Effect of Storage Temperature on the Degradation of Dimethoate in Fortified Orange and Peach Juices

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The effect of storage temperature on dimethoate degradation in fortified orange and peach juices was studied. The insecticide was aseptically injected into packed orange and peach juices and stored at 40, 15, and 0 °C. Samples were taken at regular time intervals and were examined for dimethoate residues. The residues were determined with a simple gas chromatographic method; the recoveries of dimethoate from orange and peach juices were found to be from 88 to 114% for both products. The limits of determination were 0.004 and 0.003 mg/kg, respectively. From the experimental data, rate constants, half-lives, and activation energies for the decomposition of dimethoate in orange and peach juices were evaluated. During the storage of fruit juices in refrigerated rooms (0 °C) half-lives of dimethoate were found to be largely extended, being 1733 days for orange juice and 2310 days for peach juice. Corresponding times for storage at 15 °C were 533 days for both juices and for storage at 40 °C 24 days for orange juice and 24.6 days for peach juice. The activation energy for dimethoate in orange juice was 22.3 kcal/mol and for peach juice, 21.2 kcal/mol.

Keywords: *Dimethoate; insecticides; orange juice; peach juice; degradation*

INTRODUCTION

Dimethoate [*O*,*O*-dimethyl-*S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] (Kidd and James, 1991) is an organophosphorus insecticide used to kill acaries and insects systematically and on contact. It is used against a wide range of insects and acaries of orange and peach including *Aphis spiraeccola*, *Aphis gossypis*, *Toxoptera aurantii*, *Hyalopterus amygdaii*, and *Brachycaudus persicaecola* and also against acaries such as *Tetranychus* spp., *Aculus pelecassi*, *Panonychus citri*, *Bryobia prunicolla*, *Panonychus ulmi*, and *Tetranychus urticae*. In Greece dimethoate is the pesticide of choice for late treatment of orange and peach orchards against insects such as *Ceratitis capitata* and *Rychitis bachus*.

Dimethoate is a moderately toxic compound in EPA toxicity class II. It is a cholinesterase inhibitor, and it is moderately toxic by ingestion, inhalation, and dermal absorption. Dimethoate is reportedly nonirritating to the skin and eyes of laboratory animals (U.S. Public Health Service, 1995; Kidd and James, 1991). Via the inhalation route, the reported 4 h LC_{50} for laboratory animals is >2 mg/L, indicating slight toxicity (Kidd and James, 1991). Concerning chronic toxicity, in an experiment with humans given oral doses of 5, 15, 30, 45, and 60 mg/day for 57 days, cholinesterase inhibition was observed only in the 30 mg/day and higher dosage groups (Gallo and Lawryk, 1991). Concerning also teratogenic and mutagenic effects, it was found that dimethoate has a teratogenic effect in rats and cats (U.S. Public Health Service, 1995; Gallo and Lawryk, 1991). Mutagenic effects due to dimethoate exposure were seen in mice but are considered unlikely in humans under normal conditions (Gallo and Lawryk, 1991).

Dimethoate is of low persistence in the soil with degradation half-lives of 4−16 days and up to 122 days (Howard, 1991; Wauchope et al., 1992). A representative value may be on the order of 20 days. Dimethoate is highly soluble in water and may be subject to considerable leaching. It degrades by hydrolysis especially in alkaline waters (Howard, 1991; Wauchope et al., 1992). Dimethoate is not toxic to plants (Kidd and James, 1991), and it is commonly used for protection of such fruits as oranges, lemons, and peaches from insect attack. Due to the processing procedures in juice production natural (essential oils) or synthetic compounds (pesticides) from fruit peel may gain access to juices produced. Dimethoate as a systemic pesticide (*The Pesticide Manual*, 1997) will also get entrance into the juice after each application. As far as we know the fate of dimethoate in fruit juices during their storage has not been studied up to now. The objective of this work was to study the effect of storage temperature and juice acidity on the degradation rate of dimethoate in fortified orange and peach juices.

MATERIALS AND METHODS

Sample Preparation. Samples of orange and peach juices packed in 250 mL paper boxes were used in this study. Juices were received from a packing company, EVGA, G.A., and care was taken that all of the samples of each product came from the same batch.

