

## Effects of Soils and Leaf Surfaces on the Photodecomposition of [<sup>14</sup>C]Azinphosmethyl

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The effects of environmental conditions on the photodecomposition of [<sup>14</sup>C]azinphosmethyl by light were investigated with glass surfaces (controls), three soil types, and surfaces of bean and corn leaves. The insecticide was not degraded in the dark. Exposure of [<sup>14</sup>C]azinphosmethyl on glass, in soils, or on leaves to sunlight or ultraviolet (uv) light for 8 h resulted in the degradation of the insecticide and loss of radiocarbon, most on glass surfaces and least in soils. The amounts of noninsecticidal water-soluble photoproducts due to sunlight amounted to 18.6% of the applied radiocarbon on glass surfaces, 6.5% in a sandy soil, 4.3% in a loam soil, 2.5% in a muck soil, 3.5% on corn leaves, and 1.4% on bean leaves. With increasing soil moisture content increased degradation occurred with uv light, but not with sunlight. The amounts of unextractable, bound residues produced due to irradiation increased with soils of higher organic matter and with increasing moisture contents. With sunlight, *N*-methylbenzazimide was produced only on corn leaves, while *N*-methylbenzazimide sulfide (or disulfide), benzazimide, and the oxygen analogue of azinphosmethyl were produced on both corn and bean leaves, but primarily on bean leaves. The insecticide was least susceptible to photodecomposition when used as a granular formulation.

The importance of photodecomposition of pesticides has only been fully recognized during the last decade. Thus Crosby et al. reported in 1965 on the photodecomposition of carbamate insecticides and other researchers investigated the effect of light from various sources on several insecticides (Baker and Applegate, 1974; Dejonckheere and Kips, 1974; Gaeb et al., 1975; Ivie et al., 1974; Machin et al., 1974). Many of these studies were conducted with short-wavelength uv light, and with pesticides dissolved in organic solvents. Liang and Lichtenstein reported in 1972 on the photodecomposition of azinphosmethyl primarily in water utilizing uv (2537 Å), yellow (5889 Å), and red (6563 Å) light. As a continuation of this study, the photodecomposition of this insecticide due to irradiation by sunlight and uv light was investigated under more realistic conditions, such as in soils and on plant leaf surfaces. The effects of soil type, soil moisture, surface area, and insecticide formulation on the photodecomposition of azinphosmethyl were also studied.

### MATERIALS AND METHODS

**Chemicals.** Analytical grade azinphosmethyl, [*carboxyl*-<sup>14</sup>C]azinphosmethyl (specific activity 2.7 μCi/mg), 22% azinphosmethyl emulsifiable concentrate, 7% azinphosmethyl granules, and their potential degradation products azinphosmethyl oxygen analogue, benzazimide (1,2,3-benzotriazin-4-(3*H*)-one), *N*-methylbenzazimide sulfide and disulfide, *N*-mercaptomethylbenzazimide, and anthranilic acid were supplied by the Chemagro Corp., Kansas City, Mo.

The solvents used for extractions and analyses were redistilled acetone, benzene, hexane, and chloroform and reagent grades acetonitrile, methanol, and 2-propanol.

**Materials. Soils.** Three soil types were used in the various experiments. They were Plainfield sand (pH 5.6, organic matter 0.6%, sand 94%, silt 3%, clay 3%), Plano silt loam (pH 6.0, organic matter 4.7%, sand 5%, silt 71%, clay 24%), and a muck soil (pH 4.5, organic matter 57%, sand 22%, silt 17%, clay 4%).

**Plant Material.** Bean (variety Top Crop) and corn plants (variety Funk G-4444) were grown for 2–3 weeks in vermiculite until the greens reached a length of 20 to 30 cm. Six corn plants and four bean plants per clay pot

were used for each duplicated test.

**Irradiation.** Ultraviolet light was supplied by two 15-W Westinghouse 15T8 germicidal lamps held 30 cm from the test substrates. The light produced by these lamps was rich in radiation at 2537 Å. Tests with sunlight were run on cloudless June and August days, between 9 a.m. and 5 p.m. on an area of a roof protected as much as possible from the wind. Temperatures at the test surfaces varied between 30 and 42 °C in the sunlight and 25 and 31 °C under ultraviolet light.

**Insecticide Application.** All soils, plants, water, and glass surfaces were treated with acetone solutions of azinphosmethyl in either its <sup>14</sup>C-labeled or unlabeled forms or by the direct application of a granular formulation. When the insecticide was to be mixed with the soil, the method of Lichtenstein and Schulz (1959a) was employed. The application onto glass, soil, and plant leaf surfaces involved dispensing various amounts of the compound in acetone solution onto the appropriate surface and allowing the solvent to evaporate before the tests were begun. Granules were applied by uniformly distributing 28.6-mg portions over the glass and soil surfaces or adding that amount to 100 ml of distilled water.

