Degradation of Azinphos-ethyl in Apples Stored in Different Conditions

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Residual levels of the pesticide azinphos-ethyl were studied in an experiment on field-sprayed Grand-Smith apples. The pesticide was applied according to manufacturer recommendations. Apples received a single spraying at a rate of application of 60 g of active ingredient/100 L. Residues were determined with a simple gas chromatographic method using a 30 m glass capillary column and an NP detector. The recovery of azinphos-ethyl was 87−123%, and the limit of determination was 0.005 mg/kg. The decomposition of azinphos-ethyl was studied in apples remaining on the trees after spraying and in apples harvested and stored under ambient temperature conditions, in refrigerated rooms, and in controlled atmosphere (CA) rooms. The results show that there exists a delayed residue decline during postharvest storage compared to that for apples remaining on the trees. From the experimental results, best fit curves were determined and kinetic equations, rate constants, and half-lives were calculated. Half-lives and times to reach the legal limit (0.05 mg/kg) were, respectively, 10 and 62 days for apples on the trees, 83 and 447 days for apples stored at ambient conditions, 91 and 507 days for apples stored in CA rooms, and 136 and 749 days for apples stored in refrigerated rooms.

Keywords: *Azinphos-ethyl; insecticide residues; degradation; apples*

INTRODUCTION

Azinphos-ethyl is the common name of an *O,O*-diethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate, a nonsystemic organophosphorus insecticide with a contact action and long residual activity. Azinphos-ethyl is used to protect apples and apple trees from a number of insects. It exhibits contact action on eggs, lavrae, and insects of *Lepidasalpes ulmui*, *Cemiostoma scitella*, *Phyllonorycter blancardella*, *Carpocapsa pomonella*, and most of the *Aphis* species. This pesticide is used on field crops, fruits, and vegetables (Royal Society of Chemistry, 1989). Its use is approved in all European countries.

Degradation of some synthetic organic pesticides begins as soon as they are synthesized. Breakdown of the principal components may occur due to harsh environmental conditions, prolonged period of storage, or chemical interactions (Sanz-Asensio et al., 1997).

Apples are a significant crop for Greece, cultivated mainly in the central and northern regions of the country. A traditional winter fruit harvest usually takes place during October and November. Most of the produce is stored in refrigerated rooms or in controlled atmosphere (CA) rooms and is consumed gradually until June. This long storage period has led producers to apply insecticides few days before harvesting on the basis of the understanding that during the storage period pesticides will be degraded and their residues will be below the permitted limits at the time of consumption.

The effect of postharvest storage at different conditions of temperatures, humidity, and atmospheric gas composition on azinphos-ethyl has not been studied up to now. The objectives of this work were (I) to compare the degradation rates of azinphos-ethyl in apples on the tree and during postharvest storage and (II) to determine the kinetics of degradation of azinphos-ethyl under different storage conditions.

MATERIALS AND METHODS

Field Experiment. The field experiment was carried out in 1996 in an apple orchard at Agia near Larissa, in central Greece. The experimental area comprised four plots, of eight trees each, receiving all routine horticultural practices, except of pesticide application for the last 2 months. The trees ages were ≈15 years. A proprietary aqueous emulsion of 40% azinphos-ethyl w/v was used in these experiments. The pesticide was applied at a rate of 60 g of active ingredient (ai)/100 L of water, which is the recommended application dose (RD). Three of the experimental plots received the RD, and one plot was not treated to be used as control. The emulsion was applied with a motorized mist blower, and the trees were sprayed to runoff. Spraying was performed on November 11, 1996, at the harvesting period. There was no rainfall at any time during the experimental period. The average minimum daily temperatures during the experiment were 11−15 °C and the average maximum range from 23 to 27 °C.

