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Residues and Disappearance of Triforine from Various Crops

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A convenient method for the residue analysis of triforine, piperazine-1,4-diylbis[1-(2,2,2-trichloroethyl)formamide], sensitive to 0.01 ppm is presented. Disappearance rate data and harvest residue data are presented.

The systemic fungicide triforine has been found effective for the control of a number of diseases in ornamentals, cereal grains, fruits, and vegetables. More specifically it has been found in New York orchards (Gilpatrick et al., 1972) to control scab, powdery mildew, rusts, and European red mite on apples, leaf spot fungus on cherry (Szkolnik, 1974), and brown rot in plums, peach, and tart cherry (Gilpatrick, 1973). Not only is this material useful as a protectant but it has also been reported to have curative action against a number of plant pathogenic organisms (Fuchs et al., 1971; Szkolnik, 1973). The systemic activity of triforine has been demonstrated by correlating disease control with fungicide concentrations in leaves following soil application (Gilpatrick and Bourke, 1973). The fungicidal activity has been shown to be due to the parent compound which is metabolized to a series of nonfunitoxic products.

Triforine is a colorless and odorless, crystalline substance which decomposes at 155 °C. It has been formulated as a 20% emulsified concentrate and as a 25% wettable powder, both to be applied to drip off. It has a relatively high rodent LD_{50} in excess of 6000 mg/kg body weight.

The results reported here were gathered during the development and adaptation of the analytical method to various crops and as a result of field testing.

MATERIALS AND METHODS

The field spray formulations were a 20% emulsifiable concentrate (CA70203) supplied by Celamerck, a 20% emulsifiable concentrate (W524) supplied by Niagara Chemical Company, and a 25% wettable powder (W524) also supplied by Niagara. The analytical standard used was supplied by Celamerck GMBH & Co., Ingelheim, West Germany. All reagents used were of analytical grade; the solvents were redistilled to further enhance purity. Difficulty was experienced with all solvents. Interfering peaks from the acetone and ethyl acetate could not be removed by all-glass distillation. Each solvent was checked prior to use for the interfering peak by 50 to 1 concentration and injection into the GC. Only solvents found free of contamination were used. Some lots of spectro grade solvent contained the offending peak. The interfering peak in the ethyl formate could be removed by all-glass distillation. The internal standard was a 0.4% solution of 1,2-dibromoethane in acetone.

Extraction and Cleanup. After fruits were minced and the pits were removed, they were subsampled into 50-g laboratory samples. Lab samples were blended with 200 ml of acetone in an explosion proof blender for approximately 2 min. The homogenized sample was then filtered through sintered glass and the residue reblended twice in the same manner with 100 ml of acetone.

The combined acetone extracts were mixed with 500 ml of water and 500 ml of saturated sodium chloride and then extracted with 200 ml of ethyl acetate. The aqueous phase was extracted twice more with 150 ml of ethyl acetate, and the organic phases were combined and evaporated to approximately 30 ml on a rotary evaporator at room temperature. The residue was then transferred into a 250-ml one-neck round-bottomed flask with acetone and the acetone evaporated off on the rotary evaporator at room temperature. To the 10 to 20 ml of liquid remaining in the flask, 60 ml of 10% sulfuric acid was added. This mixture was distilled at 140 °C (oil bath) under a nitrogen flow (20 ml/min) for 1 h into 10 ml of doubly distilled water, in the apparatus shown in Figure 1. The distillate was cooled by immersion in an ice brine bath.

The distillation with sulfuric acid results in the conversion of triforine to chloral hydrate which is collected and quantitized by the following procedure. The collected aqueous solution was transferred to a 250-ml separatory funnel, 30 g of sodium chloride was added, and the solution was extracted with 12 ml followed by 10 ml of ethyl formate. The organic phases were collected in a 20-ml volumetric flask and made up to volume. Ten microliters of the internal standard solution and 1 g of calcium chloride were added and the flask was left for 0.5 h.

Gas Chromatography. Gas chromatographic analysis was performed on a Microtech MT 220 chromatograph

Department of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York 14456 (J.B.B., T.R.N.) and Celamerck GMBH & Co. KG, Ingelheim, West Germany (D.E.).

