RESIDUES OF GRANULAR AND SPRAY PARATHION AND BAYTEX IN ALFALFA HAY TREATED AT MOSQUITO LARVICIDAL RATES [1, 2]

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Numerous species of pest and vector mosquitoes breed in sources located in agricultural crops. Certain types of alfalfa fields in California, for example, provide ideal niches for mosquito breeding, and these sources have to be treated with mosquito larvicides through most of the breeding season.

The possible occurrence of toxic residues above legal tolerance levels in food and forage crops due to mosquito larvicidal treatments is causing concern among agencies responsible for conducting mosquito control operations in California. Although numerous studies have been conducted of residues of parathion in alfalfa hay and other crops at agricultural pest control dosages (Fahey *et al.* 1952, Ginsburg *et al.* 1949, 1950, Hamilton 1948, Hopkins *et al.* 1952, King & Hutson 1949, King *et al.* 1948, Stansbury and Dahm 1951, Walker 1950, Westlake & Fahey 1950, and others), no information on the residue levels of parathion and other insecticides as used in mosquito control programs has been gathered.

These studies present information on the residue levels of parathion and Baytex® (*O, O*-dimethyl *O*-[4-methylthio-*m*-tolyl] phosphorothioate) applied as sprays and granules to alfalfa hay at mosquito larvicidal dosages. Both of these materials show high biological activity against mosquito larvae (Mulla *et al.* 1960, 1961), and are currently considered as choice materials in mosquito larvicidal programs in California.

METHODS AND MATERIALS. Hemet-San Jacinto Valley.—In the Hemet-San Jacinto Valley a field close to the cutting stage, with no noticeable growth during the period of residue evaluation, was chosen. Nonreplicated 1/16 acre plots were sprayed with a hand sprayer (T-Jet nozzle No. 8002 and 20 lbs./sq. in. pressure) or treated with granules applied by hand. Spray was applied at the rate of 0.5 gallon per plot or at the rate of 8 gallons per acre. Two percent granular formulations of parathion and Baytex were prepared by impregnating 16/30 A-RVM attapulgite granules with solutions of the technical material in Chevron Light solvent or acetone; the solvent comprised 10 percent of the finished formulation. The treatments were made on November 10, 1960, applying both parathion and Baytex at 0.1 lb./acre. Temperature and humidity were measured during the first 3 days of the study. Maximum temperature was 76° F. and the minimum temperature was 42° F. The average mean temperature prevailed around 58° F., somewhat cooler than necessary for fast development of mosquito larvae. Three days after treatment 0.2 inch of rain fell. Relative humidity of 90 percent or more prevailed in the field throughout most of the study period.

Kern County—The alfalfa hay in Kern County was treated on May 9, 1961 one week before cutting. All materials were applied at the rate of 0.1 pound of toxicant per acre. The average height of the stand at time of treatment ranged from 14.0 to 16.5 inches; no measurement of stand height was made on the last day of sampling but it was noticed that 5 or 6 inches of growth was added during the one-week study period. The mean density of alfalfa

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at time of treament was 0.672 g. and at the end of the sampling period it was 0.682 g. The average water content was 83 percent.

Plots were 109 x 100 feet, and each treatment was replicated twice. Sampling areas of the various treatments were separated by 100 feet. The sprays were applied at the rate of 1 gallon per acre with a Rawdon airplane, and the parathion granules were applied with a Stearman plane at the rate of 5 pounds of 2 percent granules (20/30 Volclay KWK) per acre. The flight level of the plane ranged from 25–35 feet above ground level. Baytex granules (5 percent, 30/40 coated type) were applied with a cyclone seeder at the rate of 2 pounds per acre. Maximum temperature recorded during the entire period in Kern County was 86° F. and the minimum temperature was 45° F.; mean maximum temperature was 81° F. and the mean minimum temperature was 52° F.; mean relative humidity was 60 percent.

