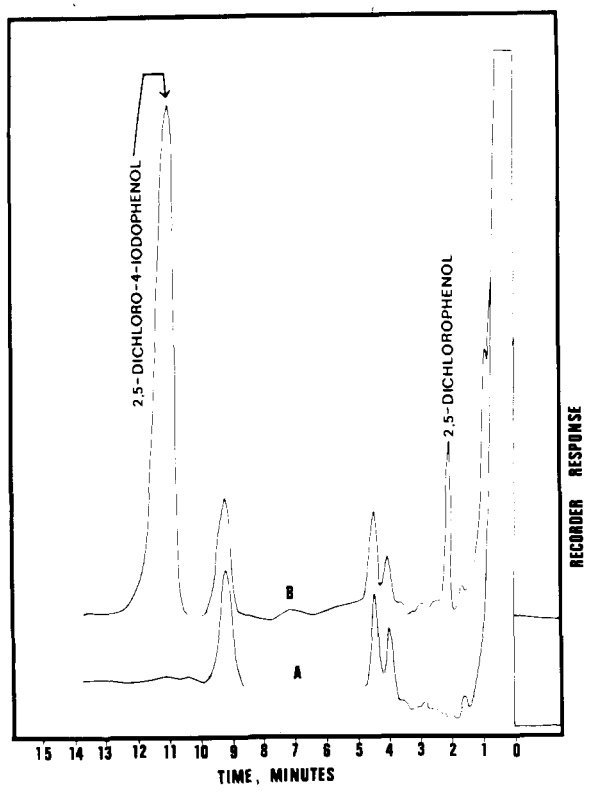

Figure 4. Chromatogram of extracts from kidney with the alcohol–water–sodium hydroxide procedure: (A) untreated tissue; (B) tissue + 0.05 and 0.02 ppm of the indicated compounds.

adding known amounts of iodofenphos, its photodecomposition product, ronnel, the oxygen analogue of iodofenphos, and its photodecomposition product to control samples of the various tissues before blending. The recovery of these compounds from fortified tissues is reported in Table I.

Phenolic Compounds. The efficiency of the procedure was tested by adding known amounts of 2,5-dichlorophenol and 2,5-dichloro-4-iodophenol to control samples of the various tissues before blending. The recovery of these compounds from fortified tissues is reported in Table II.

Figures 1 and 2 are chromatograms showing recoveries of the phosphorus compounds from fat. Figure 3 is a chromatogram showing recovery of the phenolic compounds from muscle, and Figure 4 shows recovery from kidney with the alcohol–water–sodium hydroxide procedure.

The control tissues showed no peaks at the retention times for any of the compounds. With the sample sizes and dilutions used, 0.005 ppm of iodofenphos, 0.002 ppm of its photodecomposition product, 0.003 ppm of ronnel, 0.01 ppm of the iodofenphos oxygen analogue, 0.004 ppm of its photodecomposition product, 0.01 ppm of 2,5-dichlorophenol, and 0.002 ppm of 2,5-dichloro-4-iodophenol can be detected in the various tissues.

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Photodegradation of *O*-Ethyl *O*-[4-(Methylthio)phenyl] *S*-Propyl Phosphorodithioate (BAY NTN 9306)

G. Wayne Ivie* and Don L. Bull

The ^{14}C-labeled organophosphorus insecticide, *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate (BAY NTN 9306), degraded rapidly when exposed to sunlight as deposits on cotton foliage, glass surfaces, or in water solution. The half-life of the compound was in each case less than 2 days. Exposure of water solutions of the insecticide to >280-nm artificial light resulted in very rapid degradation (half-life <2 h) to the same major photoproducts as were generated by exposure to sunlight. Degradation pathways included oxidation of the methylthio sulfur to sulfoxide and sulfone derivatives, hydrolysis of the phosphorus–*O*-phenyl ester, oxidative desulfuration of the P=S moiety, and undefined transformations leading to highly polar products that increased in quantity with time of exposure. When ^{14}C-labeled photoproducts of BAY NTN 9306 were orally administered to rats, they were rapidly excreted and no appreciable radiocarbon was retained in body tissues.

