Persistence of Bidrin Residues on and in Mature Oranges and in Laboratory-Processed Citrus Pulp Cattle Feed

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The insecticide Bidrin (3-hydroxy-*N*,*N*-dimethylcrotonamide dimethyl phosphate) has been evaluated as a residue on and in mature Valencia oranges. The persistence data indicate the RL_{50} (half-life) value for this insecticide is 13 to 16 days on the peel of oranges under the conditions described. There was no detectable translocation of parent Bidrin into the juice and pulp of the orange fruit during the 100-day study. Although unchanged Bidrin was found in laboratory-processed navel orange "pulp" cattle feed made from samples taken 15 and 30 days after treatment, none was detected in the corresponding 100-day Valencia orange samples. Losses of Bidrin from peel to finished cattle feed averaged 70%. The minimum detectable level was about 0.1 p.p.m., which corresponds to 12 μg. in the measuring cell.

THE compound 3-(dimethoxyphosphinyloxy) - *N*,*N* - dimethyl - *cis*-crotonamide (Bidrin) is a promising new insecticide which has been shown to be effective against certain insect pests attacking citrus and other agricultural crops. Its metabolism has been reported (*1*, *4*). There is no published report of a complete chemical residue persistence study on and in any field-treated agricultural crops, although 2- and 7-day residues in oranges have been reported (*5*). Such information must be submitted with a petition for residue tolerances if a compound is to be registered by either federal or state governmental agencies. Therefore, a 100-day study of the magnitude and persistance of unchanged Bidrin residues on and in mature Valencia oranges treated in the field to simulate probable commercial practice was undertaken. An integral part of the study was the analysis of samples of cattle feed made from oranges of both navel and Valencia trees treated in the field with this insecticide.

Experimental

Mature Valencia orange trees (90 per acre) were sprayed August 8, 1964 with water solutions of technical grade Bidrin (7.5 pounds per gallon) at the rates of 0.5, 1.0, and 1.5 pints per 100 gallons of water. Applications were made as conventional sprays, with a high-pressure reciprocating pump and manually operated spray guns, at the rate of approximately 2500 gallons per acre.

Each plot consisted of four trees and each treatment was applied to three plots. Three untreated plots upwind were used as controls throughout the experiment. Eight fruits (two from each quadrant) were picked from each tree of the four-tree plots and the resulting triplicated 32-fruit sample units were processed separately. All plots were sampled prior to treatment, and 3, 6, 13, 20, 27, and 34 days after treatment. A composite sample was taken from all the treated plots 100 days after application for conversion to cattle feed.

No Bidrin residue was detectable in either the unwashed peel (80% moisture) or the cattle feed (7% moisture) prepared from it in these 100-day samples. Consequently, this experiment did not show the possible concentrating effect of the processing procedures on detectable residues, such as those found to occur at least 34 days after Bidrin application in the field. To establish this effect an additional plot of navel orange trees was sprayed January 20, 1965, as described above at the rate of 1.5 pints per 100 gallons of water. Fruit samples 15 and 30 days following treatment were processed into cattle feed.

Treatment of Samples. Unwashed fruits were peeled and processed with benzene as described (*2*, *5*) to obtain stripping solutions of both peel (2 ml. per gram) and pulp (1 ml. per gram) (edible) portions of the fruit. To permit evaluation of possible storage deterioration control, stripping solutions were fortified at 5 p.p.m. with Bidrin and all samples were stored at 5° C. awaiting analysis.

Portions of the samples for conversion to cattle feed were processed as previously described (*3*). Subsamples were equilibrated with benzene at the following stages of processing: (1) unwashed chopped peel, (2) ground peel and pulp of oranges which had previously been washed with a 1% solution of Triton X-100, juiced, and chopped, and (3) finished cattle feed. Control samples were fortified with 5 p.p.m. of Bidrin after equilibration and stored with the other samples at 5° C. until analyzed.

The analytical method described by Murphy, Gaston, and Gunther (*5*) was used without modification. Aliquots of stripping solutions representing 25 to 200 grams of sample were analyzed. The cleanup procedure involved an acid reflux and distillation of impurities, followed by alkaline hydrolysis and steam distillation of the resulting dimethylamine. The amine was determined colorimetrically on a spectrophotometer as cupric dimethyl dithiocarbamate formed by the addition to this amine of carbon disulfide and alkaline cupric ion.