Alfalfa samples were taken both in the Hemet-San Jacinto Valley and Kern County areas within one-half to one hour (indicated as 0 days in the tables) and at intervals after treatment. Plots were sampled in crisscross manner with 20–30 bouquets of alfalfa per sample. Samples from each plot were composited and placed in polyethylene bags; they were either quick-frozen, then thawed and chopped or were chopped and then frozen until processed for analysis.

PREPARATION OF SAMPLES FOR CHOLINESTERASE ASSAY. One hundred grams of partially thawed, chopped alfalfa were extracted with 400 ml benzene in a Soxhlet extractor. The extracts were then concentrated to less than ten ml by removing the benzene on a steam cone through a Snyder column or by inserting a vacuum hose into the flask while maintaining the sample at a temperature below the boiling point of benzene. The concentrated samples were made up to 25 ml. (4 gm./ml) and were refrigerated or frozen until analyzed.

Oxidation.—Baytex was converted to its oxygen analogs and parathion to paraoxon by oxidation with peracetic acid. Five-ml aliquots of Baytex extract were oxidized with 6 ml of 1:5 peracetic acid (1 part 30 percent hydrogen peroxide and 5 parts glacial acetic acid) for 20 minutes at 75° C. Five ml of parathion extract were oxidized with 6 ml peracetic acid for 10 minutes at 60° C. The tubes were cooled, 5 ml of water were added to each, and the layers were allowed to separate in the cold room until the benzene layer cleared. In order to fall within the sensitivity range, oxidized samples of 0-day Baytex spray and parathion spray were diluted 1:9 with acetone. No dilution was necessary for the subsequent spray or granular treated alfalfa samples.

Aliquots were then removed and placed in reaction tubes calibrated to hold 50 ml; the solvent and actetic acid were evaporated with an air stream while being heated on a steam plate. The samples were dried until the odor of acetic acid could no longer be detected. Five ml of water were added to each tube, stoppered, and heated on the steam bath for 10 minutes. After cooling, 5 ml of human blood serum were added to each tube. Cholinesterase inhibition was measured by the technique described by Giang and Hall (1951). Inhibition was read between 20 and 68 percent. Samples giving less than 10 percent inhibition were considered to have no detectable residues. Minimum detectable residues for Baytex and parathion were 0.19 p.p.m. and 0.01 p.p.m. respectively, using 20-gram samples.

Standard Curves.—The Baytex standard curve (Fig. 1) was prepared from a sample of untreated alfalfa fortified at 10 p.p.m. and carried through the extraction and oxidation procedures previously described. Aliquots containing from 1 to 24 μgm of oxidized Baytex were incubated with human blood serum and the degree of inhibition was determined. The standard curve was obtained by plotting percent inhibition versus μgm Baytex/50 ml on 3-phase semi-log paper. The resulting standard curve showed 8 to 12 percent more toxicity than that obtained by using pure Baytex oxygen

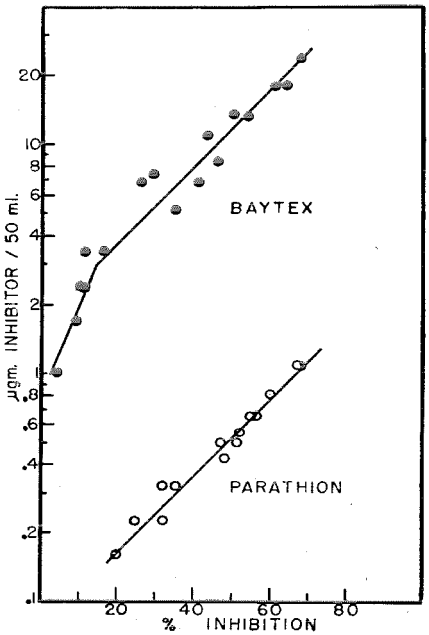

FIG. 1.—Standard cholinesterase inhibition curve for parathion and Baytex. To convert the µg inhibitor to p.p.m., the former values should be divided by 50.

analog sulfoxide, indicating that the oxidation proceeded mostly to this compound.

