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Field Study for Degradation of Methyl Parathion in Apples Cultivated with Integrated Crop Management System

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Occurrence of pesticide residues on agricultural products has become quite a common problem nowadays (Tsakiris et al, 1998). The solution of this problem has two different parts, the field part dealing with minimization of the residues and the analytical part dealing with inspection. On the field part, the integrated crop management system contributes to production of fruit that are either free from pesticide residues or with residues well below the maximum residues limits (MRLs). This system is a part of management of the rural environment with specific regulations on applicators safety and fruit production. For the detection of residues new analytical methods are developed, like for example solid phase extraction (SPE) and solid phase micro extraction (SPME), coupled to gas chromatography-mass spectrometric (GC-MS) analysis giving high levels of recovery, great specificity and with low analysis time requirements.

Methyl parathion (O, O-dimethyl O-nitrophenyl phosphorothioate) is one of the most common insecticides used in apple plant-protection. It is a widely used potent anticholinergic compound found as the active ingredient in several plant protection products. Commercially available formulations of methyl parathion are usually in the form of dust, emulsifiable concentrates (EC) and microcapsules. Up to now there are 25 different formulations registered in Greece (Gianopolitis 2000). Most of them (22) are emulsifiable concentrates (EC), classified as poisonous to humans and toxic to the aquatic organisms. Two of them are microencapsulated formulations and classified as highly toxic to aquatic organisms. Only one is granular (GR) and is classified as toxic for humans and aquatic organisms. Pure methyl parathion is a colorless crystalline solid with melting point of 37-38 ^{0}C. The technical product is light to dark tan, with about 80% purity (Kidd 1991). Poisonings due to currently used pesticides are not uncommon in Crete (Tsatsakis 1998). The majority of severe intoxications (fatal and non-fatal) were attributed to intentional suicide or homocide attempts, while accidental poisonings have also been reported (Tsatsakis et al. 2001). The great importance of methyl parathion is that it usually used very close to harvesting due to its efficiency on major insects infecting the apples. There is therefore a potential danger to overcome the Maximum Residues Limits due to improper handling of the substance.

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The main objective of this study was to study the degradation process of methyl parathion on apples under local climatic conditions and hence enhance the integrated crop management system with new information on fruit production and plant protection plans, in order to produce fruits of high quality, short of pesticide residues. Following the applications, samples were obtained from both treated and untreated plots every second day, for analysis. The levels of methyl parathion were determined by GC-MS.

MATERIALS AND METHODS

The field study was performed according to a protocol containing information about the study objective; plot design, applications, samplings, storage and shipment of residue samples, residue analysis and final report (Anonymous 1997). All the procedures were according to Good Laboratory Practice applied to field studies, following specific guidelines known as SOPs (Standard Operated Procedures). A motorized backpack sprayer was used for the application after a proper calibration. The test field was divided in two plots T1 and C. T1 was the application plot and C was the control plot. The calculations for estimating the volume of the spraying solution are shown in table 1. The product used contained 40% of the active ingredient. During the application the temperature was 17 ^{o}C, relative hydration 67%, soil conditions were fine and foliage was dry. The calculation of spraying time was done according to the results of calibration. Both treated and non-treated plots were labeled on trees and in front of each plot. The operator did both the application and mixing loading.

The first sampling was done when the fruits dried, immediately after the first application. Records such as temperature (min max), number of fruits in each sample and diameter of the selected fruits were kept for each sampling day. Samples were taken every second day for 16 days. Each sample was packed separately and labeled. The total weight of each sample was at least 1 Kg and consisted from 12 apples. The samples were chopped and blended before deep-freezing without any prior process. The directions of the FDA were followed exactly for the sampling procedure (Clifton 1996). An imaginary grid divided into approximately 100 areas was superimposed on the plot. The grid was extended by height until the highest part of each sampling area. The samples were obtained randomly. The sample places of each sampling were marked in proper sheets. Then the fruits were packed intact and each sample was numbered. A different pair of gloves was used for each sample. The sampling was done at two different levels. The first level started from the beginning of the foliage up to the middle and the second started from the middle of the foliage up to the end. This procedure was easy and homogeneous because all the trees in the orchard had the same height. Furthermore a marking tape was used in order to define the areas.