Dimethoate solution was added by injection to each box to a final concentration of 1.6 mg/kg. This concentration was selected to correspond to that found on fruits on the trees (C. Pappas, N. B. Kyriakidis, and P. E. Athanasopoulos, unpublished results) just after spaying, following Good Agricultural Practice (GAP). Initial values measured were slightly different from those applied but less than the relevant recovery values. Commercial product of dimethoate (Rogor L 40% EC Enichem) was diluted with distilled water, and the appropriate amount was injected into each box under aseptic conditions, in a

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laminar flow cabinet. A wax drop was applied to fill the needle opening to prevent any contamination of the product and to eliminate air entrance.

Storage Conditions. The samples of orange and peach juices were divided in three lots of 15 boxes for each product. One lot was stored (a) at 0 ± 1 °C, the second (b) at ambient temperature (≈15 °C), and the third (c) in a cabinet at 40 ± 1 °C. These temperatures were selected to correspond to (a) retail market cabinets and home refrigerators, (b) ambient temperature, and (c) an accelerated test. Preliminary measurements showed the low degradation rate of this pesticide in the selected juices, so the temperature of 40 °C was chosen as an accelerated test. Samples were taken every 15 days for juices stored at 0 °C, every 10 days for juices stored at 15 °C, and every day up to the fourth and then every 2 days for juices stored at 40 °C. Sampling periods were 105 days for juices stored at 0 °C, 110 days for juices stored at 15 °C, and 20 days for juices stored at 40 °C.

Analytical Procedures. Acidity was evaluated by titration of 10 mL of juice with 0.1 N NaOH solution and phenolphthalein as an indicator. Results were expressed as citric acid.

All samples were analyzed by a general method suitable for gas chromatographic analysis with a nitrogen−phosphorus detector (NPD) (Ministry of Welfare Health and Cultural Affairs, 1988) modified with regard to the timing of the program. According to the method, 50 g of juice was mixed with 100 mL of ethyl acetate and 50 g of anhydrous sodium sulfate. The mixture was blended for 2 min, and the extract was filtered through 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; the temperature was further increased from 210 to 270 °C at a rate of 10 °C/min with a residence time of 2 min and from 270 to 285 °C at a rate of 13 °C/min 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 of 2 μL of the extract (in triplicate) were injected, and quantitation of the insecticide was performed by automatic integration of the peak areas. Certified standards of dimethoate were used for external calibration.

Degradation Kinetics. To determine degradation kinetics, plots of concentration against time were constructed for each data set, and the maximum square of correlation coefficients found was used to determine the equations of best fit curves. For all six cases studied exponential relations were found to apply, corresponding to first-order rate equations. Confirmation of the first-order rate kinetics was further made graphically from the linearity of the plots of ln C against time.

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

$$C_t = C_0\, e^{-kt} \quad (1)$$

where C_t represents the concentration of pesticide at any time t, C_0 represents the initial concentration, and k is the rate constant in days^{-1}. The half-life ($t_{1/2}$) was determined from the k value for each experiment, being $t_{1/2} = \ln 2/k$.

RESULTS AND DISCUSSION

Determination and Recovery. The method of analysis was simple. The responses of the detector for ethyl acetate solutions with dimethoate calibration standards were linear in the studied range of 0.1−2.5 mg/kg. The equation of the best fit curve for dimethoate was $Y = 0.23 + 76X$ ($N = 9$) with a correlation coefficient of 0.989.

Table 1. Kinetic Parameters for the Degradation of Dimethoate in Orange and Peach Juices

storage temp, °C	dimethoate degradn[a]	correl coeff R^2	rate constant (k), days^{-1}	degradn half-life ($t_{1/2}$), days
	Orange Juice			
40	$C = 1.621e^{-0.0289t}$	0.982	0.0289	24.0
15	$C = 1.576e^{-0.0013t}$	0.979	0.0013	533
0	$C = 1.558e^{-0.0004t}$	0.984	0.0004	1733
	Peach Juice			
40	$C = 1.644e^{-0.0282t}$	0.975	0.0282	24.6
15	$C = 1.531e^{-0.0013t}$	0.931	0.0013	533
0	$C = 1.546e^{-0.0003t}$	0.955	0.0003	2310

[a] C = concentration (mg/kg) of active ingredient of dimethoate formulations; t = time (days).