Sampling, Processing, and Storage. Sampling was performed by randomly collecting apple fruits from various places of the experimental plot, according to the FAO/WHO (1986) recommendations. Samples were taken 24 h after the pesticide applications. This time was considered enough for the emulsion to adhere and dry on the fruit. Samples were taken according to the following storage schedule. Fifty (200−250 g each) fruits were stored at ambient conditions, 80 in a refrigerated room, and 85 in a CA room. Samples intended for ambient conditions and for refrigerated room storage were transported and stored in our laboratory facilities, whereas

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samples for CA were stored in a commercial CA room near the orchard.

Storage conditions were as follows: (ambient temperature) temperature of 18 ± 5 °C, relative humidity (RH) of ~60%; (refrigerated room) temperature of 0 ± 0.5 °C, RH of 85%; (CA room) temperature of 0 ± 0.5 °C, RH of 92−95%, air composition (ultralow-oxygen atmosphere) of 0.9−1% oxygen, 2.5% carbon dioxide, and 96.5% nitrogen.

Collected samples were analyzed according to the following plan: Apples were removed directly from the tree every 3 days. Apples stored in ambient conditions were sampled every 3 days in the beginning and every 5 days later. Apples stored in the refrigerated room were sampled every 10 days for the first 2 months and every 15 days afterward. Apples stored in the CA room were sampled every 30 days.

Samples of five fruits (~1 kg) were chopped and blended.. Part of the homogenized material was extracted and filtered. The extract is water free and remains stable at -18 °C for months. The usual storage time in this work was from 1 to 5 days from the extraction process.

Analytical Procedure. All samples were analyzed by a properly modified general method suitable for gas chromatographic analysis with a nitrogen−phosphorus detector (NPD) (Ministry of Welfare, 1988). According to the method, 50 g of the homogenized sample was mixed with 100 mL of ethyl acetate and 50 g of sodium sulfate. The mixture was blended for 2 min, and the extract was filtered on Whatman No. 1 filter paper containing 2 g of sodium sulfate into a conical flask. During filtration all parts were kept under crushed ice to avoid undue evaporation of ethyl acetate. The clear filtrate was injected into the chromatograph.

Gas Chromatographic Determination. A Hewlett-Packard gas chromatograph was used, equipped with a splitless injector, an NPD, and a 30 m × 0.5 mm i.d. × 0.88 μm film thickness glass capillary column (Hewlett-Packard) coated with cross-linked 5% 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 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. Samples (in triplicate) of 2 μL of the extract were injected, and quantification of the insecticide was performed by automatic integration of the peak areas. Certified standards of azinphos-ethyl were used for external calibration.

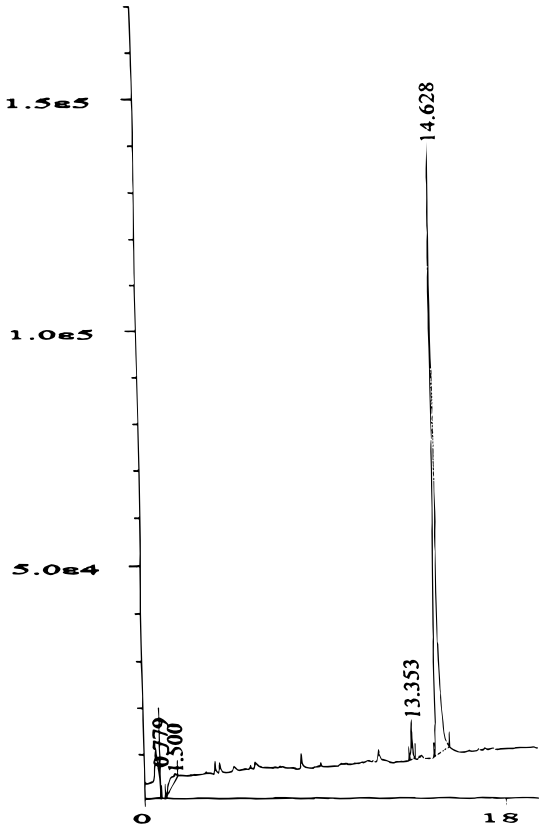

Figure 1. Fortified control apple sample with 0.1 mg/kg azinphos-ethyl. Chromatogram area corresponds to 0.92 mg/kg.

