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Residues and Half-Lives of Acephate, Methamidophos, and Pirimiphos-Methyl in Leaves and Fruit of Greenhouse-Grown Tomatoes

George F. Antonious[1] and John C. Snyder[2]

[1]Kentucky State University, Atwood Research Facility, Frankfort, Kentucky 40601, USA and [2]Department of Horticulture and Landscape Architecture, University of Kentucky, Lexington, Kentucky 40546-0091, USA

Pirimiphos-methyl, acephate and methamidophos are active ingredients in the organophosphorus insecticidal formulations bearing the trade names Actellic, Orthene and Monitor respectively (Figure 1). These insecticides are used in many parts of the world to control a range of pests on an array of crops, including vegetables. However, toxicities of these insecticides to mammals are quite different. Pirimiphos-methyl has low mammalian toxicity, having an acute oral LD_{50} > 2000 mg/kg (Leahey and Curl 1982) and no cholinesterase inhibiting metabolites (FAO/WHO 1983). Acephate, a systemic insecticide, is toxic to mammals at 900 mg/kg and methamidophos, also systemic, is toxic at 13 mg/kg (Ware 1989). In plants 5 - 10% of the applied acephate is metabolized to methamidophos (Leary 1974, Magee 1974, Leidy et al 1978, Hadjidemetriou et al 1985, Levine and Felsot 1985). The persistence of the methamidophos resulting from metabolism of acephate has not been well characterized in tomato.

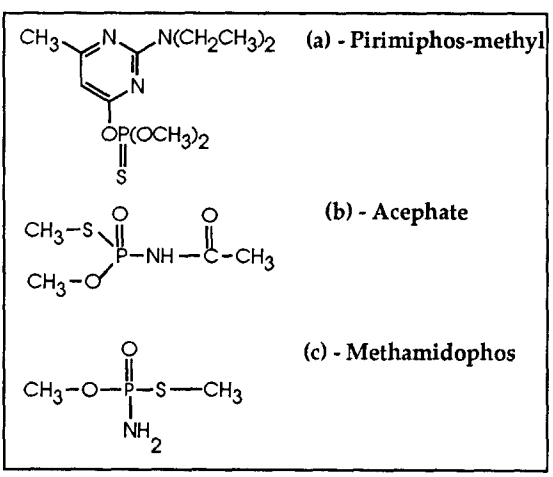

Figure 1. Structures of pirimiphos-methyl [O-(2-diethylamino-6-methyl pyrimidin-4-yl)O,O-dimethyl phosphorothioate], acephate (O,S-dimethyl N-acetyl phosphoramidothioate) and methamidophos (O,S-dimethyl phosphoramidothioate).

In the United States, acephate and methamidophos but not pirimiphos-methyl are

Send reprint requests to John C. Snyder at the above address

used on vegetables. However, pirimiphos-methyl is used in many other parts of the world for insect control on many vegetable crops. The objectives of this study were to compare rates of dissipation of the three insecticides on a common vegetable crop, tomato, and to determine the extent of acephate decomposition to methamidophos in the greenhouse environment. A greenhouse provides an environment allowing comparison of the longevity of these insecticides, not confounded by factors such as rainfall.

MATERIALS AND METHODS

Tomato plants (*Lycopersicon esculentum* Mill.) cv. Olympic were grown in silty loam soil in 15 cm diameter plastic pots in the greenhouse. The plants were arranged in a randomized complete block design consisting of three blocks and 8 plants per block. When the first 5 or 6 fruit on each plant were ripe (fruit diameter ≈ 7 cm), five plants in each block were treated with one of the insecticides at the rates indicated below; the remaining three plants served as untreated controls. Plants were watered as needed and fertilized at three-day intervals with a 200 ppm solution of Peters 20-20-20, a water soluble fertilizer. Greenhouse temperatures averaged 37 and 41 °C and relative humidity averaged 46 and 62% during June and July, 1991, respectively. Insecticides were applied as sprays to runoff with a 7.5 liter compressed air sprayer at 40 psi.

Actellic 5E was applied at the rate of 7.5 ml/L of water (4.5 g ai/L); both the commercial and the standard (97% purity) materials were obtained from ICI Agrochemicals. Orthene 75WP was applied at the rate of 6.44 gm/L of water (4.8 g ai/L); the commercial and standard (99.94% purity) materials were obtained from Chevron Chemical Co. Monitor 40EC was applied at the rate of 10 ml/L (4.0 g ai/L); the formulated and standard (76% purity) materials were obtained from Mobay Corporation. Actellic and Monitor were also applied at one-half the rates indicated above.