The organophosphorus (OP) compound *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate (BAY NTN 9306) has given good control of certain phytophagous insects that attack cotton and other crops; moreover, its mammalian toxicity is considerably below that of many of the OP insecticides in current use. It thus appears that the efficacy and toxicological characteristics of 9306 are such that the compound may find widespread application in a variety of insect control situations. A thorough evaluation of the metabolic and environmental behavior of 9306 is needed since the compound and its derivatives can be expected to interact with a variety of nontarget

Veterinary Toxicology and Entomology Research Laboratory (G.W.I.), and Cotton Insects Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, College Station, Texas 77840.

organisms. In previous investigations at these laboratories, we have considered the metabolic fate of 9306 in laboratory rats (Bull and Ivie, 1976), a lactating ruminant (Ivie et al., 1976), and in cotton plants and soil (Bull et al., 1976). The studies reported here were made to evaluate the fate of 9306 upon exposure to sunlight on surfaces and in water solution.

MATERIALS AND METHODS

Chemicals. Radiocarbon labeled 9306 (2.2 mCi/mmol, with uniform labeling in the phenyl ring) was supplied for these studies by Chemagro Agricultural Division, Mobay Chemical Corp., Kansas City, Mo. The labeled compound was of >99% radiochemical purity as indicated by thin-layer chromatographic (TLC) analysis, radioautography, and subsequent quantitation of the radioactive areas of the plate by liquid scintillation counting (lsc). Certain nonradioactive analogues of 9306 were also supplied by Chemagro. These compounds and their chemical and trivial names were those used in previous tests (Bull and Ivie, 1976).

Degradation on Leaf and Glass Surfaces. Greenhouse grown cotton plants in the fruiting stage (Stoneville 213 variety) were used to study the sunlight degradation of 9306 on leaf surfaces. Areas (4 × 4 cm) on the dorsal side of the larger leaves were marked off with a felt tip marker for treatment. Only those leaves of which the dorsal surface could be easily exposed to direct sunlight were treated. The treatment solution was prepared by mixing the radioactive 9306 with an unlabeled emulsifiable concentrate formulation of 9306 containing 50% active ingredient. The final treatment mixture, a milky suspension in water, was such that application of 20 μl to each 16-cm^2 treatment area gave a deposit of 42 μg of 9306/cm^2. The treated plants were then exposed outdoors (late summer) for as long as 14 days. The plants were moved periodically to maintain the treated leaves in full sunlight, which prevailed during most of the study periods. There was very little cloudiness and no rain so the daily exposure to sunlight ranged from 9 to 12 h. In all cases samples were moved to a protected area at night.

At appropriate intervals, the treated leaves were clipped from the plants, the untreated portion of the leaf was removed and discarded, and the residual radiocarbon on the treated surface was recovered from the leaf by swirling for 2–3 min in 10 ml of methanol. The leaf was then washed with an additional 10 ml of methanol, the two methanol rinses were combined, and the radiocarbon was quantitated by lsc. The extract was then concentrated under a gentle stream of nitrogen and analyzed by two-dimensional TLC. The residual radiocarbon on or in the treated leaf was quantitated by oxygen combustion (Bull and Ivie, 1976).

The sunlight photodegradation of 9306-^{14}C was also studied on frosted glass microscope slides (2.5 × 7.5 cm). Frosted glass was preferred over smooth glass because it permitted more even spreading of sample and greater surface area for exposure. Radiolabeled 9306 was mixed with technical 9306 (unlabeled), and 1-mg deposits were spread as evenly as possible over the surface of the slide via 25 μl of methanol carrier. The samples were then exposed to direct sunlight and analyzed at intervals by rinsing the slide thoroughly with methanol. The extracts were subsequently concentrated and analyzed by TLC.