**Extraction.** All soils were extracted by the method described by Lichtenstein et al. (1973), which involves the use of two extractions with (1:1) methanol–acetone followed by a single extraction with (1:1:1) methanol–acetone–benzene. Residues on glass surfaces were removed by quantitatively rinsing with the same solvents. Insecticide granules were quantitatively transferred into an Erlenmeyer flask and extracted with the same solvents utilizing a magnetic stirrer. Benzene and water phases were eventually obtained from each extract.

Corn and bean leaves from each pot were removed and cut into 0.5-cm sections which were then extracted three times with acetonitrile in a Waring Blendor. The combined filtrates were concentrated on a vacuum evaporator to approximately 10 ml. The concentrates were diluted with 100 ml of water and partitioned in separatory funnels with three 100-ml portions of benzene and then with two 100-ml portions of chloroform. The chloroform was necessary to quantitatively extract benzazimide from the water. The benzene and chloroform extracts were concentrated separately at 40 °C in a vacuum evaporator and were adjusted to volume with the appropriate solvent. In experiments where water had been treated with analytical

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grade or emulsifiable concentrates of azinphosmethyl, extractions were made directly with benzene and then with chloroform. In the tests with azinphosmethyl granules in water the following procedure was used: At the end of the irradiation period, the water was decanted from the granules through filter paper. The filtered granules were transferred quantitatively to Erlenmeyer flasks and extracted twice with a mixture of methanol-acetone (1:1) and then with a mixture of methanol-acetone-benzene (1:1:1) on a rotary shaker. The combined extracts were added to the water which was filtered off earlier and the entire solution was concentrated to approximately 100 ml for subsequent partitioning with benzene and chloroform.

Soils and plant leaf material, after they had been extracted as described, were prepared for determination of the residual radioactivity by oxidation to  $^{14}\text{CO}_2$  in a Packard Model 305 Sample Oxidizer as described by Flashinski and Lichtenstein (1974).

**Analytical.** Radiocarbon assays of the benzene and chloroform extracts, the extracted water, and the trapped  $^{14}\text{CO}_2$  from the oxidized samples were performed with a Packard Tri-Carb Model 3320 scintillation spectrometer as described by Lichtenstein et al. (1972).

In some experiments the benzene extraction phases were analyzed for azinphosmethyl and its oxygen analogue by gas-liquid chromatography (GLC) utilizing a Tracor Model 550 gas chromatograph equipped with a Melpar flame-photometric detector for the detection of phosphorus-containing compounds. A 140 cm  $\times$  2 mm i.d. Pyrex glass column packed with 60/80 mesh Chromosorb W coated with 5% DC-11 silicone grease was modified by vapor phase deposition of Carbowax 20 M (Ives and Giuffrida, 1970) for the detection of these compounds.

Qualitative thin-layer chromatography and autoradiography of the combined benzene and chloroform extraction phases were performed according to methods described previously (Liang and Lichtenstein, 1972).

One-dimensional TLC was used to isolate azinphosmethyl oxygen analogue from the co-extracted plant pigments to facilitate GLC analysis. Individual extracts were spotted beside reference standards on 5  $\times$  20 cm precoated silica gel plates. The plates were developed in hexane-acetone-2-propanol (6:3:1) and the portion of the plate containing the standards was sprayed with the chromogenic reagent. Unsprayed areas corresponding to the visualized azinphosmethyl oxygen analogue spot were scraped from the plate and quantitatively extracted with benzene for GLC analyses.

Bioassays with mosquito larvae (*Aedes aegypti* L.) were used to test the toxicity of the organic solvent and water extraction phases. Aliquots of the benzene or chloroform extraction phases, representing 7.5  $\mu\text{g}$  of the originally applied insecticide, were transferred to 100 ml of tap water. The solvent was removed by vacuum evaporation at 30  $^\circ\text{C}$  and the water was readjusted to 100 ml. Duplicate 10-ml aliquots of each solution were placed into test vials and 15 third instar mosquito larvae were added to each vial. Mortality counts were made over a 72-h period. Portions of the water extraction phases representing either 20 or 10% of original applied azinphosmethyl were also subjected to vacuum evaporation to remove any toxic solvents which may have been present. After readjusting the volumes with water, 15 mosquito larvae were added to each of the duplicate 10-ml portions and mortalities were observed over a 72-h period.

