Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 91 (2005) 235–240
Food Chemistry
www.elsevier.com/locate/foodchem

Degradation of methamidophos on soultanina grapes on the vines and during refrigerated storage

Panagiotis E. Athanasopoulos *, Christos Pappas, Nikolaos V. Kyriakidis, A. Thanos

Department of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, Votanikos, Athens 118 55, Greece

Received 2 July 2003; received in revised form 23 October 2003; accepted 23 October 2003

Abstract

Decomposition of the pesticide methamidophos was studied in an experiment in field-sprayed grapes of the Soultanina variety. Residues were studied by a simple gas-chromatographic method, using a glass capillary column and NP detector. The decomposition of methamidophos was studied in grapes remaining on the vines after spraying in open and covered vineyards, in grapes harvested and stored in a refrigerated room and in grapes dried into raisins. The recovery of methamidophos was 96.2–112%. The relative standard deviation (RSD) was between 1.4 and 6.2%. The detection limit was 0.02 mg/kg. The results show that, there exists a delayed residue decline during post-harvest storage compared to that for grapes remaining on the vines. From the experimental results, best fit curves were determined and kinetic equations, rate constants and half-lives were calculated. Half-lives found were 16 days for grapes on uncovered vines, 22 days for covered vines and 267 days for grapes stored in a refrigerator. Concentration of methamidophos in raisins was three times higher than that in grapes.

1. Introduction

Methamidophos is the common name of O,S-dimethyl-phosphoramido-thioate. It is a highly active, systemic, organophosphate insecticide, acaricide, aphicide, with contact and stomach action. Methamidophos is a potent acetylcholinesterase inhibitor (Hussain, 1987). It is effective against chewing and sucking insects and is used to control aphis, flea beetles, worms, whiteflies, trips, cabbage loopers, potato tuberworms, mite, leafhoppers and many others. Crop uses include broccoli, Brussels sprouts, cauliflower, many other vegetables, grapes, peaches and other crops. It is also highly toxic to mammals, birds and bees (Thomson, 1992). Commercially available formulations include soluble concentrate, emulsifiable concentrate, wettable powder, granules, ultra-low volume spray and water-miscible spray concentrate. It is compatible with many other pesticides, but must not be used with alkaline materials (HSDB, 1990).

Methamidophos is highly toxic via oral, dermal and inhalation routes of exposure. In humans and animals, methamidophos is rapidly absorbed through the stomach, lungs and skin. It is eliminated primarily in the urine. The LD_{50} values for male and female rats are 21 and 16 mg/kg body weight, respectively. Inhalation LD_{50} in rats is 9 mg/kg (RTECS, 1990).

Concerning teratogenic effects, some fetal liver pathologic changes were observed when pregnant rabbits were exposed to methamidophos (Juarez & Sanchez, 1989). Methamidophos has tested positive for genotoxicity, or ability to induce changes to chromosomes in some tests, but was negative in others. It may be weakly mutagenic (Baker, Scott, & Wilkinson, 1990).

Methamidophos is taken up through the roots and leaves. In studies of methamidophos degradation in tomato plants, the half-lives in fruits and leaves were measured as 4.8–5.1 days and 5.5–5.9 days, respectively, (Hussain, 1987).

Methamidophos, under the trade name Tamaron, is the pesticide of choice for the protection of grapes against a number of insects, such as *Lobesia botrana*, *Clysia ambiguella*, *Frankliniella occidentalis* and acarinu such as *Tetranynchus curticae*, *Phyllocoptes vitis* and

* Corresponding author.

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.10.018

Eriophyes vitis. Farmers use it during the last stages of grape ripeness. Also, in several areas in order to avoid damage to grapes from rain or strong sun-light, farmers cover their vineyards with polyethylene sheets. Previous experience has shown that, these changes may dramatically change the degradation rates of methidathion and pyrazophos (Athanasopoulos, Kyriakidis, & Pappas, 2000; Kyriakidis, Athanasopoulos, Thanos, Pappas, & Yialitaki, 2000). The degradation of methamidophos on the grapes of the open and covered vineyards and during refrigerated storage has not yet been studied. The objective of this work was to study the rate of degradation of methamidophos under these conditions.