Degradation in Water. Radiolabeled 9306 in acetone solution was added to a boiling flask, and the solvent was removed by a gentle stream of nitrogen. An appropriate amount of distilled water (pH 7.0) was added and the surface deposit of 9306-^{14}C was solubilized by rotation of the flask for about 1 h to give a 0.5-ppm solution in water, below the reported solubility limit for 9306 (Flint, 1976). Then, 25-ml portions of the solution were transferred to quartz test tubes (Precision Cells, Hicksville, N.Y.), and the tubes were capped with Teflon coated stoppers, and then exposed to sunlight. At intervals, samples were acidified to pH 2.0 and were extracted four times with equal volumes of ethyl acetate. Radiocarbon in both ethyl acetate and aqueous phases was quantitated by lsc; then the organic phase was dried over sodium sulfate, concentrated, and analyzed by TLC.

A 1-l. solution of 9306-^{14}C prepared as described above was subjected to artificial light photolysis. The photochemical apparatus was a 450-W, high-pressure mercury vapor Hanovia lamp, housed in a water-cooled fused quartz immersion well, and 1-l. capacity reaction vessel (Ace Glass, Vineland, N.J.). A Pyrex absorption sleeve was used to prevent <280-nm light from reaching the sample. During exposure, the stirred sample was aerated by bubbling compressed air through the solution. At intervals, 10-ml portions of the sample were removed, acidified, extracted, and analyzed as described above.

Analysis and Product Characterization. Resolution of photoproducts was accomplished by using two-dimensional TLC. The silica gel precoated plates were Brinkman Silplate F-22 (20 × 20 cm, 0.25-mm gel thickness, with fluorescent indicator). The photoproduct mixture was spotted as a 1–2-cm band in one corner of the plate, and the plate was developed in a solvent mixture of heptane–chloroform–methanol (9:4:1) and then in the second dimension in hexane–ethyl acetate–methanol (2:2:1). The plates were then exposed to x-ray film (Kodak No-Screen) for 1 week. After development of the film, the radioactive areas of the gel were scraped, and the radiocarbon was quantitated by lsc. Identification of the radioactive photoproducts was accomplished by two-dimensional TLC with the authentic 9306 analogues. When sufficient quantities of photoproducts were available, the compounds identified by TLC were confirmed by GLC–mass spectral analysis by using the instrumentation and procedures previously reported (Bull and Ivie, 1976).

Fate of ^{14}C-Labeled 9306 Photoproducts in Rats. A 0.5-ppm water solution of 9306-^{14}C was irradiated with artificial light as described above for 4 h. Extraction of the photolysis mixture with ethyl acetate separated the photoproducts into nonpolar (ethyl acetate soluble) and polar (water soluble) components. The composition of the ethyl acetate soluble photoproducts was essentially the same as indicated in Table III for an identical 4-h exposure. The number or nature of the water-soluble photoproducts was not determind. The photoproduct mixtures were transferred to dimethyl sulfoxide solution and were administered orally to adult female Sprague-Dawley rats. Two rats were treated with the polar products and two with the nonpolar materials. The treatment in each case was 0.1 mg/kg 9306 equivalents. The two rats in each group were housed in a common metabolism cage, and total urine and feces samples were collected for 5 days after treatment. At this time the animals were killed, and selected tissues were analyzed for radiocarbon residues by oxygen combustion. Radiocarbon excreted into the urine of the treated animals was quantitated by lsc of aliquots of fresh urine and that in feces by oxygen combustion.