Leaves and ripe fruit (1 kg) were collected from each block at one hour, 1,3,7,10,14,21,28,35,42,50 and 56 days after spraying. Leaves (50 g) were picked from each block at random from the top 10 cm of plants and from a 10 cm mid-stem section. Samples were placed in glass jars, covered and forwarded immediately to the laboratory.

Sub-samples (25 g leaves or 150 g fruit) were blended at high speed for 3 minutes with methanol (200 ml) for pirimiphos-methyl extraction and with ethyl acetate (200 ml) for acephate and methamidophos extraction. After filtering with Whatman No. 1 paper, the extracts were dried over anhydrous Na_2SO_4 and then concentrated to a known volume with a rotary-vacuum evaporator at 35 °C. A glass chromatographic column (1.5 x 20 cm) packed with 6 g silica gel-magnesium oxide (2:1) mixture was used for clean-up of pirimiphos-methyl residues (Antonious and Abdel-All 1988). An aliquot of the concentrated extract (equivalent to 12.5 g leaves or 75 g fruit) was transferred to the column that was

pre-wetted with hexane-CH_2Cl_2 (4:1 v/v). The column was then eluted with 150 ml of 20% acetone in hexane (v/v). The eluate was evaporated on a rotary evaporator at 35 °C. Residues of pirimiphos-methyl were redissolved in methanol for gas chromatographic analysis (GC). For clean-up of acephate and methamidophos a 22 mm id x 20 cm chromatographic column packed with Florisil (10 g) under a 3 cm layer of anhydrous Na_2SO_4 was used (Iwata et al 1985). Acephate and methamidophos were eluted with 120 ml of methanol-acetone (1:4 v/v). Both acephate and methamidophos were redissolved in ethyl acetate for GC analysis.

A Hewlett-Packard gas chromatograph equipped with a flame ionization detector was used for quantitation. For pirimiphos-methyl the following chromatographic conditions were used: column, 10 m x 0.53 mm id bonded FSOT polydimethylsiloxane (Alltech Associates, Inc.); injector temperature, 130 °C; column temperature, 130 °C; detector temperature, 300 °C; carrier gas, He at 2.3 $ml \cdot min^{-1}$. Retention time was 10.26 minutes. Conditions for analysis of methamidophos and acephate were: column, 15 m x 0.53 mm id Teflon© tubing, coated with polydimethylsiloxane 95% phenyl:5% methyl (Alltech Associates, Inc.); oven temperature, 90 to 250 °C at 10 °C/min.; detector temperature, 220 °C; injector temperature, 220 °C; carrier gas, He at 20 ml/min. Retention time was 2.39 minutes for methamidophos and 2.95 minutes for acephate. To obtain reproducible results using this column, large amounts of acephate and methamidophos standard materials (250 ng/µl) were injected to initially condition the chromatographic column and achieve good detector response.

Insecticide recoveries were determined, just prior to residue analyses, by fortification of untreated tomato tissues at 1.0 and 10.0 µg/g. Insecticide standard materials were prepared at appropriate concentrations, based on their purity, in the same solvent used for extraction and were then added in duplicate directly to the tissue in the blender jar prior to blending. Extraction, clean-up and detection procedures were followed as described above.

Initial retention of insecticides was analyzed by ANOVA and means were compared by Fisher's protected LSD. Half-life was calculated using the methods of Anderson (1986) and statistically compared after covariance analysis using t-test.

RESULTS AND DISCUSSION

The procedures used for extraction and quantitation of the 3 insecticides were reliable and provided good recoveries and excellent limits of detectability (Table 1). Detectability was 2-fold greater than that reported by Frank, et al (1991) for acephate. Recoveries were highest for pirimiphos-methyl (Table 1) and tended to be less for the more water soluble insecticides, acephate and methamidophos, especially when attempting to recover them from tomato fruit tissue which has a high water content.

Initial retention of the insecticides one hour after spraying was, as is typical for most insecticides, greater on leaves than on fruits (Table 2). For the two insecticides which were applied at two rates, retention on fruits and leaves was proportional to rate of application. Among insecticides, even though approximately similar amounts were applied, initial retention on leaves was proportionally greatest for pirimiphos-methyl and least for methamidophos. Initial retention on leaves was inversely proportional to the solubility of the insecticides in water (methamidophos > acephate > pirimiphos-methyl). On fruits retention of pirimiphos-methyl was equal to that of acephate and both were greater than that of methamidophos. The differences in initial retention between fruits and leaves probably relates to their different surface to weight ratios and perhaps, different surface properties.