Table I. Sensitivity and Recovery of Triforine in Various Crops

Crop	Apparent background, ppm	Level spiked, μg/50 g	Recovy mean value, %
Apple	0.01	4	75
Apple juice	0.01	4	85
Apple pomace	0.01	4	100
Applesauce	0.01	4	90
Blueberry	0.01	4	73
Prunes	0.04	4	90
Peaches	0.01	4	75
Barley grain	0.02-0.01	1-5	78
Barley straw	0.02-0.01	0.5-2	83
Barley green plant	0.02-0.01	2-40	79
Beans	0.01	5-25	79
Cherries	0.02-0.01	2-100	82
Cucumber	0.03-0.01	2-25	87
Currant	0.05-0.01	10-100	76
Plums	0.01	5-40	87
Pumpkin	0.01	5	88
Wheat grain	0.02-0.01	1-10	80
Wheat straw	0.02-0.01	0.5-2	79
Green plants	0.01	5-40	79

Table II. Residues of Triforine on McIntosh Apples as Determined by Two Independent Laboratories

Sample no.	Sample date	Residue, ppm	
		Geneva	Ingelheim
770	8/19/71	0.48	0.51
771	8/26/71	0.18	0.13
772	9/02/71	0.11	0.16
773	9/08/71	0.12	0.12
774	9/16/71	0.08	0.09
775	9/23/71	0.06	0.08
776	9/29/71	0.06	0.08

equipped with a ^{63}Ni electron capture detector. The 6 ft × 0.25 in. glass column packed with 3% XE-60 on 80–100 mesh Gas-Chrom Q was maintained at 65 °C and flushed with nitrogen at the rate of 50 ml/min. The detector and injector were maintained at 160 and 125 °C, respectively.

A series of 20-ml standard solutions was prepared in ethyl formate. These contained 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, and 0.15 μg of chloral hydrate per milliliter. To each of these solutions 10 μl of a 0.4% solution of 1,2-dibromoethane was added. A standard curve was prepared by plotting the ratio of the height of the chloral hydrate peak and the height of the dibromoethane peak against the chloral hydrate concentration.

Sample residues of triforine were calculated according to the equation:

$$R = \frac{1.31WV}{G} \times F$$

where W is the concentration of chloral hydrate taken from the standard curve; V is the volume of solution used (20 ml); G is the weight of sample used (in grams); and F is the recovery factor.

In all untreated crop samples a small chromatographic peak, indistinguishable from chloral hydrate, was found. This peak was quantitated and is reported as apparent background in Table I. The apparent background is constant for a crop and can be subtracted from the calculated concentration to give the actual concentration.

In experiments in which disappearance was being studied the data were used to calculate curves of best fit using least-squares exponential functions. This involved fitting the analytical data to the first-order reaction equation $C_t = C_0e^{-kt}$ where C_t is the concentration at time t, C_0 is the residue concentration immediately after ap-

Table III. Triforine Residues in Apples and Apple Products after Foliar Application of 20% EC (W524)

Product	Year	No. applications	Application rate, oz of act. ingred./acre	Days after last application	Harvest residue, ppm	Disappearance rate constant
Apple						
McIntosh	1971	9	12.8	41	0.05	0.044
Cortland	1971	9	12.8	41	0.08	0.044
McIntosh	1971	9	10.8	41	0.06	0.046
McIntosh	1971	9	12.0	56	0.06	
Delicious	1971	9	12.0	56	0.06	
Delicious	1971	13	12.8	37	0.08	0.037
Delicious	1971	13	9.6	37	0.06	0.042
McIntosh	1972	9	6.4	30	0.07	0.057
McIntosh	1972	9	9.6	30	0.10	0.056
McIntosh	1972	9	12.8	30	0.24	0.041
Cortland	1972	9	6.4	30	0.05	0.068
Cortland	1972	9	9.6	30	0.12	
Juice						
McIntosh	1972	9	7.2	21	0.02	
McIntosh	1972	9	9.6	21	0.04	
McIntosh	1972	9	12.8	21	0.04	
Pomace						
McIntosh	1972	9	7.2	21	0.16	
McIntosh	1972	9	9.6	21	0.30	
McIntosh	1972	9	12.8	21	0.19	
Sauce						
McIntosh	1972	9	7.2	21	0.01	
McIntosh	1972	9	9.6	21	0.01	
McIntosh	1972	9	12.8	21	0.02	
Juice						
Cortland	1972	9	7.2	21	0.03	
Cortland	1972	9	9.6	21	0.03	
Pomace						
Cortland	1972	9	7.2	21	0.19	
Cortland	1972	9	9.6	21	0.31	
Sauce						
Cortland	1972	9	7.2	21	0.01	
Cortland	1972	9	9.6	21	0.01	