RESULTS

Sunlight Photodecomposition on Surfaces. *Cotton Leaves.* ^{14}C-Labeled 9306 was rapidly degraded by exposure to sunlight as surface deposits on cotton foliage (Table I). After 2-days exposure, less than 20% of the

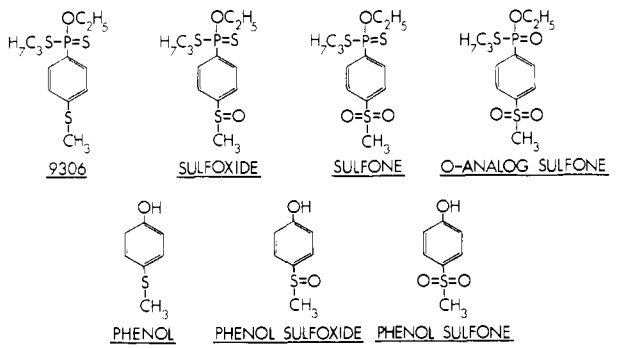

Figure 1. Structures and trivial names of characterized BAY NTN 9306 photoproducts formed during sunlight exposure as surface deposits or in water solution.

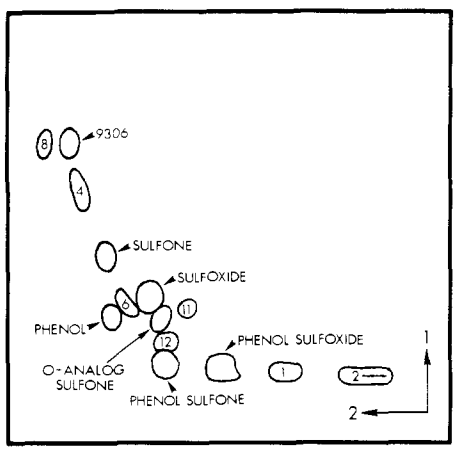

Figure 2. TLC resolution of BAY NTN 9306 photoproducts formed during sunlight exposure as deposits on cotton foliage. Compounds designated by numbers are uncharacterized photoproducts (Table I).

original 9306 deposit was recovered unchanged and by 4 days after treatment, only trace amounts of the parent compound remained. The major degradation pathways were oxidation of the methylthio sulfur to sulfoxide and then sulfone derivatives, and cleavage of the phosphorus–*O*-phenyl ester to phenolic compounds (Figure 1). The phenols, however, did not acumulate to any appreciable extent. Based on the observed accumulation of uncharacterized photoproducts, particularly unknowns 1 and 2 (Table I, Figure 2), and upon the accumulation with time of radiocarbon which was not extractable from the treated leaves, it seems likely that the phenols underwent additional degradation to more polar products. Oxidative desulfuration of the P=S moiety occurred to a limited extent, as evidenced by the appearance of small amounts of the *O*-analogue sulfone in the later samples. The major uncharacterized photoproducts, unknowns 1 and 2 (Figure 2), did not cochromatograph with any of the authentic standards available, and efforts to resolve these compounds by GLC–mass spectroscopy were unsuccessful.

Glass. The photodegradation of technical grade 9306-^{14}C exposed to sunlight on glass surfaces was quite similar to that of an emulsifiable concentrate formulation of 9306-^{14}C on cotton foliage. The major photoproducts extracted from glass surfaces (Table II) were the same as those on cotton foliage (Table I), and the relative distribution of products with time of exposure was remarkably similar. Studies in which glass slides were treated with 9306-^{14}C and then held in the dark for 7 days indicated that the surface deposits of 9306 underwent very little degradation in the absence of light. The only degradation product observed was a small quantity of the

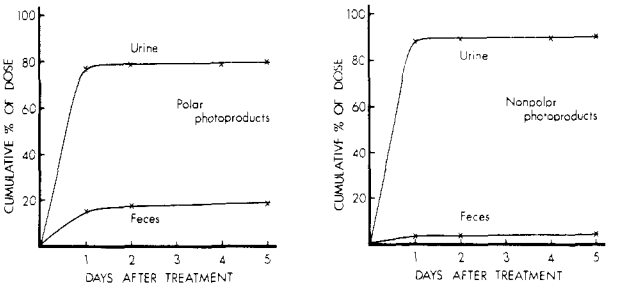

Figure 3. Radiocarbon excretion following oral treatment of rats with polar and nonpolar photoproducts of BAY NTN 9306-^{14}C.

sulfoxide derivative (Table II).