## EXPERIMENTAL SECTION

**The Effects of Sunlight on the Decomposition of  $^{14}\text{C}$ Azinphosmethyl on Glass Surfaces.** To test the

effect of light on the photodecomposition of  $^{14}\text{C}$ azinphosmethyl in soils or on plant leaf surfaces, the insecticide was first deposited on glass surfaces for comparison purposes. To that effect 100  $\mu\text{g}$  of  $^{14}\text{C}$ azinphosmethyl (0.27  $\mu\text{Ci}$ ) was deposited as described on the inner bottom surface (64  $\text{cm}^2$ ) of each of two glass petri dishes. These dishes were then exposed outdoors to sunlight for 8 h as described. Controls were handled the same way except that they were covered with a cardboard box to exclude light. After the 8-h exposure period, the insecticide residues were removed and analyzed as described.

**Photodecomposition of  $^{14}\text{C}$ Azinphosmethyl in Three Soil Types.** The effects of soil type on the photodecomposition of azinphosmethyl were tested with the sandy, loam, and muck soils described above. Of each air-dried soil 310 g was treated and mixed with 620  $\mu\text{g}$  (1.67  $\mu\text{Ci}$ ) of  $^{14}\text{C}$ azinphosmethyl as described. Six 50-g portions of each soil containing 100  $\mu\text{g}$  (0.269  $\mu\text{Ci}$ ) of  $^{14}\text{C}$ azinphosmethyl each were placed in rectangular glass dishes with bottom surface areas of 268  $\text{cm}^2$ . Two dishes containing either the sandy, the loam, or the muck soils were then exposed to sunlight, to ultraviolet light, or kept in the dark for 8 h as described. Controls without soil (glass surfaces with an area of 268  $\text{cm}^2$ ) were prepared in duplicate with 100  $\mu\text{g}$  of  $^{14}\text{C}$ azinphosmethyl and also exposed for 8 h to sunlight, to ultraviolet light, or kept in the dark. To maintain field moisture levels as much as possible, distilled water was periodically added to the soils during irradiation.

After that the soils were removed from the dishes and extracted as described. Ultimately benzene and water extraction phases were obtained as well as unextractable or bound residues in the soils.

**Effects of Soil Moisture and Soil Surface on the Photodecomposition of  $^{14}\text{C}$ Azinphosmethyl.** The effects of soil moisture and surface area on the photodecomposition of  $^{14}\text{C}$ azinphosmethyl were tested with the loam soil in dry, moist, and water-logged conditions. Air-dried soil was treated with 2 ppm of  $^{14}\text{C}$ azinphosmethyl as described. Fifty-gram portions of this soil were then placed into six beakers with a bottom surface of 16  $\text{cm}^2$ . Two of the soils were kept dry, two were kept moist (15–20% moisture), at an approximate field moist condition by adding distilled water as needed, and two were "flooded" until 1.5 cm of water covered the soil surfaces. Two beakers without soils or water containing a deposit of 100  $\mu\text{g}$  of  $^{14}\text{C}$ azinphosmethyl (0.27  $\mu\text{Ci}$ ) served as controls. These eight beakers were then exposed for 8 h to uv light as described. Simultaneously, eight beakers prepared as described above were exposed for 8 h to sunlight and another eight were kept in the dark. The same experiment but with a 17 times larger surface area was repeated with rectangular glass dishes with a bottom surface area of 268  $\text{cm}^2$ . The depth of 50 g of air-dry loam soil in containers with a surface of 16  $\text{cm}^2$  was 29 mm and that in those with a surface area of 268  $\text{cm}^2$  was 1–1.5 mm. At the end of the exposure of soils and glass surfaces, extraction and analyses for radiocarbon were conducted as described.

**Photodecomposition of  $^{14}\text{C}$ Azinphosmethyl on Plant Leaves by Sunlight.** The degradation of  $^{14}\text{C}$ azinphosmethyl due to irradiation was studied with leaves of corn and bean plants. For that purpose four pots, each containing six corn plants (20–30 cm greens), and four pots, each containing four bean plants (20–30 cm greens), were utilized.  $^{14}\text{C}$ Azinphosmethyl (100  $\mu\text{g}$ ) (0.27  $\mu\text{Ci}$ ) in 0.1 ml of acetone was distributed as equally as possible with a microsyringe over the upper epidermis of 14 corn leaves

or of 12 bean leaves per pot. Thus, a total of 56 corn leaves and 48 bean leaves were treated with the insecticide. All treatments were performed in subdued light. After the solvent had evaporated, two pots each of treated corn and beans were taken outdoors and placed in the sunlight at 29–31 °C for 8 h. In addition, two pots each with treated corn or bean plants were covered with black cloth to exclude light and placed next to the uncovered pots. For comparison purposes tests were conducted with 64-cm<sup>2</sup> glass surfaces as described initially. It would have been interesting to also study the effect of sunlight on the photodecomposition of [<sup>14</sup>C]azinphosmethyl on leaf surfaces with time. This, however, was not feasible under the experimental conditions using a minimum of an uninterrupted 8-h exposure to sunlight. To study these phenomena over a longer period and possibly under field conditions would have introduced other environmental factors, thus making the interpretation of the data more difficult.