2. Materials and methods

2.1. Field experiments

The spraying experiments were conducted in a vineyard in Assos, a village near Korinthos, in north Peloponese, on vines receiving all routine horticultural practices except pesticide application over the last three months before spraying. Vines on this vineyard were 3 m apart in the same line and 2 m apart from line to line. Vines were about 1.5 m high.

The experimental vineyard consisted of 40 lines, each of which included 40 vines. Half of the lines were covered by means of polyethylene sheets. This is a common practice for late table grapes in this region of the country. Two pesticide applications were conducted, one on covered and the other on uncovered grapes on 16/9/99, at the ripe stage of their development. A proprietary aqueous emulsion of 60% methamidophos, under the trade name Tamaron 60 SL (Bayer), was used in these experiments. According to the relevant EC Directive 98/82 (1998), the maximum residue limit (MRL) is 0.01 mg/kg. The pre-harvest interval for this insecticide in grapes, according to manufacturer instructions, is 21 days. Dilutions used were 100 ml Tamaron/100 l of solution, which is the recommended application dose (RD). This dose corresponds to 60 g of active ingredient/100 l of solution.

2.2. Sampling, storage and drying procedures

Sampling was performed by randomly collecting grapes from various parts of the experimental plots according to the FAO/WHO (1986) recommendations.

Samples of grapes of about 3 kg each were collected 3 h after the pesticide application of both sprayed and unsprayed vines for analysis. Samples were also collected every 5 days from both covered and uncovered vines for up to 50 days.

Samples of grapes intended for storage, of about 40 kg each, were collected 3 h following the pesticide application, to allow enough time for the emulsion to dry. The grapes were divided in 2 kg sub-samples and were packaged into plastic boxes, following common commercial practice. A polyethylene bag was fixed inside the plastic box, the grapes were packed in the bag and on top of them a special carton sheet, containing metabisulfite, was put. This impregnated paper prevents any rot of the grapes (*Botrytis inerea*). Sulfur dioxide (SO_2) is an effective fungistat (Marois, Bledsoc, Fubler, & Luvisi, 1985) and is applied by repeated fumigation (Athanasopoulos & Thanos, 1998) of grapes or by means of in-package SO_2 generators (Nelson, 1983; Kokalos, 1986). The plastic boxes were store in a refrigerated room at 0 ± 0.5 °C. Every 10 days, 2 kg samples were removed from the refrigerated room and sent to our laboratory for analysis.

Samples, also of about 6 kg, were collected from the sprayed vines every 10 days and dried in an air blast dryer. The resulting raisins were analyzed for pesticide residues.

Collected samples were analyzed according to the following plan: grapes were removed directly from the vines 3 h after spraying and then every 5 days. Grapes stored in the refrigerated room were sampled every 10 days. Prepared raisins were also sampled every 10 days.

2.3. Raisin preparation

Raisins were prepared in a raisin factory near Korinthos. A special automatic drying machine was used for their preparation. Grape samples, collected after spraying, were cleaned from insect-attacked berries and woody remnants, to be processed into raisins. Then, and in order to avoid pesticide residue losses, berries were carefully separated from the stems with scissors and they were transferred into the drying-shed for drying and transformation into raisins. For 1 kg raisins, 4 kg of grapes were used. Prepared raisins, after packaging, were transferred the laboratory for methamidophos residue analysis.

2.4. Analytical procedure

All grape samples, as well as for pesticide residues, were also analyzed for pH, Total solids (°Brix), acidity, and density (°Baumé). The pH was measured with a digital pH-meter with an accuracy of 0.1 pH unit. Brix was measured with a portable Brix meter, having a measuring accuracy of 0.1 Brix degree. Acidity was measured with a standard 0.1 N NaOH solution, using phenolphthalein as indicator. Acidity measured was expressed as % citric acid. The Baumé was measured with a Baumé meter.

2.5. Gas-chromatographic analysis

All samples were analyzed by a general method suitable for gas-chromatographic analysis with a nitro-

gen-phosphorus detector (NPD) properly modified as concerns the timing of the heating programme (Ministry of Welfare, 1988). The methamidophos samples were extracted three times with 3 ml of ethyl acetate. The extracts were filtered through a small Whatmann No. 1 filter paper containing a small amount of sodium sulfate into a 10 ml volumetric flask that was made to volume with ethyl acetate. During filtration, all parts were kept under crushed ice to avoid undue evaporation of ethyl acetate. The clear filtrate was injected into the chromatograph.