Calculations to parts per million were by standard methods and included correction for the laboratory per cent recovery determined with each group of samples. No correction was made for the apparent residue found in the control samples, because demonstrations of consistency of control values are highly significant.

Results and Discussion

Residue values for Bidrin on and in the peel and pulp of Valencia oranges treated with three dosage levels are collated in Table I. The results of duplicate analyses of orange peel stripping solutions are included to show the actual variation encountered using the described method. Pretreatment samples showed a maximum "apparent" or background residue of 0.1 p.p.m. (an apparent 12 μg. in the measuring cell) in both peel and pulp. The apparent residue on and in the peel of the control samples increased by 0.1 p.p.m. (possibly because of minor spray drift, since citrus trees are sprayed only during windless periods) before returning to the pretreatment level in 20 days. The 3-day results of the treated samples show that normally uniform spray application and a correct deposit ratio were accomplished by the field application.

The degradation RL_{50} (half life) of Bidrin on the peel was calculated as described (*2*) to be 3 to 5 days (3, 5, and 5

Table I. Persistence of Apparent Bidrin Residues (P.P.M.) on and in Peel[a] and Pulp[b] of Field-Sprayed Valencia Oranges[c]

	½ Pint/100 Gal.		1 Pint/100 Gal.		1½ Pints/100 Gal.		Control	
Elapsed Days	Peel[d]	Pulp[e]	Peel[d]	Pulp[e]	Peel[d]	Pulp[e]	Peel[d]	Pulp[e]
Pretreatment	0.1	0.1	0.1	0.1	0.1	<0.1[f]	<0.1[f]	0.1
	0.1	<0.1[f]	0.1	<0.1[f]	<0.1[f]	<0.1	0.1	<0.1[f]
	0.1	0.1	0.1	0.1	<0.1	0.1	0.1	0.1
3	1.9, 2.1	0.1	3.3, 3.8	0.1	5.5, 5.9	0.1	0.2	<0.1
	2.2, 2.2	<0.1	4.4, 4.6	<0.1	6.3, 7.2	0.1	0.1	0.1
	2.0, 2.2	<0.1	4.4, 4.2	<0.1	6.0, 5.6	0.1	0.2	0.1
6	1.4, 1.2	0.1	1.8, 2.0	0.1	3.5, 3.4	0.1	0.1	0.1
	1.1, 1.1	<0.1	1.9, 2.0	<0.1	3.4, 3.3	<0.1	0.2	0.1
	1.0, 1.2	<0.1	2.2, 2.2	0.1	2.5, 2.7	<0.1	0.2	<0.1
13	0.8, 0.7	<0.1	1.6, 1.6	0.1	2.0, 2.2	0.1	0.1	0.1
	1.7, 1.0	0.1	1.6, 1.7	0.1	2.0, 1.9	0.1	0.1	0.1
	0.9, 1.0	0.1	1.2, 1.4	0.1	2.1, 2.1	0.1	0.1	0.1
20	0.5, 0.5	0.1	1.1, 1.1	0.1	1.5, 1.5	0.1	0.2	0.1
	0.7, 0.6	0.1	1.0, 1.1	0.1	1.2, 1.2	0.1	0.1	0.1
	0.7, 0.7	0.1	1.2, 1.2	0.1	1.3, 1.4	0.1	0.1	0.1
27	0.5, 0.5	0.1	0.9, 0.9	0.1	1.3, 1.5	0.2	0.2	0.1
	0.4, 0.5	0.1	0.8, 0.8	<0.1	1.2, 1.2	0.1	0.1	<0.1
	0.5, 0.5	0.1	0.8, 0.7	<0.1	1.4, 1.2	0.1	0.1	0.1
34	0.3, 0.4	<0.1	0.7, 0.7	0.3	0.7, 0.7	0.1	0.1	0.1
	0.4, 0.4	0.1	0.6, 0.4	0.1	0.7, 0.6	<0.1	<0.1	0.1
	0.4, 0.4	0.1	0.4, 0.4	0.1	0.6, 0.6	0.1	0.1	0.1
100 (composite[g])	Peel av. = 0.2	Pulp av. = 0.1					0.2	0.1