After the 8-h exposure period, the 14 corn leaves or 12 bean leaves from each pot were removed and extracted as described. Analyses for radiocarbon were finally performed with the benzene, chloroform, and water extraction phases as well as with the extracted leaf material. Quantitative and qualitative analyses of the benzene extraction phases after preliminary cleanup by TLC were performed by GLC. Two-dimensional TLC and autoradiography were conducted with the combined benzene and chloroform phases. To determine the presence of insecticidal materials, bioassays were conducted as described with the benzene extraction phases and the combined chloroform and water extraction phases.

**Effects of Formulations of Azinphosmethyl on Its Photodecomposition under Environmental Conditions.** An experiment was conducted to determine the effects, if any, of commercial formulations of azinphosmethyl exposed to sunlight. A 22% emulsifiable liquid formulation and a 7% mixture on clay granules were compared with authentic, analytical grade azinphosmethyl. The stability of the three forms was tested on glass surfaces, in distilled water, and in the loam soil. The liquid formulation and analytical grade chemicals were also compared on bean leaf surfaces. Since the treatments were made with nonradioactive azinphosmethyl, it was necessary to use high (2 mg per treatment) application levels to facilitate analyses of the minor degradation products. Acetone solutions of the pure azinphosmethyl were used to treat four 268-cm<sup>2</sup> glass dishes, the top surfaces of four 100-g portions of loam soil, four 100-ml portions of distilled water, and the upper surfaces of 24 bean leaves in each of four replicate growth pots. The identical treatments were also made with solutions of the emulsifiable liquid formulation. In addition, four replicates of glass surfaces, loam soil, and water were each treated with 28.6 mg of the 7% granules by distributing the granules as uniformly as possible over the glass and soil surfaces. Two of the four replicates of each variable were covered with cloth to exclude light while the remainder were exposed to sunlight for 8 h. All samples were extracted as described and the benzene extraction phases were analyzed by GLC as described.

## RESULTS AND DISCUSSION

**The Effects of Sunlight on the Decomposition of [<sup>14</sup>C]Azinphosmethyl on Glass Surfaces.** The degradation of [<sup>14</sup>C]azinphosmethyl with sunlight was studied for comparison purposes (controls) on glass surfaces. Results (Figure 1) showed that no degradation of the insecticide occurred in the dark, since 93% of the applied

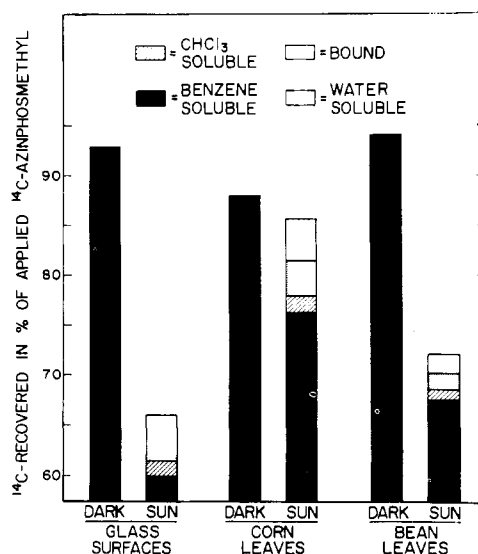


Figure 1. Photodecomposition of [<sup>14</sup>C]azinphosmethyl on plant surfaces by sunlight during an 8-h exposure period. Glass surfaces served as controls.

<sup>14</sup>C was associated with the benzene extraction phase in the form of unaltered azinphosmethyl, as determined by GLC. In sunlight, however, the total radiocarbon recovered from the benzene, chloroform, and water extraction phases was only 66% of the applied dose, indicating a loss of azinphosmethyl or of volatile photoproducts during the 8-h exposure period. Only 54.3% of the applied azinphosmethyl was recovered by GLC from the benzene extraction phase. Most important though was the fact that chloroform-soluble (1.5% of applied) and water-soluble (4.5% of applied) <sup>14</sup>C-labeled compounds had been produced. These materials were nontoxic to mosquito larvae. Employing two-dimensional TLC with the combined benzene and chloroform extraction phases, it was shown that degradation products were produced in sunlight only. They included benzazimide, *N*-methylbenzazimide, *N*-methylbenzazimide sulfide (or disulfide), a trace of azinphosmethyl oxygen analogue, and an unknown compound at *R*<sub>f</sub> 0.56 in the first dimension and 0.54 in the second. With the exception of the oxygen analogue of azinphosmethyl, all of these metabolites were nontoxic to mosquito larvae. This study, therefore, indicated that the effects of soils or plant surfaces on the photodecomposition of azinphosmethyl could probably be judged by the amount of noninsecticidal photoproducts or metabolites produced, by their appearance in the chloroform and water extraction phases, and by the amount of insecticidal substances remaining in the benzene extraction phase.