2.6. Gas-chromatographic determination

A Hewlett-Packard gas chromatograph was used, equipped with an NPD and with a 30 m × 0.5 mm i.d. × 0.88 μm film thickness glass capillary column HP-5. For FPD, the column used was a 30 m × 0.25 mm i.d. × 0.88 μm film thickness glass capillary column, Rtx-50, coated with cross-linked 50% phenyl methyl silicone. The injection port temperature was 250 °C and the detector temperature 290 °C. The column temperature was programmed as follows: the initial temperature of 120 °C was increased at a rate of 20 °C/min up to 210 °C with a residence time of 2 min. From 210 to 270 °C a rate of 10 °C/min was used with a residence time of 2 min, and from 270 to 285 °C a rate of 13 °C/min was used with a residence time of 5 min at the final temperature. Helium carrier gas at a flow rate of 7 ml/min was used. This column heating programme is generally used in our laboratory for the analysis of organophosphate pesticides. Samples of 2 μl of the extract were injected. Quantitation of the insecticide in the examined samples was made by comparing the detector response for the sample to that measured before and after each injection of calibration standard.

2.7. Degradation kinetics

To determine degradation kinetics, plots of concentration against time were made for each data set and the maximum square of correlation coefficients found was used to determine the equation of the best fitting curve. An exponential relation was found to apply, for all four cases studied, corresponding to first order rate equations. Confirmation of the first order rate kinetics were further made graphically from the linearity of the plots of lnC against time.

The rate constant k, was calculated from the first-order rate equation.

$$C_t = C_0 e^{-kt},$$

where C_t represents the concentration of pesticide at time t, C_0 represents the initial concentration (both concentrations expressed in mg/kg) and k is the rate constant in days^{-1}. The half-life ($t_{1/2}$) was determined from the equation $t_{1/2} = \ln 2/k$ where k is the rate constant.

3. Results and discussion

3.1. Determination and recovery of methamidophos

The method of analysis was simple and fast. The response of the detector for methamidophos was linear in the studied range of 0.1–7 mg/kg, the equation of the best fit curve being $Y = 0.253 + 2 \times 10–6X$ ($N = 10$, 3 replications) and the correlation coefficient 0.913. The method limit of detection calculated as the product of the standard deviation at the lowest validation level with the Student t-values (Ministry of Welfare, 1988), was found to be 0.01 mg/kg. The limit of determination used in this study was 0.02 mg/kg. The efficiency of the method was evaluated by spiking control samples with methamidophos at various concentration levels. Average recoveries were from 96.2% to 112%. Relative standard deviations (RSD) for residue determination were from 1.4% to 6.2%, values being within the accepted range (U.S. EPA, 1984).

Lai-Ming-Law and Siu-Kay-Wong (1996) determined methamidophos residues in food remnants with a method that was found to be applicable also to the determination of acephate, dimethoate, omethoate and trichlorfon.

Regarding changes of chemical and physicochemical parameters during sampling it can be seen from Table 1, that pH values varied from 3.3 to 3.8, Brix values varied from 17.5 to 27.3, acidity of grapes (in citric acid) varied from 4.8 to 9 and Baumé values varied from 12.3 to 15 (see Table 2).

3.2. Degradation of methamidophos

Results of degradation of methamidophos in grapes, covered, uncovered and stored in refrigerator, are presented in Table 3 and Figs. 1–3. Values reported are means from samples taken from three different plots and then analyzed (in triplicate). In all the cases studied, methamidophos residues were found to follow pseudo-first order kinetics. Half-lives of methamidophos degradation were, 16 days for uncovered grapes, 22 days for covered grapes and 267 days for grapes stored in the refrigerator. According to the EC Directive 98/82, the maximum residue limit (MRL) for methamidophos in grapes is 0.01 mg/kg. Calculated times needed for the attainment of the legal limit of the pesticide sprayed on the fruits were, 135 days (4.5 months) for the uncovered grapes, 180 days (6 months) for covered grapes and 2295 days for grapes stored in the refrigerator. Methamidophos should have a pre-harvest interval of 21 days according to manufacturer recommendations. It can be