Table 1. Mean Recovery and RSD for Azinphos-ethyl on Apples at Various Fortification Levels

concn (mg/kg)	recovery (%)	RSD (%)
0.1	87.3	12.6
0.2	122.9	9.6
0.5	108.8	18.6
0.8	117.8	3.4
1.0	92.1	2.2
1.5	112.3	2.3
2.0	92.5	1.0
2.5	110.6	1.7

RESULTS AND DISCUSSION

Determination and Recovery. The method of analysis was simple and fast. The response of the detector for azinphos-ethyl (standard azinphos-ethyl in ethyl acetate) was linear in the studied range of 0.005−2.5 mg/kg, the equation of the best fit curve being $Y = 0.025 + 108X$ ($N = 9$) and the correlation coefficient 0.997. Figure 1 shows a gas chromatogram of a sample fortified with azinphos-ethyl apple. Quantitation of the insecticide in the examined samples was made by comparing the detector response for the sample to that measured daily for the calibration standard within the linear range.

The efficiency of the method was evaluated by spiking control samples with azinphos-ethyl at various concentration levels. The results of the recovery study are presented in Table 1. As seen from this table average recoveries were from 87 to 123%. Relative standard deviations were from 1.0 to 18.6%, values within the accepted range for residues determination (Greve, 1984). The method limits of determination evaluated as the product of the standard deviation at the lowest validation level with the Student t values [U.S. EPA, 1984 (ref p 9)], which at 99% confidence level and for 2 degrees of freedom of 6.96 was found to be 0.005 mg/kg.

For azinphos-ethyl, maximum residue limits in and on fruits and vegetables are considered for the parent compound only and not metabolites (82/528/EEC still in use). In addition, azinphos-methyl is not converted to its oxon on plant surfaces (Tietz et al., 1957; Magill and Everett, 1966). Therefore, only residues of the parent compound (azinphos-ethyl) were studied.

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 ln C against time.

Table 2. Kinetic Parameters for the Degradation of Azinphos-ethyl during Apple Storage

storage condition	eq for azinphos-ethyl degradn[a]	correl coeff, R^2	rate constant k, days^{-1}	degradn half-life $t_{1/2}$	time to reach legal limit, days[b]
tree	$C = 3.179\ e^{-0.067t}$	0.9522	6.7×10^{-2}	10	62
ambient	$C = 2.139\ e^{0.0084t}$	0.9653	8.4×10^{-3}	83	447
CA	$C = 2.359\ e^{0.0076t}$	0.9652	7.6×10^{-3}	91	507
refrig	$C = 2.278\ e^{0.0051t}$	0.9038	5.1×10^{-3}	136	749

[a] C = concentration (mg/kg) of active ingredient in azinphos-ethyl formulations, t = time (days). [b] Calculations based on equation solutions for the attainment of the higher permitted limit for residues of azinphos-ethyl (C = 0.05 mg/kg).

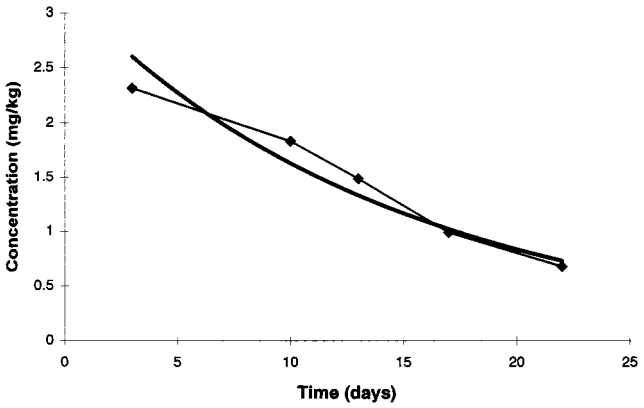

Figure 2. Degradation of azinphos-ethyl on apples from the trees.