Alfalfa was fortified with parathion at 1 p.p.m. and processed through the same procedure. Aliquots contained from 0.16 to 1.1 µgm oxidized parathion. The degree of inhibition by these aliquots was found to be about 50 percent of that obtained by comparable amounts of pure paraoxon or parathion oxidized in the absence of plant extract. It appears that some parathion is lost in the extraction procedure, or that alfalfa interferes with the complete conversion of parathion to paraoxon. This discrepancy in residues of the samples, however, is corrected by using a standard curve obtained by the procedure just described.

PREPARATION OF SAMPLES FOR CHEMICAL ASSAY. The samples were chopped in a bread slicer to 1/2-inch lengths. Five hundred grams were then placed in a 2-quart wide-mouth jar and 1000 ml of benzene and 25 ml of 10 percent HCl were added. The jar was capped with a pliofilm liner, equilibrated for 40 minutes (Gunther and Blinn 1955) and then filtered through Sharkskin paper into a screw-cap bottle for storage in a 5–10° C. refrigerator.

Prior to analysis, the stripping solutions were decolorized by shaking a 400-ml portion with a 20-g mixture of 2 parts of Attaclay and 1 part of Hyflo-Supercel; the clear supernatant was then decanted. The solution was analyzed by the method of Averell and Norris (1948) as modified by Gunther and Blinn (1955).

PROCEDURE FOR BIOASSAY. The same extracts prepared for cholinesterase assay (ChE) and chemical analysis were tested against 4th instar larvae of *Culex p. quinquefasciatus* Say in tap water. In order to find the range of sensitivity, extracts of 0-day samples of spray treatments of Baytex and parathion were diluted 1:9 with benzene. Dilutions were not necessary for the remaining samples.

Aliquots of each sample in the amounts giving over 50 percent kill of the larvae were transferred to Whatman No. 1 filter paper. After the benzene had evaporated, the filter paper containing the residue was transferred to a 6-oz. paper cup containing 100 ml of tap water and 25 fourth instar mosquito larvae. The larvae were maintained at 78±2° F. and the mortality was recorded 24 hours after exposure.

The amount of toxicant present in the aliquot was determined from standard dosage-mortality lines of Baytex and parathion established against the larvae. No mortality was obtained in the control treatments. Dosage-mortality lines of paraoxon and Bayer 35681, the oxygen analog of parathion and the oxygen analog sulfoxide of Baytex, respectively, were also determined, and the general ranges of activity were similar to those of Baytex and parathion.

The sensitivity of the bioassay procedure for the detection of parathion and Baytex was 0.06 p.p.m. and 0.3 p.p.m., respectively. Each extract was evaluated on 2 or 3 separate days and replicated 2 or 3 times.

PENETRATION STUDIES. Six borosilicate glass circular trays were placed in the path of the flying aircraft or swath of each treatment. Nine hundred ml of water were placed in each tray and the trays were hidden with the plants to simulate natural conditions. One hour after treatment, water from the trays was transferred to quart jars and each tray rinsed with two 20-ml portions of acetone. The acetone portions were also transferred to the quart jars.

The water was bioassayed against 4th instar larvae of *Culex p. quinquefasciatus* within 2 hours after treatment in the manner described earlier (Mulla *et al.* 1960, 1961).

The amount of toxicant in the water was calculated from larval mortality, using dosage standard lines of parathion and Baytex.

RESULTS AND DISCUSSION. Hemet-San Jacinto Valley (Hand Applications).—Initial residues incurred on alfalfa hay due to the application of sprays of parathion and Baytex at the rate of 0.1 pound toxicant per acre are presented in Table 1. Residues of parathion, as well as Baytex, dissipated rather rapidly. This trend of high initial residues and subsequent rapid degradation has been established for parathion and malathion on several crops (Dogger & Bowery 1958, Fahey *et al.* 1952, Ginsburg *et al.* 1949, 1950, Hamilton 1948, Hopkins *et al.* 1952, King & Hutson 1949, King *et al.* 1948, Walker 1950, and Westlake & Fahey 1950). The higher residue levels of parathion, 4 days and 8 days after treatment, determined by the ChE assay method may be due to the presence of certain metabolites which show greater activity *in vitro* as cholinesterase inhibitors as compared with the activity in the mosquito bioassay procedure. A similar high *in vitro* activity, but lower reactability to chemical assay and bioassay of parathion after exposure to ultraviolet light, was reported by Frawley *et al.* (1958).