Table 1
Measurements of pH, Brix, acidity and Baumé in samples of grapes taken from the vines and the refrigerator

Samples conditions	Days after spraying	pH	Brix	Acidity as citric acid (%)	Baumé
Samples from vines	1	3.45	18	9	13.5
	10	3.62	20	6.75	14
	20	3.76	25.8	5.2	14.8
	30	3.83	27.2	4.8	15
Samples from refrigerator	10	3.32	17.5	8.8	12.3
	20	3.35	18.3	7.5	12.6
	30	3.41	22.3	6.2	13.1
	40	3.69	25.7	6	13
	50	3.71	26.3	6.2	13.3
	60	3.70	26	5.9	13.2
	70	3.74	26.6	6.1	13.5
	110	3.81	27.3	5.5	14.6

Table 2
Recovery of methamidophos from fortified samples of grapes

Concentration (mg/kg)	Number of fortification samples	Recovery NPD (%)	RSD (%)
7	3	104	4
4	3	107	3.7
3	3	98.5	5.6
2	3	101	2.5
1.5	3	97.8	3
1	3	97.5	1.4
0.8	3	96.2	1.6
0.4	3	101	2.3
0.2	3	109	6.2
0.1	3	112	2.1

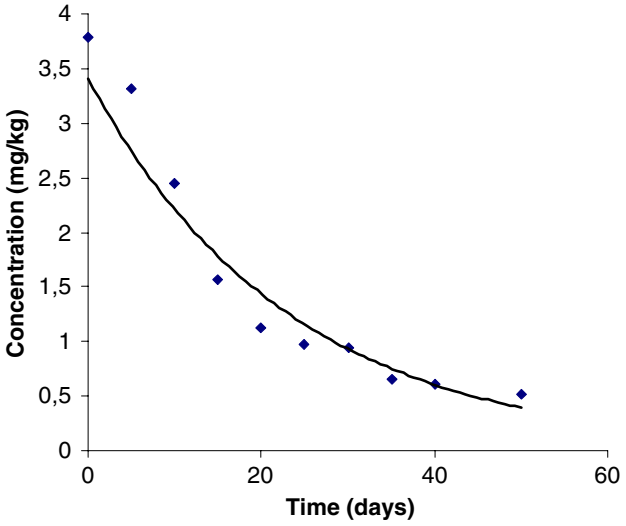

Fig. 1. Degradation of methamidophos on uncovered grapes on vines.

seen that, according to the results of these experiments, the actual time needed for methamidophos to attain the legal limit on grapes is about 6 times higher for uncovered, 9 times higher for covered and about 100 times higher for grapes stored in a refrigerator.

It should be noted that the application period was September with a relatively high ambient temperature (28–33 °C), that should enhance pesticide decomposition. Taking into consideration that time needed for the attainment of legal limit was very much extended, we believe that the use of methamidophos as a pesticide for the protection of grapes should be reconsidered.

Half-lives have been calculated for decomposition of methamidophos in other agricultural products and in the environment (soil and water): Aguilera-del-Real, Valverde-Garcia, and Camacho-Ferre (1999) determined residue levels of methamidophos in peppers, cucumbers and cherry tomatoes grown in commercial greenhouses, up to 6 weeks after being sprayed with a 60% methamidophos preparation. Half-life times were 8.68 days (cucumber), 13.28 days (pepper) and 2.77 days (tomato). Methamidophos is taken up through the roots and leaves. In studies of methamidophos in tomato plants (Antonious & Snyder, 1994), the half-lives in fruit

Table 3
Kinetic parameters for the degradation of methamidophos in soultanina grapes on the vines and during refrigerated storage

Degraded condition	Methamidophos regression equation[a]	Correlation coefficient (R^2)	Rate constant (k, days^{-1})	Degradation half-life ($t_{1/2}$, days)[b]	Time to reach legal limit (days)[b]
Uncovered	$C = 3.4e^{-0.0432t}$	0.944	0.0432	16	135
Covered	$C = 3.2e^{-0.032t}$	0.916	0.032	22	180
Refrigerator	$C = 3.9e^{-0.0026t}$	0.925	0.0026	267	2295

[a] C = concentration (mg/kg) of active ingredient of methamidophos formulations, t = time (days).
[b] Calculations based on solving equations for the highest permitted limit for residues of methamidophos ($C = 0.01$ mg/kg).