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

$$C_t = C_0\, e^{-kt} \tag{1}$$

where C_t represents the concentration of pesticide at time t, C_0 represents the initial concentration (both concentrations are expressed in milligrams per kilogram), and k is the rate constant in days^{-1}. The half-life ($t_{1/2}$) was determined from the equation for each experiment. The kinetic rate constant can be calculated from the first-order rate equation

$$dt = -k(dC/C) \tag{2}$$

that after integration between t_1 and t_2 yields

$$k = \ln(C_1/C_2)/\Delta t \tag{3}$$

where Δt is the time interval $t_1 - t_2$ and C_1 and C_2 are the pesticide concentrations at times t_1 and t_2, respectively. Kinetic equations, rate constants, and half-lives calculated for the degradation of azinphos-ethyl under the different conditions studied are presented in Table 2.

Degradation of Azinphos-ethyl. Results of application of azinphos-ethyl on apples are presented in Figures 2−5 and in Table 2. The values of the insecticide residues are referred to the whole fruit including the skin. Regarding the application on apple trees, results are presented up to the 22nd day (Figure 2) due to a producers' strike at that time. Relative standard deviations (RSD) for measurements of apples from the trees were from 1.5 to 4.0%. RSD for measurements on apples stored under different conditions were from 0.5 to 4.0%.

It can be seen that in all cases degradation follows first-order kinetics. Half-lives of the insecticide degradation are 10 days for the tree but extend to 83 days for storage under ambient temperature conditions, to 91 days for storage under CA conditions, and to 163 days

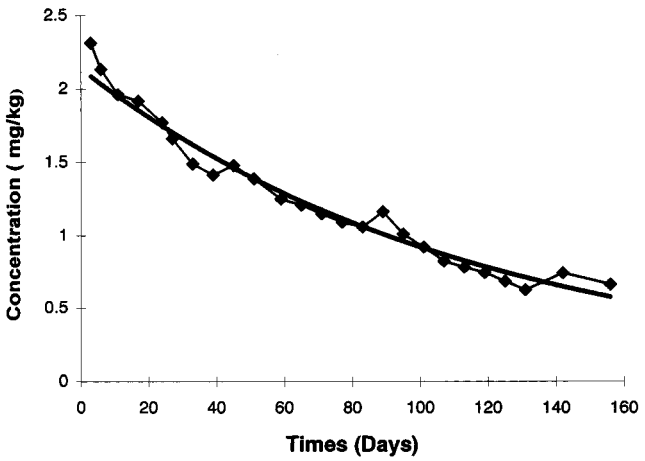

Figure 3. Degradation of azinphos-ethyl on apples stored under ambient temperature conditions.

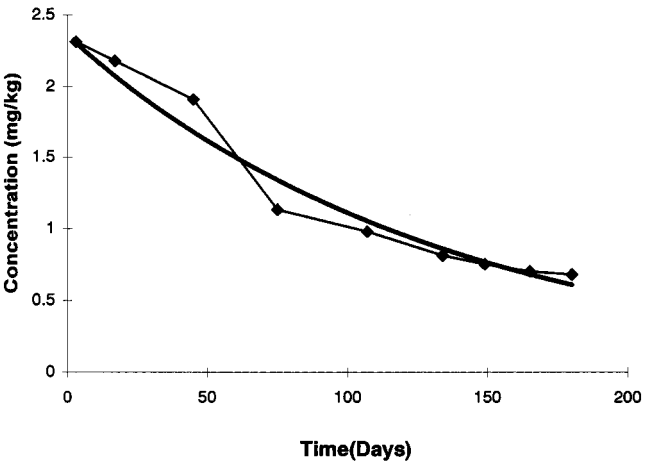

Figure 4. Degradation of azinphos-ethyl on apples stored under CA conditions.