Photodegradation in Water. Dilute water solutions of 9306-^{14}C also degraded rapidly in sunlight; the half-life of a 0.5-ppm solution was only about 0.5 day (Table III). The expected photoproducts arising from oxidation and hydrolytic reactions were observed, except that derivatives formed by oxidative desulfuration of the P=S moiety were not detected. The most obvious reactions were those leading to a rapid accumulation of uncharacterized polar products that were not extractable from the aqueous phase with ethyl acetate (Table III). Samples held in the dark for 7 days indicated that 9306 exhibits considerable instability as a water solution at pH 7.0. Only about half of the radiocarbon in these samples was recovered as unchanged 9306; the major degradation reaction was ester cleavage to the unoxidized phenol (Table III).

Exposure of 0.5-ppm water solutions of 9306-^{14}C to >280-nm artificial light gave the same major photoproducts that were observed following exposure to sunlight in water (Table III), though the rate of degradation was much faster in artificial light–the half-life of 9306 was only about 1 h, and very little of the unchanged compound remained after 4 h. Degradation of 9306 by artificial light, like that by sunlight, resulted in a rapid accumulation of highly polar photoproducts that were not extractable from the water solution.

Fate of ^{14}C-Labeled 9306 Photoproducts in Rats. The labeled photoproducts of 9306 were rapidly excreted by rats following oral administration (Figure 3). Elimination was essentially complete within 1 day after treatment though trace residues were excreted during the remainder of the 5-day posttreatment period. The large proportion of radioactive material excreted through the urine after treatment with both polar and nonpolar photoproducts indicated that both groups of photoproducts were readily absorbed from the gastrointestinal tract. However, greater amounts of radiocarbon appeared in the feces of rats treated with the polar compounds (18% of dose vs. 4% for the nonpolar treatment). Thus, there may be somewhat less absorption from the gut or perhaps greater biliary excretion of the more polar photoproducts. The chemical nature of the radioactive residues appearing in the urine and feces of the treated animals was not studied.

Analysis of tissue samples collected at sacrifice of the animals 5 days after dosing confirmed that radiocarbon excretion following each treatment was essentially complete. Brain, fat, kidney, liver, and muscle samples were analyzed from each animal; in all cases, the radiocarbon levels were <0.005 ppm 9306 equivalents, the sensitivity of the combustion method used.

DISCUSSION

The studies reported here indicate that 9306 is readily susceptible to sunlight photodegradation as surface de-

Table I. Degradation of BAY NTN 9306-^{14}C as Deposits on Cotton Foliage Exposed to Sunlight

Exposure, days	Radiocarbon as indicated product, %															
	9306[a]	Sulfoxide[a]	Sulfone[a]	O-Analogue sulfone	Phenol	Phenol sulfoxide	Phenol sulfone	Unknowns 1	2	4	6	8	11	12	Leaf residue	Loss
0	98.2	0.3	0	0	0	0	0	0	0	0	0	0	0	0	1.5	0
0.5	72.6	4.6	0.2	0	0.4	0.7	0.6	0.8	1.6	0	0.2	0	0	0	9.4	8.9
1	61.3	6.9	1.3	0	0.5	0.9	0.9	1.2	3.2	0	0	0.5	0	0	16.8	6.5
2	17.8	20.4	12.3	0	0	1.8	1.7	4.9	5.6	0	0	2.2	0	0	30.6	2.7
4	1.3	13.0	21.7	1.3	0	0	0	6.0	13.6	0.8	1.4	0	0.7	0.9	36.9	2.4
7	0.4	2.9	16.0	3.6	0	3.6	0.2	6.4	27.7	0	0	0	0	0	31.7	7.5
14	0.2	1.8	9.5	2.7	0	5.0	0.4	5.4	15.1	0	0	0	0	0	38.6	21.3

[a] Products characterized by two-dimensional TLC and GLC–mass spectroscopy. Other products identified by two-dimensional TLC only.