[a] Based on weight of peel only, mature Valencia oranges have 18.7 ± 6.3 wt. % peel from 297 measurements. Results of duplicate determinations of treated samples presented to illustrate reproducibility of analytical method.
[b] Based on weight of pulp (edible portion) only.
[c] Three plots each consisting of 4 trees treated with indicated dosages Aug. 7, 1964.
[d] All values corrected for recovery (average 97 ± 14% in range 0.2 to 5.0 p.p.m.) from 21 fortified controls to establish laboratory recovery values.
[e] All values corrected for recovery (average 87 ± 13% in range 0.2 to 0.5 p.p.m.) from 10 fortified controls to establish laboratory recovery values.
[f] With absorbance readings normally reliable to about 0.020 unit, and a slope of 28 μg./0.100 unit (*5*), present laboratory subsample size allows detection of about 6 μg. of Bidrin, or about 0.04 p.p.m. In this table values above 0.05 p.p.m. round off to 0.1 p.p.m.; those below 0.05 p.p.m. designate as less than 0.1 p.p.m.
[g] Duplicate samples removed from composite fruit sample picked from plots of 3 treatment rates. Peel value represents average of 4 analyses (0.1 to 0.2 p.p.m.) and pulp value represents identical result from 2 analyses.

days for the three dosages) for about the first 10 days, confirming the earlier preliminary study (*5*), whereas the persistence RL_{50} of Bidrin in the peel was similarly found to be 13 to 16 days (13, 15, and 16 days for the three dosages) over the next 90-day period. The results for the 100-day samples show that no detectable Bidrin remained in a sample composited from all of the treated plots. There was no significant increase in the apparent (background) Bidrin content in the pulp of oranges at any of the treatment levels or at any harvest date, which indicates that Bidrin was not translocated in detectable amounts through the peel into the edible pulp portion of the fruit, unless rapidly metabolized. Loss of a methyl group from the nitrogen in the Bidrin molecule results (*1*, *4*) in a metabolite, des-*N*-methyl Bidrin, which is not detected by the analytical method used in this investigation; the *N*-hydroxymethyl-*N*-methyl metabolite (*1*, *4*) presumably would not respond to this method, either. The fact that the present method does not respond to the cholinesterase-inhibiting metabolites of Bidrin could be a serious deficiency in some applications; in these instances separate cholinesterase assays should be run on aliquots of key

Table II. Bidrin Residues[a] during Various Stages of Production of Laboratory-Processed Navel Orange "Pulp" Cattle Feed

	Bidrin Residues, P.P.M.			
Elapsed Days	Unwashed peel[b]	Ground peel[c]	Finished cattle feed[d]	% Moisture in Cattle Feed
Pretreat	<0.1	...	...	...
	<0.1	...	...	
	<0.1	...	...	
15	9.2	4.7	3.5 \	10.0
	8.0	4.7	3.2 } 80%	
	8.5	4.3	3.6 / loss	
Control	0.1	0.3	1.0	
30	4.1	2.7	4.1 \	11.5
	3.2	3.0	4.6 } 60%	
	4.6	2.9	3.9 / loss	
Control	0.2	0.2	0.9	
100[e] (Valencia oranges)	0.1	<0.1	0.1	7.0
	0.1	<0.1	<0.1	
	0.1	<0.1	0.2	
Control	<0.1	<0.1	...	

[a] P.p.m. values are average of duplicate laboratory analyses, corrected for percentage laboratory recovery but not for "apparent" residue in controls.
[b] Unwashed orange peels chopped before solvent equilibration. Fortification of 10 samples in range 0.5 to 10.0 p.p.m. gave average recovery of 87.7 ± 10%.
[c] Oranges washed, juiced, and chopped before solvent equilibration. Fortification of 8 samples in range 0.5 to 10.0 p.p.m. gave average recovery of 95.4 ± 6%. Moisture, 75–80%.
[d] Ground peel (footnote [c]) treated with lime, pressed, and dried before solvent equilibration. Fortification of 6 samples in range 1.0 to 10.0 p.p.m. gave average recovery of 104 ± 8%.
[e] Triplicate samples removed from composite Valencia orange sample picked from plots of all 3 treatment rates applied August 7, 1964. Included to show whether residues below limit of detectability might be concentrated during processing involved.

sample extractives with compensation for the partitioning ratios of these metabolites in benzene-water systems.