for storage in a refrigerated room. RSD for rate constants (k) and half-lives ($t_{1/2}$) are between 14 and 17%. Compared to tree conditions, to reach the same level of insecticide residue under different storage conditions requires ~7-fold more time for storage under ambient temperature conditions, ~8-fold more time for storage under CA conditions, and ~11-fold more time for storage under refrigerated conditions. Solutions of the relevant equations (Table 2) for C = 0.05 mg/kg (the maximum legal limit of the insecticide on foods) (British Crop Protection Council, 1987) can give the corresponding time (t, days). These time calculations give 62 days for degradation on the trees, 447 days for degradation under ambient temperature storage, 507 days for degradation under CA storage, and 749 days for degradation under refrigerated storage. From these results it can be seen that the time recommended by the insecticide manufacturer for safe collection of fruits after spraying (20 days) is not adequate under the experi-

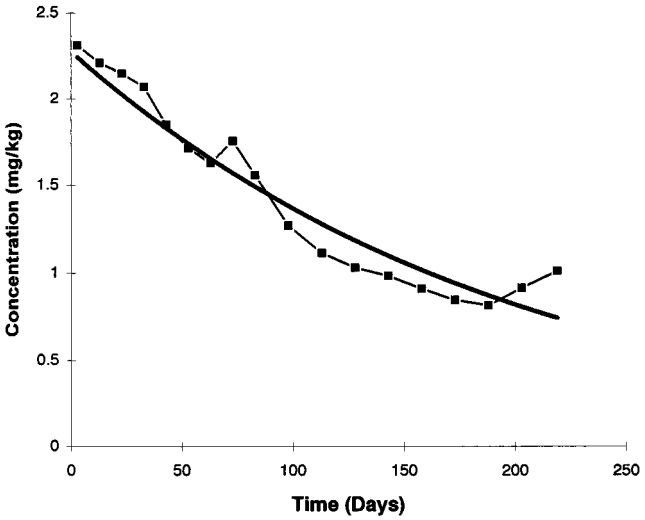

Figure 5. Degradation of azinphos-ethyl on apples stored under refrigerated conditions.

mental conditions used. Also, the time found to reach safe levels of the insecticide residues is >1 year for storage under ambient and CA conditions and >2 years for refrigerated storage. Another interesting point is that half-lives and times needed to reach legal limits for the insecticide under ambient and CA conditions are very similar. CA rooms have a very low oxygen percentage and a low temperature but a rather high RH. On the other hand, ambient temperature conditions exhibit a lower RH but a higher temperature. Refrigerated rooms have a low temperature (equal to that of CA rooms) but a lower RH. The processes responsible for the degradation of pesticides in fruits and vegetables can be classified as physical, chemical, and biological. Heat and cold occasionally contribute to pesticide degradation (Barcelo et al., 1994). Water in the form of solution or humidity, a principal reactive agent of chemical degradation, is responsible for considerable breakdown of pesticides in solution or in contact with fruit surfaces. For many pesticide molecules, hydrolysis is a primary route of degradation (Lartiges and Garrigues, 1995). Our findings may suggest that high humidity may be the reason for increased degradation rates for storage under CA conditions. Pesticide decomposition mechanisms could be hydrolytic or oxidative. Accordingly, it could be assumed that the mechanism for azinphos-ethyl degradation during apple storage may be mainly hydrolytic rather than oxidative. Literature on the decomposition of azinphos-ethyl is limited. The average half-life for azinphos-methyl on and in oranges was 46 days in Florida, where rainfall averaged 10−15 in. On the other hand, the half-life of oranges under the arid conditions prevailing in California was between 340 and 400 days (Gunter et al., 1963). This hypothesis can be verified by the use of model systems.

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