Table II. Degradation of BAY NTN 9306-^{14}C as Deposits on Glass Surfaces Exposed to Sunlight

Exposure, days	Radiocarbon as indicated product, %												
	9306[a]	Sulfoxide[a]	Sulfone[a]	O-Analogue sulfone[a]	Phenol	Phenol sulfoxide[a]	Phenol sulfone[a]	Unknowns 1	2	8	9	10	Loss
0	99.0	0.2	0	0	0.7	0	0	0	0	0	0	0.1	0
0.5	88.7	4.8	0.7	0	0.4	0.6	0	0.8	3.3	0	0.5	0	0.2
1	73.3	11.5	2.2	0	0	1.3	0	1.8	6.9	0.8	1.0	0	1.2
2	10.2	37.3	18.6	0	0	3.4	2.3	3.0	6.3	2.1	1.4	0	15.4
4	0.3	1.2	13.8	5.0	0	0	4.2	6.0	33.6	0	1.0	0.6	34.3
7	0.3	0.3	6.6	3.1	0	0	9.8	3.5	40.7	0	0.8	0.1	34.8
7 (dark)	99.0	1.0	0	0	0	0	0	0	0	0	0	0	0

[a] Products characterized by two-dimensional TLC and GLC–mass spectroscopy. Other products identified by two-dimensional TLC only.

Table III. Degradation of BAY NTN 9306-^{14}C on Exposure of 0.5 ppm of Water Solutions to Sunlight and Artificial Light

Exposure	Radiocarbon as indicated product, %														
	9306	Sulfoxide	Sulfone	Phenol	Phenol sulfoxide	Phenol sulfone	Unknowns 1	2	3	4	5	6	7	Water soluble
Sunlight, days														
0	90.4	1.7	0.2	5.2	1.3	0	0.9	0.3	0	0	0	0	0	0
0.5	47.8	15.0	2.2	1.5	5.0	0	3.1	4.7	1.2	1.2	0.6	0.8	0	16.9
1	31.2	13.4	0.8	2.7	10.6	0	4.2	6.2	2.0	0.5	0.6	0.5	0	27.3
2	8.8	14.9	0.7	0	9.9	0.9	5.3	4.4	3.0	0	0	0.6	1.0	50.5
4	3.3	13.0	0	0	7.8	1.4	3.8	6.1	2.6	0	0	1.3	0	60.7
7	0.7	6.5	0	0	3.8	1.2	0	10.1	4.2	0	0	0.7	0	72.8
7 (dark)	47.8	1.6	0	38.3	3.9	0	5.8	0.8	0	0	0	0	0	1.8
Artificial light, h														
0	97.3	2.7	0	0	0	0	0	0	0	0	0	0	0	0
0.5	67.6	7.9	6.2	0	3.4	0.6	1.8	2.9	0	0	0	0	0	9.3
1	47.3	12.5	6.0	0	3.5	1.5	3.3	5.1	0	0	0	0	0	20.7
2	21.2	17.4	3.1	0	3.5	1.6	5.1	5.0	0.8	0	0	0	0	42.3
4	3.1	17.9	1.5	0	1.9	0.8	6.1	4.2	1.1	0	0	0	0	63.4
4 (dark)	91.4	3.6	0	3.0	1.7	0	0.3	0	0	0	0	0	0	0

posits of the technical and formulated material and in water solution. The major identified photoproducts arise through established oxidative and hydrolytic mechanisms. Oxidation of the phenyl-*S*-methyl sulfur to sulfoxide and sulfone derivatives appeared to be the initial step in the degradation of 9306. Comparable photochemical oxidation of thioether moieties is known for both OP (Wendel and Bull, 1970) and carbamate (Abdel-Wahab et al., 1966) insecticides. Likewise, oxidative desulfuration and aryl ester cleavage of OP insecticides are previously reported photochemical pathways (Koivistoinen and Merilainen, 1962; Ohkawa et al., 1974).