TABLE 1.—Residues of parathion and Baytex in alfalfa hay treated manually with spray and granular formulations of the toxicants in the Hemet-San Jacinto Valley.

Days after treatment	Residues (fresh weight basis) in p.p.m. (spray treatments) [a]			
	Parathion [c]		Baytex [c]	
	ChE	Bioassay	ChE	Bioassay
0	>20.0	26.0	18.4	21.9
2 [b]	5.2	4.0	1.3	1.9
4 [b]	4.3	3.8	1.7	1.7
8	4.3	1.0	0.7	0.8

[a] No residues were detected in untreated alfalfa or in the granular treated hay. All materials applied at 0.1 lb./acre.

[b] 0.2 inch of rain fell between the 2- and 4-day intervals.

[c] Mean recoveries of Baytex added at 0.2 p.p.m. and 0.4 p.p.m. to the alfalfa were 80% and 87%, respectively. Mean recovery of parathion added at 1 p.p.m. to the alfalfa was 91%.

The granular formulations of both parathion and Baytex resulted in no residues that could be detected by the ChE inhibition method or bioassay. Therefore, any residues, if present, were below 0.01 p.p.m. and 0.19 p.p.m. for parathion and Baytex, respectively.

Kern County (Aircraft and Hand Application).—The level of initial residues in the plots sprayed by aircraft was not as high as that incurred in the Hemet-San Jacinto Valley where the sprays were applied by hand sprayers. As was the case in this area, the residues of parathion and Baytex sprays in Kern County dissipated rapidly (Table 2). Here, again, as in previous studies, the residues of parathion determined by the ChE assay were greater than those determined by bioassay. This, too, could be explained in terms of oxidation products of parathion which have greater ChE inhibition properties than parathion but not such high activity against insects. The oxidation products may also degrade rapidly in water, a

TABLE 2.—Residues (in p.p.m. on fresh weight basis) of parathion and Baytex on alfalfa hay treated in Kern County, California.

Days after treatment	ChE	Bioassay of ChE extract	Chemical [c]	Bioassay of chemical extract
		Parathion Spray [d]		
0	4.5	3.1	3.3 [a]	3.4
1	1.7	1.0	1.9	1.5
2	1.7	0.7	1.1	0.9
4	0.6	0.2	N.D.*	0.5
7	0.3	0.1	N.D.	0.3
		Parathion Granules (2%)		
0	0.02	N.D.*	N.D.*	N.D.*
1	0.05	N.D.	N.D.	N.D.
2	0.02	N.D.	N.D.	N.D.
4	0.02	N.D.	N.D.	N.D.
7	0.03	N.D.	N.D.	N.D.
		Baytex Spray [d]		
0	8.0	8.4		
1	2.3	2.5		
2	1.3	1.2		
4	1.2	0.5		
7	1.0	0.3		
		Baytex Granules (5%) [b]		
0	0.2	N.D.*		
1	0.2	N.D.		
2	0.4	0.34		
4	0.3	N.D.		
7	0.3	N.D.		
Control	N.D.	N.D.	N.D.	N.D.

* None detectable.
[a] Sensitivity 0.2 p.p.m.
[b] Applied with Cyclone seed spreader. All other treatments were made by aircraft.
[c] Mean recovery 98%.
[d] For recoveries see footnote in Table 1.

factor which is not so important in ChE assay. In the bioassay procedure, the larvae are exposed for 24 hours, while the ChE assay is completed in about an hour.

The parathion samples were also assayed by the Averell-Norris (1948) method. There is reasonable agreement in the residue determinations by the three methods, as shown in Table 2.