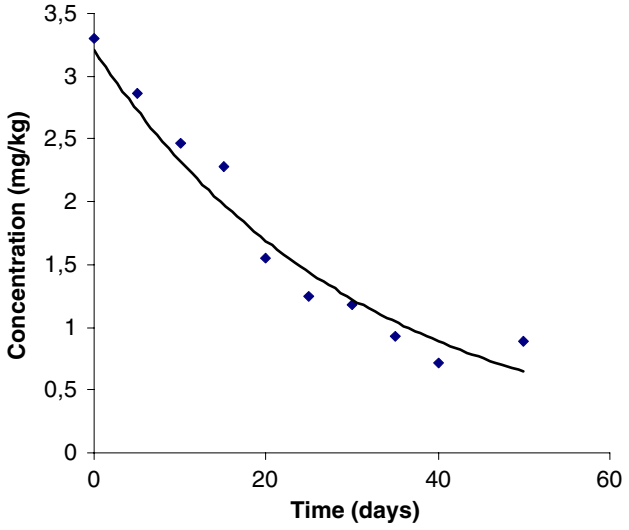

Fig. 2. Degradation of methamidophos on covered grapes on vines.

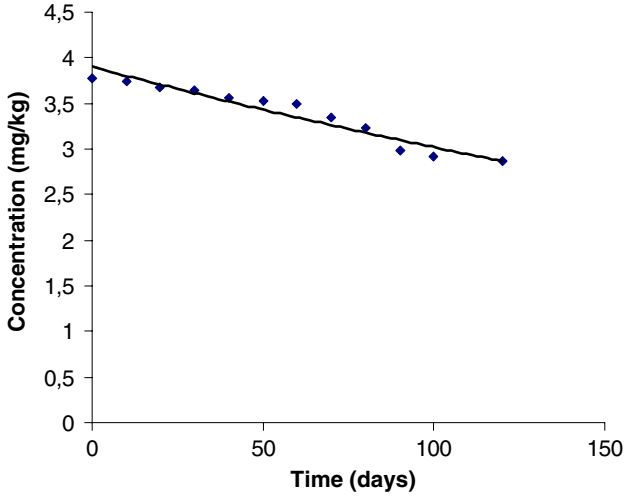

Fig. 3. Degradation of methamidophos on grapes stored in refrigerator.

and leaves were measured as 4.8–5.1 days and 5.5–5.9 days, respectively.

In aerobic soils, the half-life of methamidophos is as follows: 1.9 days in silt, 4.8 days in loam, 6.1 days in sand, and 10–12 days in sandy loam (U.S. EPA, 1989). The half-life of the chemical in water is 309 days at pH 5.0, 27 days at pH 7.0 and 3 days at pH 9.0. The chemical will break down in the presence of sunlight, and has a half-life of 90 days in water at pH 5 when there is sunlight (U.S. EPA, 1989).

Regarding the pesticide residues on raisin prepared from the samples of grapes, it can be seen (Table 4) that these were increased by 12–43% compared to initial values of pesticide on grapes. During raisin preparation, (1) water losses were in the order of 75% and (2) the whole process of raisin preparation took place in about

Table 4
Methamidophos losses during drying of grapes

Days	Residues in grapes	Expected residues in raisins	Found residues in rainins	Losses of pesticide (%)
0	3.5	14	4.48	68.0
5	2.7	10.8	3.23	70.1
10	2.05	8.2	2.3	71.9
15	1.26	5.04	1.78	64.7
20	1.14	4.56	1.63	64.2
25	1.53	4.48	1.53	65.8

2 h. Taking these facts into consideration, a fourfold increase of pesticide residues during raisin preparation was expected. From Table 4 it can be seen that a pesticide loss took place during the drying process, due probably to evaporation of the pesticide. Losses measured were from 64.2% to 71.9%.