The identified photoproducts of 9306 (Figure 2), which are formed through straightforward oxidative and hydrolytic reactions, are clearly not the terminal photodegradation residues to be expected in the environment because more polar products are rapidly generated both on surfaces and in water solution. While these polar derivatives remain uncharacterized, studies with rats indicate that they will not likely result in appreciable toxicological hazards to mammals. The compounds were rapidly excreted by rats and were not retained in the tissues. These studies thus indicate that 9306 will be of short persistence in the environment, and that the persisting terminal residues will likely be of little toxicological significance.

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Effects of Light on the Fate of Carbofuran during the Drying of Alfalfa

Thomas E. Archer

Carbofuran and related compounds as residues on alfalfa hay exposed to drying by sunlight, ultraviolet light, and air in the dark under controlled conditions were investigated using gas–liquid chromatography (GLC) and thin-layer chromatography (TLC) for the purpose of determining the fate, form, and removal of these residues from the hay. Maximum loss of carbofuran and related compounds calculated as total carbofuran occurred approximately 10 days after application from all the samples. Maximum losses of residue from lot I, lot II, and lot III were 83.6, 81.3, and 89.8%, respectively. Hydroxycarbofuran, 3-oxocarbofuran, 3,7-diol, and the 3-keto-7-phenol increased in all of the lots. The hydroxycarbofuran increased most dramatically in the dark drying experiment. This increase was evident because less volatility with plant moisture occurred as it was formed since the percent moisture loss from the alfalfa during days 2 to 10 in the dark experiment was less than in the ultraviolet light and sunlight experiments.

Carbofuran (Furadan) is used for the control of alfalfa weevil larvae and adults, Egyptian alfalfa weevil larvae, pea aphid, and lygus bugs. Furadan 4 Flowable (4 lb of active ingredient/gal) is applied to alfalfa as a foliar application in rates ranging from 0.5 pt to 1 qt/acre 7 days to 28 days preharvest.

Tolerances are established for combined residues of the insecticide carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate), its carbamate metabolite (2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl *N*-methylcarbamate), and its phenolic metabolites (2,3-dihydro-2,2-dimethyl-7-benzofuranol, 2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranol, and 2,3-dihydro-2,2-dimethyl-3,7-benzofurandiol) in or on alfalfa as follows: 40 ppm in or on alfalfa hay (of which not more than 20 ppm is carbamates); 10 ppm in or on fresh alfalfa (of which not more than 5 ppm is carbamates).

It has been shown of six insecticides applied before and after alfalfa weevil adults oviposited on alfalfa in the spring of 1969 in New York that carbofuran gave by far the best control in both experiments (Summers et al., 1971). The metabolism of carbofuran residues in alfalfa and bean plants and in the dairy cow has been discussed (Knaak et al., 1970a,b). The persistence of carbofuran and 3-hydroxycarbofuran on alfalfa has been discussed by Shaw et al. (1969). Fahey et al. (1970) showed that carbofuran residues on green alfalfa were not found 14 days after treatment at the rates of 0.5 and 1.0 lb/acre in 20 gal of spray in Indiana, and dehydration of the alfalfa in a drum-type commercial dehydrator reduced the carbofuran residues by 65%. The effect of drying harvested mature alfalfa plants by sunlight, ultraviolet light, and air in the dark on residues of DDT and related chlorinated hydrocarbon residues (Archer, 1969), toxaphene residues (Archer, 1971), and endosulfan residues (Archer, 1973) has been published.

The present investigations were undertaken to determine the effect on the residues of drying in the dark and under natural and artificial light conditions harvested alfalfa containing carbofuran and related compounds residues (Figure 1). The dark and artificial ultraviolet light experiments were conducted as relative experiments to the normal sunlight drying to determine the fates of the pesticide residues on nonliving alfalfa plants under con-

Department of Environmental Toxicology, University of California, Davis, California 95616.