The results obtained from the various stages of cattle feed processing are collated in Table II. Comparison of the residues in unwashed peel with those in the ground peel shows the loss of Bidrin from the detergent wash of the 15- and 30-day amples to be 42 and 28%, respectively.

The ground peel contained 75 to 80% water as compared to 7.0 to 11.5% in finished cow feed. If no Bidrin were lost after the ground peel stage, the residues in the cow feed from the 15- and 30-day samples would average 16.8 and 10.7 p.p.m., respectively. However, the 15-day sample lost 80%, while the 30-day sample lost only 60% of the Bidrin present in the ground peel stage. Possible explanation of this apparent discrepancy is the higher final moisture content of the 30-day samples; the laboratory processing procedure is analytically reproducible. Thus, there may be a critical moisture content above which very little Bidrin is lost but below which Bidrin is lost very rapidly. However, it is clear that laboratory processing of orange fruits into citrus "pulp" cattle feed does eliminate a significant amount of any Bidrin residue which may be present initially.

There was no detectable (~0.1 p.p.m.) storage (5° C.) deterioration of fortified control peel, juice, and citrus pulp cattle feed over the 4-month duration of this program.

The winter application on mature navel oranges (Table II) afforded significantly greater peel residues than the same dosage in a summer application on mature Valencia oranges.

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HERBICIDE SAMPLING APPARATUS

Collection Technique for Aerosol and Gaseous Herbicides

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An air-sampling system for the differential collection of aerosol and gaseous fractions of airborne herbicides consists of a rotating disk impactor for collecting aerosol droplets down to approximately 3 microns in diameter, followed by a midget impinger to collect the gaseous fraction. The impactor was specially designed and constructed of glass, Teflon, and stainless steel to prevent contamination of the collection fluid with substances that interfere with electron capture gas chromatography. Incoming air impinges on the impaction disk, which slowly rotates through a fluid well containing *n*-decane. The impacted droplets wash off into the collection fluid. The disk then passes through a Teflon squeegee to remove the adhering droplets presenting a smooth surface containing a fluid film upon which the air stream impinges. The collection efficiency of the system for gaseous and aerosol forms of 2,4-D esters has been studied under laboratory conditions. The system was used at two field sampling sites for approximately 3 months.

IN THE EVALUATION of atmospheric drift of herbicides a method was required for continuous differential collection of aerosol and gaseous fractions. Since a modified midget impinger was used in our earlier studies of total atmospheric herbicide (*1*), a suitable system was needed to collect the aerosol fraction and pass the gaseous fraction to the midget impinger.

The requirements of this aerosol collection device included: continuous sampling for 24-hour periods without presence of an operator, a relatively low air sample flow rate of 1 liter per minute for compatibility with a midget impinger, a minimum collection efficiency of 90% for aerosols in the range of 1 to 50 microns, at least 90% of the smaller droplets and gases not collected, aerosol sample in a form amenable to electron capture gas chromatographic analysis, the sample collected in 5 to 20 ml. of absorption liquid, and the complete unit portable and operable from a 12-volt d.c. source.

Aerosols can be collected by a number of methods including filtration, centrifugation, electrostatic precipitation, and impaction. Any of these techniques could be used to collect aerosols containing compounds of 2,4-D, simultaneously separating them from concomitantly occurring gases. However, the direct application of these techniques to the collection of a 24-hour sample of aerosols containing the more volatile esters of 2,4-D would suffer from continuing loss of the collected esters to the sampled air stream during the remainder of the sampling period. A survey of existing aerosol collection devices (*2, 3*) indicated that no device meeting the above requirements was available; therefore, a suitable sampler was designed.

Schadt *et al.* (*6*) described a rotary, electrostatic precipitator in which sulfuric acid aerosol was deposited on a rotating, stainless steel disk which dipped into a flowing stream of water. This concept was modified to provide a rotary impactor constructed of glass and Teflon