Removal of Parathion and Related Products from Lettuce Trim

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Parathion applications have been recommended for the control of leaf-feeding aphids and leafminers on lettuce at the rate of one-half pound of actual material per acre not less than seven days before harvest (CALIF. AGR. EXPT. STATION BULL. 1973). Several workers have studied the residues of parathion on lettuce under various conditions (STORHERR et al. 1964, COFFIN 1966 and POLIZU et al. 1963). STOBWASSER 1961 reported that when lettuce containing residues of parathion of 0.035 and 0.105 percent was washed using conditions similar to those customary in households the residues were reduced by 40%. Very little other work, if any, has been reported in the literature on the removal of parathion residues from lettuce.

The purpose of the present investigation was to study decontamination procedures for parathion and related products on lettuce trim leaves which could be potential animal feed. The trim leaves (approximately 51.6% of the field lettuce head weight) were separated from the edible head, chopped, mixed and subjected to the various pesticide removal procedures.(Tables I through VI).

Materials and Methods

Lettuce Plots. In June 1974 in Yolo County, California, rows of Climax lettuce 40 in. wide and 48 ft. in length were selected for duplicate spray applications of Thiophos® Parathion 4 E.C. at the rates of 1/2 (Plot B) and 1 lb. (Plot C) active ingredient per acre. Another plot of similar dimensions upwind from the others was established as a control plot (Plot A). The lettuce selected was at the stage of maturity 7 days before harvest since the recommended spray application is 1/2 lb. active ingredient/acre 7 days before harvest. The first sampling of the control plot was taken before spraying the other plots. At the harvest sampling all the lettuce from each plot was completely harvested and random subsamples were immediately extracted for analysis. Samples were taken daily from time of pesticide application to time of harvest. The sprayed plots were duplicated and the analytical data represent average values for the duplicate plots. The percent moisture content of the field lettuce head was 92.5 $\pm$ 1.0, the trimmed lettuce head was 94.8 $\pm$ 0.4 and the lettuce trim was 91.2 $\pm$ 0.1.

Spray Application. The lettuce was sprayed with Thiophos® 4 lb. E.C. using a Hudson Climax® 6335 Simplex Sprayer, 8.5-

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liter capacity equipped with a Hudson 149-403 spray control valve and a nozzle extension. A 0.95 cm inner diameter flexible neoprene, teflon-lined rubber tubing for chemical inertness was attached between the pressure tank and the Roto-Spray Nozzle®.

Parathion Applied. The parathion applied was in the formulation of American Cyanamide Co. designated as Thiophos® Parathion 4 E.C. (1 gal. contains 4 lbs. parathion).

Active ingredients:

Parathion (0,0-diethyl 0-p-nitrophenyl phosphorothioate) 46.7%

Xylene-range aromatic hydrocarbon solvent 47.6%

Inert ingredients 5.7%

Plot B (1/2 lb. active parathion/acre) was sprayed as evenly as possible with 1 gal. of water containing 1.666 g of parathion using the equipment described above and Plot C (1 lb. active parathion/acre) was evenly sprayed with 1 gal. of water containing 3.334 g of parathion.

TABLE I

Parathion Residues on Lettuce Trim after Exposure to Removal Procedures

Treatment	Plant Material: Initial Sample 1/2 Lb./Acre*			Harvest Sample 1/2 Lb./Acre*		
	24°C	55°C	75°C	24°C	55°C	75°C
H_2O	9.20	7.00	4.52	0.707	0.626	0.388
0.1N NaOH	8.71	5.94	4.86	0.656	0.348	0.089
0.1N HCl	7.38	6.86	4.84	0.550	0.313	0.251
0.1N NaHCO	7.62	6.48	3.24	0.581	0.400	0.314
4% Acetic acid	7.12	5.78	5.40	0.538	0.644	0.358
95% C_2H_5OH	4.06	3.27	1.82	0.288	0.194	0.093
1N NaOH in 95% C_2H_5OH	0.538	0.067	0.083	0.030	0.031	0.032
1N HCl in 95% C_2H_5OH	4.06	1.41	1.07	0.365	0.190	0.083
	Cooking Broth					
H_2O	0.020	0.016	1.86	0.002	0.001	0.142
0.1N NaOH	0.007	0.015	0.082	0.001	0.001	0.006
0.1N HCl	1.04	1.78	2.65	0.072	0.083	0.025
0.1N $NaHCO_3$	0.042	0.034	1.57	0.001	0.001	0.183
4% Acetic acid	1.36	3.73	3.24	0.072	0.142	0.253
95% C_2H_5OH	7.50	7.19	6.23	0.398	0.449	0.467
1N NaOH in 95% C_2H_5OH	2.17	0.116	0.010	0.034	0.007	0.001
1N HCl in 95% C_2H_5OH	8.94	10.4	6.73	0.674	0.571	0.593

*Initial residues 11.3 ppm; harvest residues 1.01 ppm.