References

Aguilera-del-Real, A., Valverde-Garcia, A., & Camacho-Ferre, F. (1999). Behavior of methamidophos residues in peppers, cucumbers and cherry tomatoes grown in a greenhouse: Evaluation by decline curves. *Journal of Agricultural and Food Chemistry, 47*(8), 3355–3358.

Antonious, G. F., & Snyder, J. C. (1994). Residues and half-lives of acephate, methamidophos, and pirimiphos-methyl in leaves and fruit of greenhouse-grown tomatoes. *Bulletin of Environmental Contamination and Toxicology, 52*, 141–148.

Athanasopoulos, P. E., Kyriakidis, N. B., & Pappas, C. (2000). Disappearance of the pesticide pyrazophos from Greek soultanina grapes on the vines and during storage. *Italian Journal of Food Science, 12*(3), 353–356.

Athanasopoulos, P., & Thanos, A. (1998). Quality characteristics of sultanina table grapes store in pilot plant scale. *Fruits, 53*, 199–206.

Baker, J., Scott, P. A., & Wilkinson, C. F. (Eds.). (1990). *The effect of pesticides on human health volume XVIII of advances in modern environmental toxicology*. Princeton, NJ: Scientific Publishing Co.

EC Directive 98/82 (1998). OJ.L. 292/27.

FAO/WHO (1986). *Recommended methods of sampling for the determination of pesticide residues*. (2nd ed.), Codex Alimentarius Commission of the joint FAO/WHO Food Standards Program, Food and Agricultural Organization/World Health Organization, Rome, (Part VI Vol. VIII).

HSDB: (Hazardous Substances Data Bank) (1990). National Library of Medicine, Bethesda, MD (CD–ROM Version), Micromedix Inc., Denver, CO.

Hussain, M. A. (1987). Anticholinesterase properties of methamidiphos and acephate in insects and mammals. *Bulletin of Environmental Contamination and Toxicology, 38*, 131–138.

Juarez, L. M., & Sanchez, J. (1989). Toxicity of the organophosphorus insecticide methamidophos to larvae of the freshwater prawn macrobrachius rosenbergii and the blue shrimp penaeus stylirostris stimson. *Bulletin of Environmental Contamination and Toxicology, 43*, 302–309.

Kokalos, T. J. (1986). Post-harvest decay control of grapes by using metabisulfite in cartons enclosed in plastic bags. *Americal Jouranl of Enology and Vitamins, 37*, 149–151.

Kyriakidis, N. B., Athanasopoulos, P. E., Thanos, A., Pappas, C., & Yialitaki, M. (2000). Decomposition of methidathion on Greek soultanina grapes on the vine trees and during storage. *Journal of Agricultural and Food Chemistry, 48*, 3095–3097.

Lai-Ming-Law, M., & Siu-Kay-Wong (1996). Determination of methamidophos residues in food remnants. *Food-Additives and Contaminants, 13*(6), 687–694.

Marois, J. J., Bledsoc, A. M., Fubler, W. O., & Luvisi, D. A. (1985). Control of Botrytis sinerea on grape berries during post-harvest storage with reduced levels of sulfur dioxide. *Plant Disease, 70*, 1050–1052.

Ministry of Welfare Health and Cultural Affairs (1988). In P. A. Greve, (Ed.), *Analytical methods for residues of pesticides in foodstuffs* (Part 1, p. 3). The Netherlands: Rijwijk.

Nelson, K. E. (1983). Effects of in-package sulfur dioxide generators, package liners and temperature on decay and desiccation of table grapes. *American Journal of Enology and Vitamins, 3*(4), 10–16.

RTECS: Registry of Toxic Effects of Chemical Substances (1990). National Institute for Occupational Safety and Health, Cincinnati, OH.

Thomson, W. (1992). *Agricultural chemicals book I: Insecticides*. Fresno, CA: Thomson Publications.

U.S. Environmental Protection Agency (EPA) (1984). Fed. Reg. 49, 198.

U.S. Environmental Protection Agency (EPA) (1989). Pesticide Environmental Fate On Line Summary: Methamidophos U.S. EPA Environmental Fate and Effect Division, Washington, DC.