The efficiency of the method used was evaluated by spiking juice samples with the insecticide at concentration levels of 0.25, 0.5, 1, 1.3, 1.8, 2, 2.2, and 2.5 mg/kg. Average recoveries were from 88 to 114%. Relative standard deviations were from 2 to 8.4%. The values were within the accepted range for residue determination (Greve, 1986). Quantitation of the insecticide in the examined samples was made by comparing the detector responses for the samples to that measured before and after each sample with calibration standards. 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), at 99% confidence level and for 2 degrees of freedom of 6.96, were found to be 0.004 mg/kg for orange juice and 0.003 mg/kg for peach juice.

Acidity and pH of Orange and Peach Juices. The volumetric acidity of orange juice was found to be 0.8 g in citric acid/100 mL of juice and that of peach juice, 0.3 g in citric acid/100 mL of juice.

pH values at the three temperatures used were as follows: orange juice at 0 °C, pH 3.98, at 15 °C, pH 3.60, and at 40 °C, pH 3.23; peach juice at 0 °C, pH 4.3, at 15 °C, pH 4.00, and at 40 °C, pH 3.7.

The hydrogen ion concentrations that correspond to the above pH values are, respectively (orange) 1.05×10^{-4}, 2.5×10^{-4}, and 5.9×10^{-4} g·ions/L; and (peach) 0.5×10^{-4}, 1×10^{-4}, and 2×10^{-4} g·ions/L.

Degradation of Dimethoate in Orange and Peach Juices. Results of degradation of dimethoate in fortified orange and peach juices are presented in Table 1 and Figures 1−3. In all cases studied, dimethoate degradation was found to follow first-order kinetics. Half-lives of the insecticide degradation in orange and peach juice were 24 and 24.6 days for storage at 40 °C, but they were extended to 533 days for both when they were stored at 15 °C and to 1733 and 2310 days for storage at 0 °C, respectively. It can be seen that half-lives at 15 °C for orange and peach juices were 22-fold higher than that at 40 °C. Half-lives at 0 °C compared to half-lives at 40 °C were 72- and 94-fold higher, respectively. An interesting point is that half-lives of pesticide degradation in orange and peach juices are very similar. The lower pH and the higher acidity of orange juice are major differences between orange and peach juices. Dimethoate is relatively stable in aqueous media at pH 2−7 (*The Pesticide Manual*, 1997). Differences in acidity between orange and peach juices do not affect the

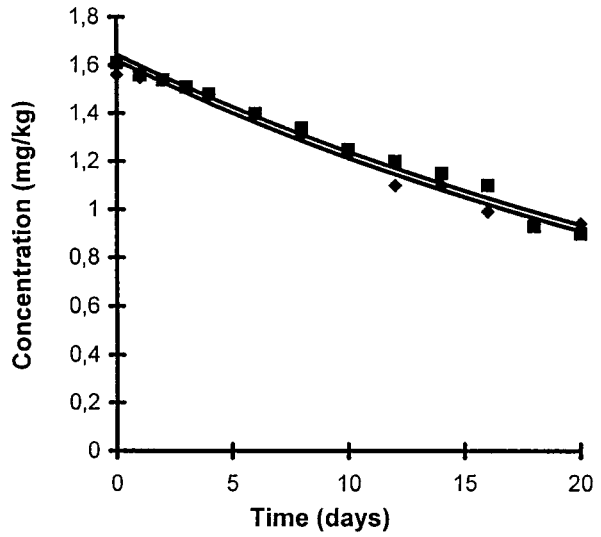

Figure 1. Degradation of dimethoate in orange and peach juices, at a temperature of 40 °C: (◆) orange juice; (■) peach juice. Reported values are means from triplicate analysis.