**Photodecomposition of [<sup>14</sup>C]Azinphosmethyl in Three Soil Types.** Sandy, loam, and muck soils treated with the insecticide were irradiated with uv and sunlight as described, utilizing glass surfaces as controls. Results obtained are summarized in Table I. In the absence of light no degradation of [<sup>14</sup>C]azinphosmethyl had occurred, since practically all (over 98%) of the applied radiocarbon was still recovered from the benzene extraction phases of the glass surfaces or the soils. As shown above by GLC, this radiocarbon was associated with unaltered [<sup>14</sup>C]azinphosmethyl. Irradiation of glass surfaces or of soils for 8 h, however, had a pronounced effect. With glass surfaces, a total of only 51% (uv light) or 65% (sunlight) of the applied radiocarbon was recovered, probably due to volatilization of azinphosmethyl or its degradation products. This loss was to some extent reduced with the sand of low organic matter content and no losses occurred with the

Table I. Effects of Soil Types on the Photodecomposition of [<sup>14</sup>C]Azinphosmethyl

		[ <sup>14</sup> C]Azinphosmethyl exposed to light on 268-cm <sup>2</sup> surfaces <sup>a</sup>			
		Glass (control)	Sandy soil	Loam	Muck
Exposed for 8 h to	Extraction phases	<sup>14</sup> C recovered from extraction phases, in % of applied <sup>b</sup>			
Uv (254 mμ)	Benzene	28.2 ± 2.4	38.3 ± 0.6	79.4 ± 1.4	82.0 ± 0.1
	Water	23.1 ± 3.3	20.1 ± 0	5.5 ± 0	2.6 ± 0.1
	Bound <sup>c</sup>		1.2 ± 0.2	10.1 ± 1.5	12.6 ± 1.3
	Total	51.3 ± 5.7	59.6 ± 0.8	95.0 ± 2.9	97.2 ± 1.5
Sunlight	Benzene	46.7 ± 8.9	75.3 ± 0.1	83.5 ± 0.2	91.1 ± 0.5
	Water	18.6 ± 1.3	6.5 ± 0.1	4.3 ± 0.1	2.5 ± 0.1
	Bound		0.8 ± 0.5	9.1 ± 0.6	5.5 ± 0.6
	Total	65.3 ± 10.2	82.6 ± 0.7	96.9 ± 0.9	99.1 ± 1.2
Dark (control)	Benzene	97.8 ± 1.6	99.6 ± 0.8	96.8 ± 0.8	98.6 ± 0.4
	Water	0.2 ± 0.2	0.2 ± 0	0.5 ± 0.1	0.4 ± 0.1
	Bound		0	0.4 ± 0.2	0.5 ± 0.1
	Total	98.0 ± 1.8	99.8 ± 0.8	97.7 ± 1.1	99.5 ± 0.6

<sup>a</sup> [<sup>14</sup>C]Azinphosmethyl (100 μg) (0.27 μCi) was applied to glass surfaces or mixed with 50 g of air-dried soil. Soils were kept moist with water. <sup>b</sup> Results are the average of duplicate experiments. <sup>c</sup> <sup>14</sup>C determined by liquid scintillation counting (lsc) after combustion of the extracted soils.

loam and muck soils, where a total of 95 to 99% of the applied radiocarbon was recovered. Water-soluble, noninsecticidal degradation products had been produced by irradiation, primarily on glass surfaces but also in the sandy soils, where 20.1 and 6.5% of the applied radiocarbon was water soluble after irradiation with uv light and sunlight, respectively. With soils of higher organic matter content, the amounts of water-soluble <sup>14</sup>C recovered were reduced and were smallest in the muck soil. It was interesting to note that with irradiated sandy soil practically no unextractable or bound residues were formed, while with irradiated loam and muck soils appreciable amounts of unextractable radiocarbon were found. This yielded 10 to 12% of the applied dose as bound residues after irradiation with uv light, and 5.5 to 9.1% after irradiation with sunlight. Since in the dark, no bound residues were noticed in soils, irradiation may have affected the binding of [<sup>14</sup>C]azinphosmethyl, or what seems more likely (Katan et al., 1976), irradiation enhanced the appearance of [<sup>14</sup>C]azinphosmethyl degradation products which in turn were "bound" to the soil. If the differences in the availability of active binding sites between the three soil types are considered, it could be possible that some of the products which appear as "water-soluble" in extracts of the Plainfield sand would be unextractable from the soils with larger proportions of organic matter.

