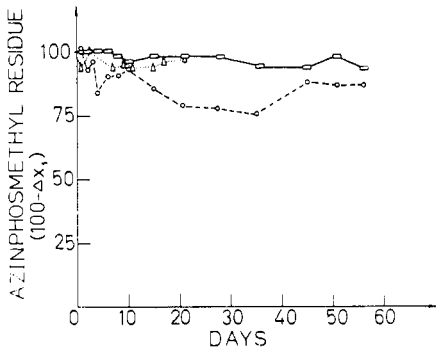

Figure 4. Calculated effect of the degrading organisms on the disappearance of Azinphosmethyl in soil. Δx_1 is the difference of per cent recovery between sterile and nonsterile wet soils: (□–□) 6°; (O- - -O) 25°; (Δ···Δ) 40°.

logical factor were not separated clearly under the conditions in our experiment, a period of time being necessary for the development of an effective population of Azinphosmethyl degrading organisms in the soil. However, from Table I it can be seen that in the natural soil the Azinphosmethyl half-life ($T_{1/2}$) is shorter than in the sterile soil. In the "dry" soil the difference is observed only at 25 and 40°. In the "wet" soil the differences between the natural and sterile soils are greater and appear in all the three temperatures experimented upon. This may be explained by the fact that biological activity is reduced in a dry environment. When the soil has a 50% water content, the half-life at 6° is reduced by 27%, at 25° by 55%, and at 40° by 16%. It is clear that at optimum soil temperature (25°) and soil moisture, the Azinphosmethyl degrading organisms are more effective. Figure 4 shows, for example, the biological effect on the Azinphosmethyl disappearance rate in the wet soil. As was mentioned with regard to the half-life, the rate of disappearance is mainly affected at 25°.

It may be concluded that the Azinphosmethyl loss in the soil environment is affected both by chemical and biological processes; the temperature affects the lag period and the rate of degradation.

LITERATURE CITED

Hamaker, J. W., in "Organic Chemicals in the Soil Environment," Vol. 1, Goring, C. A. I., and Hamaker, J. W., Ed., Wiley, New York, N. Y., 1970, pp 253–334.

Harvey, J. C., King, G. A., Young, J. W., *Advan. Chem. Ser.* **No. 86,** 81 (1969).

Kaufman, D. D., in "Pesticides in the Soil: Ecology, Degradation and Movement," International Symposium on Pesticides in the Soil, Michigan State University, East Lansing, Mich., Feb 1970, pp 73–85.

Liang, T. T., Lichtenstein, E. P., *J. Econ. Entomol.* **65,** 315 (1972).

Schultz, K. R., Lichtenstein, E. P., Liang, T. T., Fuhremann, T. W., *J. Econ. Entomol.* **63,** 432 (1970).

Received for review July 5, 1973. Accepted January 14, 1974. Contribution from the Agricultural Research Organization, The Volcani Center, 1973 Series No. 317-E. This research was financed in part by a grant made by the U. S. Department of Agriculture, Agricultural Research Service, authorized by Public Law 480.