The formulation was found to contain by analysis and calcu-

lated as the parathion active ingredient equivalent 95.4% parathion, 3.88% aminoparathion, 0.12% paraoxon, 0.11% 0,S-diethyl-0-p-nitrophenyl phosphorothiolate, 0.05% 0,0-diethyl-S-p-nitrophenyl phosphorothiolate, 0.43% p-nitrophenol and 0.01% 0,0-diethyl phosphorothioate.

Sample Extraction. Twenty-five grams of lettuce were extracted by refluxing in 250 ml of a solvent mixture containing 5% isopropanol-95% benzene (V/V) for thirty minutes. The refluxed sample was cooled in an ice bath and the solvent was filtered through Whatman #1 filter paper. The extraction was repeated by refluxing the lettuce two times further after the addition of 2 ml of 0.1 N HCl before each reflux and the filtered solvent was pooled and stored for cleanup and analysis. The extracted plant material was discarded. All solvents used in the experiment were reagent grade freshly distilled prior to use.

TABLE II

Parathion Residues on Lettuce Trim After Exposure to Removal Procedures

Treatment	Plant Material		
	Initial Sprayed Sample 1 Lb./Acre*		
	24°C	55°C	75°C
H_2O	15.6	14.1	10.6
0.1N NaOH	17.6	4.86	2.94
0.1N HCl	15.2	11.9	8.72
0.1N $NaHCO_3$	16.2	14.2	3.50
4% Acetic acid	11.9	5.40	6.19
95% C_2H_5OH	8.36	2.50	2.42
1N NaOH in 95% C_2H_5OH	0.478	0.144	0.012
1N HCl in 95% C_2H_5OH	6.22	1.86	1.39
	Cooking Broth		
H_2O	0.022	0.004	5.73
0.1N NaOH	0.006	0.014	0.018
0.1N HCl	1.98	4.37	3.93
0.1N $NaHCO_3$	0.005	0.033	2.95
4% Acetic acid	2.14	3.58	4.20
95% C_2H_5OH	10.6	7.40	8.21
1N NaOH in 95% C_2H_5OH	0.350	0.129	0.041
1N HCl in 95% C_2H_5OH	18.1	23.8	11.2

*Initial residues 25.8 ppm; harvest residues 2.20 ppm.

Diazomethane Preparation and Methylation of the Sample Extract. The laboratory preparation of diazomethane is detailed on the label of the Diazald® reagent bottle obtained from the

Aldrich Chemical Co., Inc., 940 W. St. Paul Ave., Milwaukee, Wisc. 53233.

The extractives from 10 g of lettuce (300 ml solvent extract) were concentrated *in vacuo* at 50-60°C to approximately 1 ml and the volume was quantitatively adjusted to 5 ml with n-hexane. The extractives equivalent to 5 g of lettuce were added to 5 ml. of ethereal diazomethane solution at room temp. and allowed to stand for at least 15 min. prior to sample cleanup.

Sample Cleanup. A glass column 2.54 x 25.00 cm with a solvent resevoir at the top was packed with a plug of glass wool, 10 g anhydrous Na_2SO_4, 20 g of Florisil (activated at 270°C for 3 hrs.) and 10 g anhydrous Na_2SO_4. N-pentane (100 ml) was allowed to flow to the column top by gravity flow, and the solvent was discarded after the column was washed. The methylated sample was added to the column in 25 ml of pentane followed by two 25 ml aliquots of pentane washes. The sample was eluted from the