In general it appeared that uv light had the most pronounced effects, although this light is to a large extent adsorbed by the atmosphere before it reaches the earth (Gates, 1965). With sunlight, which represents a more realistic approach to environmental conditions, the amounts of benzene-soluble radiocarbon were smallest on glass surfaces (47% of applied) and increased from the sand (75%) to loam (84%) and to the muck soil (91%). Conversely, water-soluble radiocarbon was largest on glass plates (18.6% of applied) and smallest in the muck soil (2.5%). Since the glass surfaces of 268 cm<sup>2</sup> were 4.2 times larger than those used in the initial experiment with sunlight as described above, it seemed reasonable that 4.1 times more water-soluble radiocarbon (18.6% of applied) was produced with the larger surface. Soils reduced the effects of irradiation on the photodecomposition of [<sup>14</sup>C]azinphosmethyl and this reduction increased with increasing organic matter of these soils.

Since glass dishes contained 50 g of one of the three soil types, their volume or depth was somewhat different.

Thus 50 g of the air-dried Plainfield sand had a depth of 1 mm, the loam, 1 to 1.5 mm, and the muck, 2 to 4 mm. This volume increased slightly after the addition of some water to the soil. The decreasing degradation of [<sup>14</sup>C]azinphosmethyl in soils could, therefore, also have been related to some extent to the fact that with increasing soil volume, less of the insecticide was exposed to irradiation at the soil surface.

**Effect of Soil Moisture and Soil Surface on the Photodecomposition of [<sup>14</sup>C]Azinphosmethyl.** A loam soil treated with the insecticide as described was used in a dry, moist, and water-logged condition to study the effects of soil moisture and soil surface on the photodecomposition of [<sup>14</sup>C]azinphosmethyl.

Results indicate (Table II) that in the absence of light, no loss or degradation of [<sup>14</sup>C]azinphosmethyl occurred under all experimental conditions. With insecticide deposits on glass surfaces and exposure to light, an increased loss of total radiocarbon was noticed when the deposit had been applied to the larger surface, most with uv light (49% total loss of the applied dose) and less with sunlight (35% total loss of the applied dose). Sunlight, apparently, was less effective when the insecticide was applied to a smaller surface of glass or of the dry loam soil. These differences, however, were no longer noticeable with moist or flooded loam soils.

As shown in the preceding experimental series, the loss of total radiocarbon and the production of water-soluble compounds were reduced when the insecticide was incorporated into the soil. With greater soil surfaces no substantial increases in total losses of <sup>14</sup>C-labeled compounds or in the production of water-soluble metabolites occurred. The amounts of bound residues were increased, however, after irradiation of larger soil surfaces, and this occurred only with moist and flooded soils.

Soil moisture, however, was an important factor contributing to the fate of [<sup>14</sup>C]azinphosmethyl due to irradiation with uv or sunlight, especially when the insecticide contaminated soil area had been enlarged by a factor of 16 to 268 cm<sup>2</sup>. Thus, with uv light a pronounced increase in water-soluble radiocarbon and in bound residues occurred with increasing amounts of soil moisture. With sunlight, though, no appreciable effect in the amount of water-soluble radiocarbon occurred due to an increase in soil moisture. Binding of radiocarbon due to the effects of sunlight was also greatest in moist and flooded soils.

Table II. Effects of Soil Moisture and Soil Surface Area on the Photodecomposition of [<sup>14</sup>C]Azinphosmethyl

		[ <sup>14</sup> C]Azinphosmethyl exposed to light <sup>a</sup>							
		Glass surfaces (control), dry		Silt loam soil					
				Dry		Moist		Flooded	
		16 cm <sup>2</sup>	268 cm <sup>2</sup>	16 cm <sup>2</sup>	268 cm <sup>2</sup>	16 cm <sup>2</sup>	268 cm <sup>2</sup>	16 cm <sup>2</sup>	268 cm <sup>2</sup>
Exposed for 8 h to	Extraction- phases	<sup>14</sup> C recovered from extraction phases, in % of applied <sup>b</sup>							
Uv (254 mμ)	Benzene	75.4 ± 2.0	28.2 ± 2.4	94.6 ± 0.1	79.2 ± 3.1	83.3 ± 0.8	75.3 ± 2.4	78.7 ± 3.2	73.2 ± 0.3
	Water	18.5 ± 1.3	23.1 ± 3.3	1.7 ± 0.1	2.9 ± 0.8	4.1 ± 0.2	5.7 ± 0	4.3 ± 0.4	10.9 ± 0.1
	Bound <sup>c</sup>			4.5 ± 0.4	6.0 ± 0.1	9.8 ± 0.6	13.0 ± 0.2	14.7 ± 0.4	17.7 ± 0.6
	Total	93.9 ± 3.3	51.3 ± 5.7	100.8 ± 0.6	88.1 ± 4.0	97.2 ± 1.6	94.0 ± 2.6	97.7 ± 4.0	101.8 ± 1.0
Sunlight	Benzene	87.1 ± 4.7	46.7 ± 8.9	91.8 ± 0.3	84.4 ± 7.0	91.6 ± 0.5	79.7 ± 2.5	91.4 ± 0.2	82.2 ± 2.1
	Water	3.4 ± 1.0	18.6 ± 1.3	3.3 ± 0.5	7.2 ± 0.1	2.4 ± 0.3	3.4 ± 0.8	2.7 ± 0	2.8 ± 0.1
	Bound			5.9 ± 1.3	6.9 ± 1.6	7.5 ± 0.8	16.1 ± 1.8	6.0 ± 3.9	12.8 ± 0.5
	Total	90.5 ± 5.7	65.3 ± 10.2	101.0 ± 2.1	98.5 ± 8.7	101.5 ± 1.6	99.2 ± 5.1	100.1 ± 4.1	97.8 ± 2.7
Dark (control)	Benzene	97.2 ± 2.8	97.8 ± 1.6	99.3 ± 1.7	99.5 ± 1.8	97.9 ± 0.6	97.6 ± 0.4	98.2 ± 0.1	98.2 ± 1.0
	Water	0.1 ± 0.1	0.2 ± 0.2	0.5 ± 0.1	0.5 ± 0	0.9 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.9 ± 0.2
	Bound			0	0	0.6 ± 0	0.7 ± 0	0.5 ± 0.2	0.2 ± 0.1
	Total	97.3 ± 2.9	98.0 ± 1.8	99.8 ± 1.8	100.0 ± 1.8	99.4 ± 0.8	98.8 ± 0.5	99.2 ± 0.5	99.3 ± 1.3