Table IV. Triforine Harvest Residues on Cherries after Foliar Application

Year	Formulation	No. applications	Days after last application	Application rate, oz of act. ingred./acre	Harvest residue, ppm	Disappearance rate constant
1972	20% EC(28221)	8	12	6.4	0.21	0.06
1972	20% EC(28221)	8	12	8.0	0.12	0.13
1972	20% EC(28221)	8	12	9.6	0.12	0.14
1972	20% EC(W524)	7	8	9.6	0.52	
1972	20% EC(W524)	7	8	7.2	0.45	
1972	20% EC(W524)	7	8	4.8	0.47	
1973	20% EC(N271)	7	7	10.8	0.32	0.02
1973	25% WP(N334)	4	6	22.5	0.48	0.06
1973	20% EC(N300)	4	6	21.6	0.23	0.18
1973	20% EC(N271)	4	6	21.8	0.23	0.13
1973	20% EC(N271)	7	7	21.8	0.57	

Table V. Triforine Harvest Residues on Various Crops after Foliar Application of 20% EC(W524)

Crop	Year	No. applications	Application rate, oz of act. ingred./acre	Days after last application	Harvest residue, ppm	Disappearance rate constant
Blueberries	1971	5	8	35	0.14	
Blueberries	1972	1	16	7	0.87	0.17
Blueberries	1972	1	12	7	0.47	0.17
Prunes	1972	3	9.6	7	0.70	
Prunes	1972	3	9.6	7	0.43	0.03
Prunes	1972	3	4.8	9	0.20	0.07
Peaches	1972	9	9.6	3	0.46	
Peaches	1972	3	9.6	11	1.12	0.07
Peaches	1972	3	4.8	11	0.72	0.03
Peaches	1972	3	9.6	3	2.45	
Peaches	1972	3	4.8	3	0.82	
Peaches	1972	3	9.6	0	1.73	
Peaches	1972	3	4.8	0	1.01	
Grapes	1972	4	23.4[a]	15	0.30	0.09
Grapes	1972	4	23.4[a]	0	1.25	
Grapes	1972	4	23.4[a]	14	0.55	
Grapes	1972	4	23.4[a]	7	0.50	

[a] Average of 18.75 + 18.75 + 18.75 + 37.5 oz of active ingredient/acre.

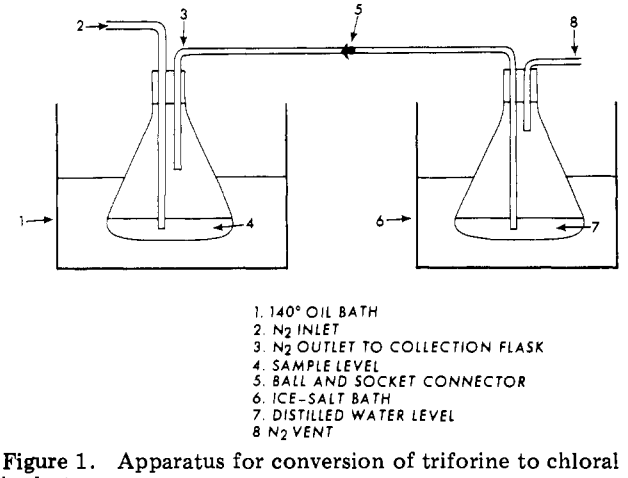

Figure 1. Apparatus for conversion of triforine to chloral hydrate.

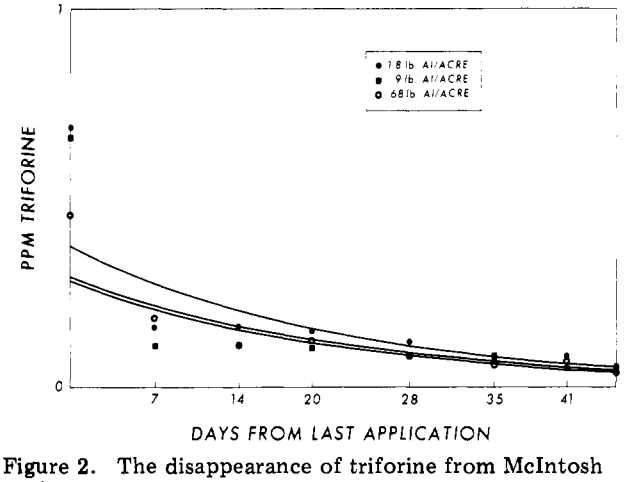

Figure 2. The disappearance of triforine from McIntosh apples.

plication, and k is the rate constant.

In general two distinct processes take place during the disposition of a pesticide from a crop surface. The first is a rather rapid physical process which includes volatization, ultraviolet degradation, and washing when rain occurs. This process may tremendously influence the disappearance, particularly just after application. The second phase is a more general chemical process which includes hydrolysis, metabolism, and oxidation. This usually results in a less rapid disappearance and becomes the predominant effect in the latter part of the disappearance period. An exponential disappearance curve prepared for a highly volatile pesticide will for these reasons show an intercept at 0 day somewhat below the concentration measured. This occurs because of the lack of necessary data during the early period which would be required to fit the curve to the rapid short-term physical processes. The reader should keep this effect in mind when studying the curves and rate constants presented here. Disappearance of triforine from McIntosh apples is shown in Figure 2.