TABLE III

Aminoparathion Residues on Lettuce Trim after Exposure to Removal Procedures

Treatment	Plant Material					
	Initial Sample 1/2 Lb./Acre*			Harvest Sample 1/2 Lb./Acre*		
	24°C	55°C	75°C	24°C	55°C	75°C
H_2O	0.435	0.717	0.222	0.043	0.047	0.018
0.1N NaOH	0.549	0.966	0.731	0.025	0.056	0.003
0.1N HCl	0.726	0.386	0.109	0.035	0.015	0.002
0.1N $NaHCO_3$	0.809	0.764	0.142	0.050	0.065	0.021
4% Acetic acid	0.300	0.351	0.201	0.012	0.050	0.006
95% C_2H_5OH	0.226	0.317	0.067	0.033	0.046	0.017
1N NaOH in 95% C_2H_5OH	0.557	0.510	0.534	0.053	0.071	0.012
1N HCl in 95% C_2H_5OH	0.032	0.070	0.069	0.025	0.065	<0.001
	Cooking Broth					
H_2O	0.448	1.26	0.764	0.053	0.036	0.010
0.1N NaOH	0.085	0.186	0.034	0.001	0.003	0.089
0.1N HCl	0.046	0.071	0.186	0.088	0.097	0.025
0.1N $NaHCO_3$	0.258	0.491	0.039	0.041	0.024	0.079
4% Acetic acid	0.074	0.128	0.087	0.008	0.023	0.028
95% C_2H_5OH	0.407	0.887	0.670	0.100	0.096	0.111
1N NaOH in 95% C_2H_5OH	0.212	0.217	0.220	0.019	0.032	0.050
1N HCl in 95% C_2H_5OH	0.159	0.222	0.257	0.047	0.075	0.056

*Initial Residues 1.13 ppm; harvest residues 0.084 ppm.

column with 390 ml of 30% diethyl ether-70% pentane (V/V) after changing the column receiver between solvents. The eluates were collected and analyzed by gas-liquid chromatography (GLC) after concentration in vacuo at 50-60°C to approx. 1 ml and transferred and adjusted to appropriate volumes in 6.5 ml MacKay-Shevky-Stafford sedimentation tubes. Parathion, aminoparathion, S-phenyl parathion, methylated p-nitrophenol and methylated O, O-diethyl phosphorothioate were eluted from the column with the ether-pentane solvent. A separate lettuce extract aliquot was cleaned-up on the Florisil column which was eluted only with the 25% methanol-75% benzene solvent (V/V) for the detection of the S-ethyl parathion. All recoveries of samples cleaned-up were quantative ranging from 70 to 90%. Samples were analyzed both with and without clean-up. The cleanup procedure facilitated analysis by GLC using the electron capture detector. An additional advantage of clean-up was the separation of the compounds into groups with the separate eluting solvents as described above.

TABLE IV

Aminoparathion Residues on Lettuce after Exposure to Removal Procedures

Treatment	Plant Material Initial Sprayed Sample 1 Lb./Acre*		
	24°C	55°C	75°C
H_2O	1.85	1.41	0.908
0.1N NaOH	1.75	1.11	0.976
0.1N HCl	1.48	1.38	1.67
0.1N $NaHCO_3$	1.92	1.38	1.14
4% Acetic acid	1.75	0.944	0.833
95% C_2H_5OH	0.727	0.278	0.312
1N NaOH in 95% C_2H_5OH	0.818	0.800	0.521
1N HCl in 95% C_2H_5OH	0.643	0.071	0.021
	Cooking Broth		
H_2O	0.148	0.165	0.182
0.1N NaOH	0.020	0.011	0.016
0.1N HCl	0.288	0.250	0.370
0.1N $NaHCO_3$	0.220	0.983	0.260
4% Acetic acid	0.250	0.222	0.058
95% C_2H_5OH	1.36	0.800	0.885
1N NaOH in 95% C_2H_5OH	0.250	0.107	0.333
1N HCl in 95% C_2H_5OH	1.94	1.79	1.25

*Initial residues 2.27 ppm; harvest residues 0.118 ppm.

Gas-liquid Chromatography (GLC). The gas chromatograph (Aerograph Model 200) was equipped with a thermionic phosphorus detector with a cesium bromide pellet. The glass coiled 8 ft. column was packed with a mixture of 10% DC 200 and 15% QF-1 on 60-80 mesh Gas-Chrom Q column support resulting in 5% DC 200 and 7.5% QF-1. The carrier gas flow (N_2) was 20 ml/min. The detector and injector temperatures were 200°C. All parathion and related compounds were quantitated on this column with the exception of p-nitrophenol. The temperatures at which the following compounds were detected are: (1) parathion, 210°C; (2) aminoparathion, 210°C; (3) paraoxon, 210°C; (4) diethyl phosphate, 150°C; (5) O, O-diethyl-S-p-nitrophenyl phosphate, 210°C; (6) O, S-diethyl-O-p-nitrophenyl phosphate, 210°C; and (7) diethyl phosphorothioate, 150°C. Diethyl phosphorothioate and diethyl phosphate were detected as the methylated compounds by GLC. A Varian-Aerograph Model 1200 gas chromatograph equipped with an electron capture detector and a 6 ft. glass column packed with 5% SE-30 and 5% Dow 710 fluid on 60/80 mesh Chromosorb W acid washed and silyla-