Table 1. Percentages of Recovery ± S.D. and Detectability Limits of Three Organophosphorus Insecticides

	Pirimiphos-methyl	Acephate	Methamidophos
Recovery (%)	95.82±1.02	90.30±1.16	92.17±2.99
Detectability limits[1]:			
Fruits	1.3×10^{-4}	1.7×10^{-4}	8.3×10^{-5}
Leaves	8.0×10^{-4}	1.0×10^{-3}	5.0×10^{-4}

[1]Detectability limit = minimum detectable concentration (µg/g)

As expected, but in contrast to initial retention, half-lives for each insecticide were very similar for leaves and fruit and were not influenced by rate of application (Table 2). Half-life was longer for acephate and methamidophos and shorter for pirimiphos-methyl. In contrast to dissipation of pirimiphos-methyl and acephate on fruit and leaves and dissipation of methamidophos on leaves (Figure 2), dissipation of methamidophos on fruit occurred in two phases (Figure 2), an early phase (0 to 7 days) with a short half-life, 1.9 days for the 2.0 g/L rate and 2.0 days for the 4.0 g/L rate and a later phase (7 to 30 days after application) with a long half-life, 12.9 days for the 2.0 g/L rate and 15.8 days for the 4.0 g/L rate, assuming both phases followed first order kinetics.

Residues of the metabolite of acephate, methamidophos, were detected in all tissues except those obtained one hour after applying acephate (Figure 2). One day after application of acephate, methamidophos was detected in fruit and leaf tissues. In fruit, between day 1 and day 35, the concentration of parent compound declined and that of its metabolite increased to 0.3 ppm 35 days after application of acephate. In leaves, during this time period, the concentration of the parent acephate also declined, but concentration of the metabolite methamidophos remained remarkably constant. Leidy, et al (1978) also reported that methamidophos residues were detected 1 day after application of acephate, but our