Residues incurred from treatments of granular formulations of parathion and Baytex were very low compared with residues from sprays. Slightly higher levels of residues of Baytex granules are probably due to poor penetration of the plant canopy by the granular particles applied with a hand applicator. Granules applied by aircraft are forced to drift through the plant canopy; this fact is borne out by the results of penetration studies explained later. Penetration of granular particles through plant canopy will also depend on the size and quality of a formulation. Granular formulations, having light particles and fine dust, are liable to lodge on plant foliage. The Baytex granules used in Kern County were of the 30/40 mesh size and, therefore, were lighter than the granules used in the Hemet-San Jacinto Valley which had 16/30 mesh particles.

In both studies it is evident that granular materials leave no residues, or negligible amounts on crops, compared

with spray treatments. Much lower residues of dieldrin were detected in forage treated with dieldrin granules compared with residues in spray-treated forage (Smith *et al.* 1961).

Deposit and Penetration Studies. Many workers have realized that granular particles penetrate plant canopy more readily than spray droplets. During the Kern County residue studies, it was decided to ascertain the degree of penetration of granules and sprays through the plant cover to water underneath, and to correlate this with the magnitude of residues determined in the crop.

Results of the penetration of sprays and granules are presented in Table 3. Parathion and Baytex sprays and parathion granules were applied by aircraft, while the Baytex granules were applied by a hand cyclone seeder. In both parathion and Baytex spray applications, an average of less than 20 percent of the active ingredients reached the water under the plant canopy. In the parathion granular treatment, 89 percent of the active ingredient reached the water.

TABLE 3.—Penetration of parathion and Baytex spray and granules through alfalfa canopy, as measured by residues of the toxicants found in water placed under the cover.[a]

Toxicant and formation	Avg. p.p.m. residue in water	Avg. % recovery
Parathion spray	0.2	22
Parathion granules	0.8	89
Baytex spray	0.1	10
Baytex granules	0.3	32

[a] Depth of water in the tray was 0.5 inch; surface of each tray was 114.75 sq. inches and the theoretical total residue on the basis of 0.1 pound per acre was calculated to be 0.92 p.p.m. in the water.

The degree of penetration in the Baytex granule-treated plot, although higher than the sprays, was not as high as for the parathion granules. This low degree of penetration can be partly explained by the lodging of particles in crowns of the plants in hand-treated crops. Slow release of the toxicant from the granules into the water is another possible factor in producing results depicting low penetration of the granular particles.

Notwithstanding the apparent low degree of penetration of Baytex granules, it is evident that even here the residues on hay due to the application of granular Baytex were very low compared with those from the spray treatment. Lodged granular particles, if present, eventually drop to the ground due to wind action, or during mowing or cutting of the plants, thus rendering the crop almost residue-free. The presence of heavy dew on the foliage may result in some release of the toxicant from the granular particles, thus yielding toxic residues in the crop. However, from the nature of the granular formulations it appears that residues incurred due to granular treatments under heavy dew conditions would still be far below the residues incurred in spray treatments.

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AIDS TO OVARIAN DISSECTION FOR AGE DETERMINATION IN MOSQUITOES

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INTRODUCTION. Polovodova (1949) and Detinova (1946 and 1962) demonstrated in *Anopheles maculipennis* that age could be determined accurately by post-ovipositional changes in the structure of the membranous tube which joins each ovariole to the inner oviduct or calyx of a mosquito ovary.

Where an ovariole has developed successfully or degenerated, local stretching of the *tunica intima* (Bertram, 1962) around the developing ovariole, followed by partial contraction after ovulation leaves a distinct nodular swelling or dilatation to mark the spot in each follicular tube. Since each dilatation marks one cycle of ovarian development, the greatest number of such dilatations within any one ovariole of an ovary, multiplied by the time taken to complete a gonotrophic cycle equals the minimum age of the mosquito.

In the course of ecological studies on *Anopheles gambiae melas* in the Gambia, West Africa, during 1959 and 1962, over 75,000 mosquitoes were dissected for age determination. This paper describes certain technical difficulties in ovariolar dissection which were met and their solution.

METHOD OF DISSECTION. The ovaries are teased out from the abdomen and freed

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