TABLE V

p-Nitrophenol Residues on Lettuce Trim after Exposure to Removal Procedures

Treatment	Plant Material: Initial Sample 1/2 Lb./Acre*			Plant Material: Harvest Sample 1/2 Lb./Acre*		
	24°C	55°C	75°C	24°C	55°C	75°C
H_2O	0.042	0.086	0.087	0.031	0.071	0.054
0.1N NaOH	0.055	0.101	0.112	0.062	0.067	0.062
0.1N HCl	0.010	0.007	0.041	0.051	0.060	0.070
0.1N $NaHCO_3$	0.027	0.067	0.009	0.117	0.141	0.078
4% Acetic acid	0.009	0.019	0.096	0.105	0.086	0.029
95% C_2H_5OH	0.038	0.045	0.099	0.089	0.073	0.068
1N NaOH in 95% C_2H_5OH	0.193	0.094	0.109	0.047	0.060	0.082
1N HCl in 95% C_2H_5OH	0.235	0.084	0.025	0.158	0.096	0.066
	Cooking Broth					
H_2O	0.017	0.012	<0.001	0.017	0.012	0.015
0.1N NaOH	0.019	0.011	0.018	0.017	0.015	0.014
0.1N HCl	0.009	0.002	<0.001	0.018	0.016	0.015
0.1N $NaHCO_3$	0.021	0.013	<0.001	0.015	0.016	0.017
4% Acetic acid	0.020	0.018	0.002	0.011	0.013	0.019
95% C_2H_5OH	0.009	0.002	<0.001	0.027	0.010	0.010
1N NaOH in 95% C_2H_5OH	0.043	0.040	0.038	0.040	0.040	0.084
1N HCl in 95% C_2H_5OH	0.026	0.025	0.025	0.013	0.032	0.106

*Initial residues 0.108 ppm; harvest residues 0.061 ppm.

ted, a carrier gas flow (N_2) of 30 ml/min. and a column temperature of 140°C was utilized to quantitate the p-nitrophenol as the methylated compound. The detector and injector temperatures were 200°C. Quantitation was accomplished by measurement of peak areas with a polar planimeter and compared with reference standards. The ppm data are based on fresh weight calculations.

Pesticide Removal Treatments for Lettuce Trim. Various physical and chemical techniques were investigated for the removal of parathion and related organophosphorus compounds from the lettuce trim as animal feed. Washing with water and ethanol separately as well as various concentrations of acid and base in these solvents were investigated. The influence of three temperatures (24°C, 55°C and 75°C) on the above solutions for removing the pesticide contaminants on the lettuce was also explored. The lettuce for treatment was covered with the solutions and treated at the above temperatures for 15 min. with intermittent stirring followed by separation of the plant material from the liquid phases for quantitative pesticide analyses.

TABLE VI

p-Nitrophenol Residues on Lettuce Trim after Exposure to Removal Procedures

Treatment	Plant Material Initial Sprayed Sample 1 Lb./Acre*		
	24°C	55°C	75°C
H_2O	0.089	0.065	0.082
0.1N NaOH	0.082	0.093	0.081
0.1N HCl	0.071	0.056	0.080
0.1N $NaHCO_3$	0.087	0.090	0.081
4% Acetic acid	0.060	0.070	0.096
95% C_2H_5OH	0.054	0.056	0.086
1N NaOH in 95% C_2H_5OH	0.046	0.041	0.078
1N HCl in 95% C_2H_5OH	0.091	0.084	0.062
	Cooking Broth		
H_2O	0.015	0.013	0.025
0.1N NaOH	0.019	0.031	0.019
0.1N HCl	0.018	0.019	0.017
0.1N $NaHCO_3$	0.028	0.024	0.020
4% Acetic acid	0.010	0.016	0.021
95% C_2H_5OH	0.016	0.014	0.023
1N NaOH in 95% C_2H_5OH	0.046	0.067	0.088
1N HCl in 95% C_2H_5OH	0.016	0.054	0.041

*Initial residues 0.140 ppm; harvest residues 0.072 ppm.