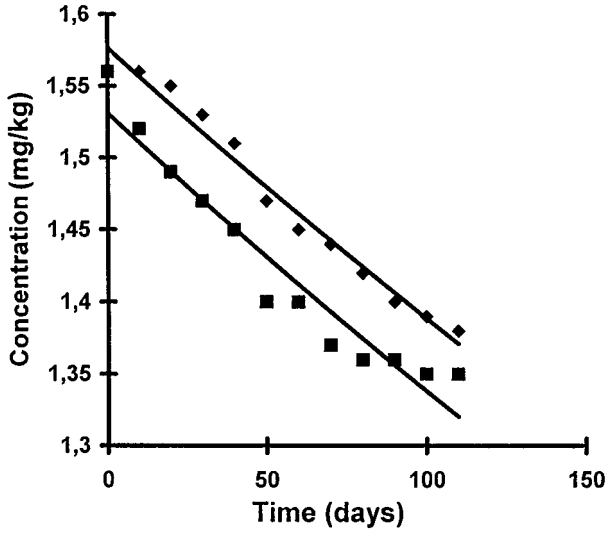

Figure 2. Degradation of dimethoate in orange and peach juices, at a temperature of 15 °C: (◆) orange juice; (■) peach juice. Reported values are means from triplicate analysis.

degradation rates, which were mostly similar in both juices. With regard to the effect of storage temperature on the degradation of both juices, it can be seen that half-lives of the pesticide were much longer at 0 °C than at 40 °C. From the pH data it can be seen that there is a significant increase of pH as the temperature decreases from 40 °C for both juices. These pH differences are much higher if they are considered from a hydrogen ion concentration point of view. Dimethoate is an (phosphorodithioate) ester and its hydrolysis will be catalyzed by acids and bases (Roberts and Caserio, 1964). It is also known that reaction rates are strongly temperature affected. Taking these two facts into consideration as well as the fact that dimethoate is "relatively" stable at acid solutions, we are proposing that perhaps the significant increase of hydrogen ion concentration and kinetic temperature effects are responsible for the very high half-lives of both pesticides at 0 °C.

Application of the Arrhenius equation for the determination of activation energies gave for dimethoate in orange juice E_a = 22.3 kcal/mol and for peach juice E_a = 21.2 kcal/mol. It is clear that these values are quite close, which means that the type of juice has no significant effect.

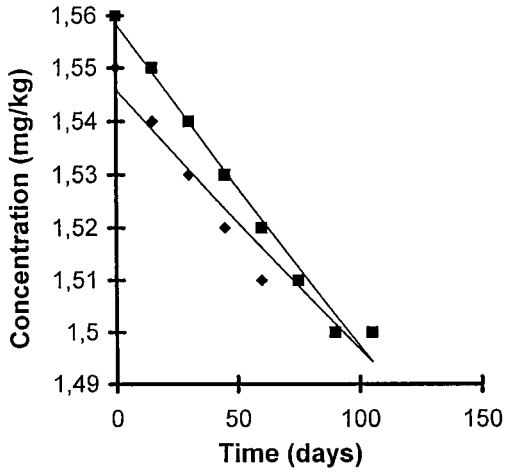

Figure 3. Degradation of dimethoate in orange and peach juices, at a temperature of 0 °C: (◆) orange juice; (■) peach juice. Reported values are means from triplicate analysis.

In a two-year trial the degradation of dimethoate in citrus (satsuma) fruits and in soil was studied after the trees had been treated at 0.1 or 0.2% active ingredient. The maximum levels of the insecticide were 5 and 11 mg/kg after the 0.1 and 0.2% rates, respectively. Complete degradation occurred within 45−57 days of treatment (Gregaga et al., 1981). Also, a study on the degradation of dimethoate residues in quick-frozen plums has shown that in plums frozen at −28 °C the dimethoate residues decreased by 50% after 7 months of storage and by 90% after 1 year. Residues in fresh fruits stored at 8 °C decreased by 50% in 3 weeks and by 90% in 6 weeks (Coch, 1976). Data from our results for fruit juices stored at 0 °C, compared with plums stored at −28 °C, have shown that degradation of dimethoate in fruit juices was much more extended, being 330 and 248 weeks, instead of 28 weeks for plums. The same trend of half-lives values can be seen in plums stored at 8 °C (3 weeks) compared to orange and peach juices (71 weeks) stored at 15 °C.

Conclusions. Differences in orange and peach juice composition do not affect the degradation rate of dimethoate in these juices. Dimethoate degradation in orange and peach juices is very slow under storage at ambient temperature and under refrigerated conditions. This has to be taken into consideration by the growers and manufacturers so that any entrance of dimethoate residues into the produced fruit juices should be avoided. Because dimethoate is a systemic pesticide, its entrance into the juice cannot be avoided if good agricultural practices are not followed.

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