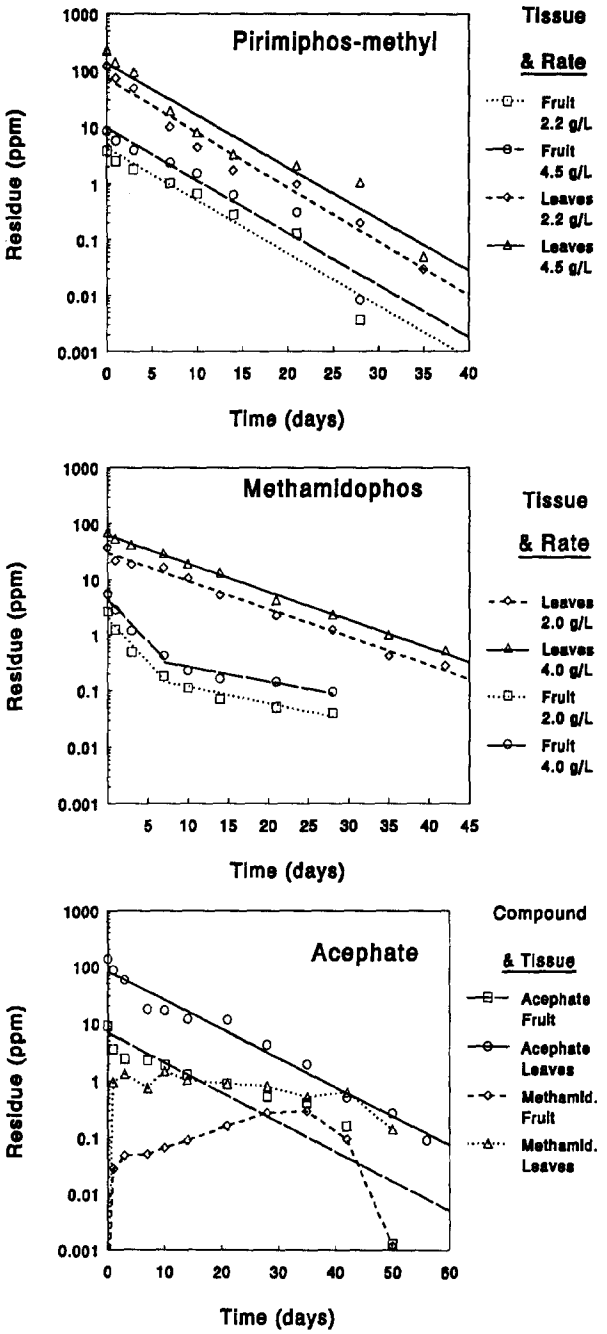

Figure 2. Dissipation of pirimiphos-methyl (upper panel) and methamidophos (middle panel) applied at two rates on tomatoes grown in a greenhouse and dissipation of acephate applied on tomatoes grown in a greenhouse and concentration of its metabolite, methamidophos (lower panel).

findings contrast with their report that concentrations of methamidophos residues in tomato fruit arising from metabolism of acephate showed no trend associated with days after application of acephate. They also reported methamidophos residues as high as 0.7 ppm and that concentrations were not directly influenced by rate or frequency of application of acephate. The reasons for the differing results are not clear but may relate to their sampling period, which was no more than 7 days after spraying compared with ours which was through 50 days after spraying and/or to differences in production systems, climates, cultivars, etc in the two experiments.

Table 2. Application Rates, Initial Residues Recovered, and Half-Life of Three Insecticides

		Residue recovered 1 hour after application[1] (mg/kg)		Half-life (days)[2]	
Insecticide	Application rate (g/L)	Leaves	Fruit	Leaves	Fruit
Pirimiphos-methyl	4.5	225 A	8.5 A	3.1 B	3.2 B
	2.2	118 C	3.9 C	3.0 B	3.0 B
Methamidophos	4.0	70 D	5.5 B	5.5 A	5.1 A
	2.0	37 E	2.6 D	5.9 A	4.8 A
Acephate	4.8	134 B	9.1 A	6.1 A	5.8 A

[1]Means within a column followed by the same letter are not significantly different at P=0.05 as determined by the protected LSD.

[2]Means within a column followed by the same letter are not significantly different as determined by t-test.

Like most vegetables, tomato is a perishable crop which must be harvested frequently and regularly. Consequently, insecticides having long post-application waiting periods are not compatible with vegetable production, especially during fruit ripening. Based on the initial retention and half-lives of insecticides on tomato fruit reported in this study and on published maximum residue limits (MRL), we have calculated waiting periods for each rate of each insecticide (Table 3). Even though pirimiphos-methyl has a short half-life, its initial retention is high and consequently, the waiting period is long using a MRL of 1 ppm (FAO/WHO 1986); the waiting period for pirimiphos-methyl at an application rate of 4.5 g/L is only acceptable if the MRL is 5 ppm. Methamidophos, the most toxic of the three insecticides, had the shortest waiting period. The waiting period for acephate is intermediate, and probably acceptable in tomato production. However, the extreme persistence of toxic methamidophos, arising from metabolism of acephate,

needs to be investigated further, especially under a wider range of production systems, environmental conditions, and multiple spray regimes.

Table 3. Approximate Waiting Periods for Residues to Reach Maximum Residue Limits (MRL) in Tomato Fruit

Compound	Maximum residue limit (ppm)	Rate of application (g/L)	Approximate waiting period (days)
Acephate	5*	4.8	3-4
Methamidophos	2*	2.0	1-2
	2*	4.0	2-3
Pirimiphos-methyl**	5	4.5	2-3
	5	2.2	0
	2	4.5	6-7
	2	2.2	2-3
	1*	4.5	14-16
	1*	2.2	6-7

*Maximum Residue Limits recommended by FAO/WHO, 1986
**Because there is no U.S. standard MRL for pirimiphos-methyl on tomato, alternative MRL's of 5, 2, and 1 ppm were assumed.

The low mammalian toxicity, lack of toxic metabolites, and its efficiency against insects as a broad spectrum, contact insecticide on vegetables and fruits (Thomson, 1979) indicate that pirimiphos-methyl may be a potential substitute for other, more toxic organophosphorus insecticides. In our experiments under greenhouse conditions, pirimiphos-methyl had a shorter half-life than the other insecticides, but its proportionally greater initial retention would result in excessive waiting periods under a MRL of 1 ppm. Under other conditions such as lower application rate or under field conditions, considering that pirimiphos-methyl loss from plant surface is mainly by volatilization (FAO/WHO, 1983) which may reduce initial retention and half-life, waiting period may be acceptable. However, efficacy at lower rates should be investigated.

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