After treatment the wash liquids were decanted into separatory funnels and the lettuce was extracted by refluxing in a mixture of benzene and isopropanol. The aqueous washes were extracted three times with hexane at pH values of approximately 1, 7 and 13 and the extracts were pooled for analyses. The 95% ethanol wash solutions were evaporated to dryness in vacuo at 50-60°C and taken to volume in hexane. The other ethanolic acid or base wash solutions were diluted with water and extracted in the same manner as the aqueous washes.

Table VII

Efficiency of Parathion Removal from the Trim of the Initial Lettuce Sample Sprayed at the Rate of 1/2 Lb. Active Ingredient Per Acre

	24°C		55°C		75°C	
Sample Treatment*	PPM	% Removed	PPM	% Removed	PPM	% Removed
None	11.3	---	11.3	---	11.3	---
H_2O	9.20	18.6	7.00	38.1	4.52	60.0
0.1N NaOH	8.71	22.9	5.94	47.4	4.86	57.0
0.1N HCl	7.38	34.7	6.86	39.3	4.84	57.2
0.1N $NaHCO_3$	7.62	32.6	6.48	42.7	3.24	71.3
4% Acetic acid	7.12	37.0	5.78	48.8	5.40	52.2
95% C_2H_5OH	4.06	64.2	3.27	71.1	1.82	83.9
1N NaOH in 95% C_2H_5OH	0.538	95.2	0.067	99.4	0.083	99.3
1N HCl in 95% C_2H_5OH	4.06	64.1	1.41	87.5	1.06	90.5

*Treated for 15 min. in the various solvents and at the various temperatures.

Results and Discussion

The parathion and related products residues that were investigated on the lettuce trim were 0,0-diethyl 0-p-nitrophenyl phosphorothioate (parathion), 0,0-diethyl 0-p-nitrophenyl phosphate (paraoxon), 0,0-diethyl 0-p-aminophenyl phosphorothioate (aminoparathion), 0,0-diethyl S-p-nitrophenyl phosphorothiolate (S-phenyl parathion), 0,S-diethyl 0-p-nitrophenyl phosphorothiolate (S-ethyl parathion), p-nitrophenol, 0,0-diethyl phosphate and 0,0-diethyl phosphorothioate.

The control field lettuce head 7 days preharvest contained trace amounts of the following compounds at ppm concentrations: parathion, 0.012; aminoparathion, 0.002; p-nitrophenol, 0.042; paraoxon, 0.001; S-ethyl parathion, <0.010; S-phenyl parathion <0.010; diethyl phosphate, <0.001; and 0,0-diethyl phosphorothioate, <0.001.

The three major compounds detected in all samples were parathion, aminoparathion and p-nitrophenol with the other compounds present, if at all, in concentrations of approximately 0.10 ppm or less. The aminoparathion and other compounds as detected in

the spray formulation were applied as contaminants and were not degradation products as shown by the formulation analysis above. Since the residues of the pesticides for the 1/2 and 1 lb./acre rate of application at harvest were similar, only the 1/2 lb. rate at harvest and both initial rates of application on the lettuce were investigated for decontamination procedures.

The effect of various washing solutions at room temperature (24°C), 55°C and 75°C for removing parathion, aminoparathion and p-nitrophenol from lettuce trimmings is shown in Tables I through VI. Since parathion is the major residue and if it is selected as the compound for discussing the average efficiency of removal of the contaminants from the lettuce trim on the 1/2 lb. and 1 lb. rates of application, it is shown that washing with water only removed approximately 29% of the contaminants, 95% ethanol removed 66%, 95% ethanol and 1N HCl removed 70% and 95% ethanol and 1N NaOH removed 97%, respectively. At 55°C these same wash solutions removed 42%, 81%, 90% and 99% of the parathion contaminant, and 50%, 87%, 93% and 99% was removed at 75°C, respectively (Tables I and II). Since 0.1N ethanolic NaOH and 1N ethanolic $NaHCO_3$ resulted in similar results as the 95% ethanol on all samples, these results are not shown in the tables. Aminoparathion appeared to be more stable to alkali treatment than parathion, and parathion appeared to be more stable to acid treatment than aminoparathion.

Table VII contains information concerning the effects of the treatment solutions and temperatures on the treated lettuce trimmings. The treatments that produced the best removal of the parathion contaminant from the lettuce trim with the least damage to the plant material were aqueous 0.1N $NaHCO_3$ at 75°C, 95% ethanol at 55°C and 75°C and 95% ethanol and 1N HCl at 55°C and 75°C. Although 1N NaOH in 95% ethanol reduced the parathion levels by 95% or higher, more damage to the plant material was produced than by the other treatments.

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