RESULTS AND DISCUSSION

Table I lists the analytical limits of sensitivity and recovery. The values given are averages of a number of

determinations and represent those used in calculating the residues reported herein. The 4-μg spike used calculates as a 0.08-ppm residue which is characteristic of the harvest residues found in the apples and apple products tested. As a further verification of the assay procedure duplicate samples of McIntosh apples from Geneva were analyzed both in the Geneva Pesticide Laboratory and the Celamerck, Ingelheim, West Germany, Pesticide Laboratory. Table II lists the residues found in this fruit at various periods after application as determined in the two laboratories. The agreement between laboratories is excellent indicating a reliable method.

The results of field testing of triforine on apples are summarized in Table III. Applications at the rates of between 8 and 16 oz of active ingredient per acre were applied to three varieties in 1971 at four different locations. Between 1 and 13 applications were applied through the growing season. Periodic samples were taken from last application to harvest and residues determined. In all cases the harvest residue was determined to be between 0.03 and 0.08 ppm. The average rate constant (k) was calculated to be 0.044. Similar experiments run during 1972 at Geneva but with shorter application to harvest intervals resulted in harvest residues from 0.05 to 0.24 ppm depending on application rate. The rate constants were slightly higher than those obtained during 1971, averaging 0.055.

Pilot plant processing of the 1972 fruit and subsequent analysis indicated the vast majority of the residue that was present in the apples at harvest (<0.24 ppm) was recovered in the pomace. Only minor quantities (<0.035 ppm) were recovered in either juice or sauce.

In 1972 and 1973 extensive trials on sour cherries were carried out in East Lansing, Mich., Geneva, Switzerland, and Lockport, N.Y. From one to eight applications at rates of between 6 and 25 fluid oz/100 gal were applied to the foliage. Harvest residues (Table IV) were found to range between 0.1 and 0.3 ppm 1 week after application depending upon application rate. The rate constant was found to average 0.10 or about twice that found in the apple experiments.

The application of a 20% emulsifiable concentrate to peaches at rates of between 8 and 16 fluid oz/100 gal resulted in harvest residues of between 0.7 and 1.1 ppm 11 days after application. Residues in blueberries sprayed at the same rate were determined to be between 0.5 and 0.8 ppm 7 days after the application. Thirty-five days past application, the residue had decreased to 0.1 ppm. Prune triforine residues of between 0.2 and 0.4 ppm were found 9 days after treatment at the above rates. Approximately 0.3 ppm of triforine was recovered from grapes sprayed four times, 15 days after the last application.

The rate constant for prunes, peaches, and grapes ranged between 0.03 and 0.09, averaging 0.06. The rate constant on blueberries was about three times that or 0.17. The specific conditions and residues from the field trials are given in Table V.

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Distribution and Dissipation of Captafol Applied to Apple Trees

Mikio Chiba* and John Northover

Seasonal changes of captafol residues on semidwarf apple trees were determined following a single application of Difolatan for early season apple scab control. Initial wood deposits of 126–128 $\mu g/cm^2$ declined to 36–2 $\mu g/cm^2$ at harvest at tree heights of 1.5 and 3.0 m, respectively. Deposits on cluster leaves declined from 6.4 $\mu g/cm^2$ (May 1) to 0.98 $\mu g/cm^2$ (June 6). The minimum deposit of captafol necessary for protection of leaves against apple scab was judged to lie between 0.1 and 1.0 $\mu g/cm^2$. During a 4-mm rainfall, rainwater collected under sprayed trees, with a wood deposit of 97 $\mu g/cm^2$ captafol, contained 0.65 $\mu g/ml$ of captafol. At harvest, whole apple residues were 0.0062 $\mu g/g$ with the highest residues associated with the peel. A simple thin-layer chromatographic technique was used to purify apple extracts prior to gas chromatographic analysis.

Captafol, *N*-(1,1,2,2-tetrachloroethylthio)-3a,4,7,7a-tetrahydrophthalimide, is an important fungicide recently introduced for the control of apple scab (*Venturia inaequalis* (Cke.) Wint.). It is applied to trees using the single application technique (SAT), at or just prior to the appearance of leaf tissue in early spring and has given adequate scab control for as long as 53 days (Northover, 1975).

The prolonged fungicidal activity of captafol is due to its persistence (Neely, 1970, 1971) and its redistribution

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