<sup>a</sup> [<sup>14</sup>C]Azinphosmethyl (100 μg) (0.27 μCi) was applied to glass surfaces or mixed with 50 g of the air-dried loam soil which was evenly placed on surfaces as indicated. After that soil was kept dry, moist, or water logged. <sup>b</sup> Results are averages of duplicate experiments. <sup>c</sup> Bound = <sup>14</sup>C determined by lsc after combustion of the extracted soils.

Table III. Effect of Plant Surfaces on the Photodecomposition of [<sup>14</sup>C]Azinphosmethyl<sup>a</sup>

Ref compds <sup>b</sup>	Corn leaves		Bean leaves	
	Dark	Sunlight	Dark	Sunlight
	Recovered by GLC from benzene extraction phases, in % of applied			
Azinphosmethyl	86.3 ± 0.7	65.3 ± 1.7	93.5 ± 3.5	52.2 ± 0.4
P=O azinphosmethyl <sup>c</sup>			N.D. <sup>d</sup>	2.8 ± 0.2
	<i>R<sub>f</sub></i> values <sup>e</sup>		Recovered by two-dimensional TLC from combined benzene and chloroform extraction phases <sup>f</sup>	
	I	II		
Azinphosmethyl	0.64	0.62	++++	+++
MMB	0.64	0.67	N.D.	N.D.
N-MB	0.56	0.62	N.D.	+
MBS	0.56	0.55	N.D.	+
Unknown	0.56	0.54	N.D.	+
P=O azinphosmethyl	0.34	0.38	N.D.	+
Benzazimide	0.33	0.56	N.D.	++
Unknown	0	0	N.D.	+

<sup>a</sup> [<sup>14</sup>C]Azinphosmethyl (100 μg) (0.27 μCi) was applied in duplicate to 14 corn leaves or to 12 bean leaves. <sup>b</sup> Reference grade chemicals: MMB = *N*-mercaptomethylbenzazimide; *N*-MB = *N*-methylbenzazimide; MBS = *N*-methylbenzazimide sulfide (or disulfide). <sup>c</sup> Azinphosmethyl oxygen analogue determined by GLC after its isolation by thin-layer chromatography. <sup>d</sup> N.D. = nondetectable. <sup>e</sup> Solvent systems, dimension I = chloroform-acetone (5:1), dimension II = hexane-acetone-2-propanol (6:3:1). <sup>f</sup> Number of plus signs indicates the intensity of spots which were visualized by autoradiography and spray reagents.

**Photodecomposition of [<sup>14</sup>C]Azinphosmethyl on Plant Leaves by Sunlight.** The degradation of the insecticide on corn and bean leaves was studied as described over an 8-h exposure period to sunlight. Results are summarized in Figure 1, indicating a pronounced effect of sunlight on the degradation or loss of [<sup>14</sup>C]azinphosmethyl. Of the applied radiocarbon 88 and 94% were recovered from corn and bean leaves, respectively, when kept in the dark and all of it was present in the benzene phases. In sunlight, however, a total of 86 and 72% of the applied radioactivity was recovered from corn and bean leaves, respectively. Apparently, volatile compounds had also been produced. This figure was 66% with glass surfaces.

Due to exposure to sunlight, chloroform and water-soluble products appeared and unextractable <sup>14</sup>C-labeled residues had been formed. These bound <sup>14</sup>C-labeled compounds amounted to 4.2 ± 0.4% of the applied dose

with corn leaves and to 1.8 ± 0.2% with bean leaves.

Exposure of mosquito larvae to residues from the benzene extraction phases, representing the equivalents of 0.75 μg of the originally applied azinphosmethyl, indicated that the time to kill 50% of the insects (LT-50) was 3.5 ± 0.2 h with extracts of corn leaves held in the dark and increased to 11.9 ± 1.1 h with those exposed to sunlight. These figures were 3.8 ± 0.2 and 14.5 ± 1.5 h, respectively, with bean leaves. This increase in LT-50 values by factors of 3.4 and 3.8 demonstrated a loss of toxicity due to irradiation. The chloroform and water extraction phases containing benzazimide and undetermined <sup>14</sup>C-labeled compounds, respectively, were not insecticidal. Analysis of the benzene extraction phases by GLC showed that nearly all the radioactivity detected by LSC was present as unaltered azinphosmethyl (Table III).

Utilizing two-dimensional TLC and autoradiography, the effect of sunlight on the degradation of [<sup>14</sup>C]azin-

phosmethyl was apparent (Table III). While with leaves kept in the dark no degradation products could be detected, exposure to sunlight for 8 h resulted in spots of various intensity indicating the appearance of *N*-methylbenzazimide on corn leaves and *N*-methylbenzazimide sulfide (or disulfide), benzazimide, and the oxygen analogue of azinphosmethyl on both corn and bean leaves. The spot developed for the latter compound was most pronounced with extracts from bean leaves. In the experiment described previously with [<sup>14</sup>C]azinphosmethyl exposed on glass surfaces to sunlight, only traces (a slight shadow at *R<sub>f</sub>* 0.34) of the oxygen analogue of azinphosmethyl could be noticed. It appears, therefore, that a factor present primarily in bean leaves enhanced the formation of the oxygen analogue from azinphosmethyl due to irradiation with sunlight.

**Effects of Formulations of Azinphosmethyl on Its Photodecomposition under Environmental Conditions.** Azinphosmethyl was applied as a liquid and a granular formulation to primarily glass surfaces, water, and a loam soil as described. After exposure of these treated materials to sunlight for 8 h, analyses were conducted for the remaining azinphosmethyl. Results obtained after application of insecticide emulsions and irradiation by sunlight were identical with those obtained with analytical grade insecticide. With granules, however, the insecticide was more persistent since nearly all of the azinphosmethyl applied was recovered from glass surfaces and soils after exposure to sunlight, while only 64% was recovered from water. Photodecomposition of granular formulations of the insecticide was largest in water, where the protective cover of the insecticide was apparently disrupted, thus making it more accessible to light. With analytical grade insecticide and emulsions, 78 and 79% of the applied azinphosmethyl, respectively, were recovered from glass surfaces, 16 and 17% from water, 99 and 96% from soil, and 90 and 93% from bean leaves. In comparison to previous tests, relatively high recoveries of azinphosmethyl were observed after its application as analytical grade material. This could have been related to its extremely high application rate of 2 mg per treatment, as opposed to 0.1 mg in all previous experiments. Lichtenstein and

Schulz (1959b) showed that persistence of insecticides in soils was increased with higher application rates when expressed in percent of the applied dose.

Results presented in this study clearly indicate that the effects of various environmental factors have to be considered when the photodegradation of agricultural chemicals is being investigated.

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## Soil Persistence and Aquatic Bioaccumulation Potential of Hexachlorobenzene (HCB)

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Soil was treated with 0.1 to 100 ppm of HCB and stored under aerobic (sterile and nonsterile) and anaerobic nonsterile conditions for 1 year in covered containers to retard HCB volatilization. No soil-incorporated HCB was lost at any treatment rate or under any storage condition. Five species of aquatic organisms were exposed to three water concentrations of HCB for 3 to 33 days in aquatic model ecosystems. Bioaccumulation potential among the organisms averaged 0.6 to  $2.0 \times 10^3$  water content for algae (*Oedogonium cardiacum*), snails (*Helisoma* sp.), daphnids (*Daphnia magna*), and mosquito fish (*Gambusia affinis*), and 6 to  $16 \times 10^3$  for catfish (*Ictalurus punctatus*). Further studies confirmed that catfish consistently accumulated more HCB than did the other four species.

The direct importance of hexachlorobenzene (HCB) to agriculture is minor. In 1971 only about 6800 kg was used

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as a seed fungicide, its only registered use. However, its indirect importance to agriculture, via environmental contamination, may be much larger. For example, HCB found in Louisiana cattle was apparently related to airborne industrial emissions, while residues in sheep from Texas and California were traced to pesticides contaminated with HCB (EPA Report, 1973). HCB residues have