A 50 gr portion of the blended pulp was used to determine the methyl parathion concentration, in triplicate. More specifically, 50 gr of the homogenized

Table 1. Calculation for the spray solution.

Name		Unit	Formula	Digits	T
Plot size	PS	m^2	-	1	280.0
Number of replicates	Rep	m^2	-	-	1
Total plot size to be treated	TPS	m^2	PS*Rep	1	280.0
Plant / plot	PP	/plot	-	-	5
Plants / ha	PH	ha	-	-	179
Total plants to be treated	TPP	m^2	PP*Rep		5
Factor	F	-	TPP/PH	7	0.0279330
Compound / ha in gr or ml /ha	Cha	/ha	-	-	2000
Spray volume / ha	Wha	L	-	-	2000
Compound/ plot or treatment in ml (if liquid)	Cp	Ml	Cha*F	4	55.8659
Spray volume / plot or treatment	Wp	Ml	Wha*F*1000	-	55866
Safety factor absolute	Sfabs	Ml	-	-	10165
Safety factor relative	SF%		Sfabs/Wp+1	4	1.1900
Amount / volume of compound to be prepared in gr or ml	Cp-sf	...	Cp*SF	2	66.48
Volume of water to be prepared	Wp-sf	Ml	Wp*SF	-	66480
Tolerance (Min Max)	T	....%	Wp*(1±T/100)	-	2793

Table 2. Fortification levels and recovery rates of methyl parathion from apple tissues

Number of replicates	Injection volume µl	Fortification level ppm	Recovery rates %	Average of recovery rates	SD
3	5	2	98, 95, 100	97.6	2.5
3	5	1	97, 99, 94	97.3	1.5
3	5	0.5	99, 98, 99	98.6	0.5

sample was mixed with 100 ml acetonitrile for 5 min, followed by the addition of 10 gr sodium chloride. A 13ml portion of acetonitrile extract was purified by a clean up step using ENVI™-C18 (6 mL, 0.5 g) solid phase extraction cartridges (SUPELCO, Bellefonte, USA). After dehydration, with sodium sulfate, and preconcetration under nitrogen stream the sample was transferred to the ENVI™–Carb (6 mL, 0.5 g) SPE tube (SUPELCO, Bellefonte, USA) and

eluted with 10 mL of acetonitrile:toluene (3:1) mixture. Because the carbon-based packing is nonporous the samples can be processed rapidly and the total preparation time per sample did not exceed two hours. Relative to traditional liquid-liquid extraction the use of SPE tubes provided superior and more uniform recovery of polar analytes and comparable results for less polar compounds. Previous studies report recoveries of various analytes between 85 to 110% (Danis *et al.* 2000; Chaput 1998; Kaphalia 1990; Fillion et al. 1995). After reconcentration and solvent exchange to acetone (final volume 1 mL), 5 μL were injected and analyzed in a Shimadzu (Kyoto, Japan) QP 5050A gas chromatograph-mass spectrometer (GS-MS). The column used was an MDN-5S fused silica capillary, 30mX0.32 mm i.d. (SUPELCO, Bellefonte, USA) column. Temperature conditions were: injector 220 ^{0}C, column program 50 ^{0}C up to 160 ^{0}C (10 min) rate 5 ^{0}C/min and from 160 ^{0}C up to 250 (20 min) 15^{0}C/min, detector temperature 250 ^{0}C. Helium with flow rate 1.5 mL/min (87.5 kPa) was used as the carrier gas. The ionization mode was Electron Impact (EI) and detector volts 1.2 kV. Mass range from 45 up to 400 m/z and scan speed 1000 amu /sec. The retention time of methyl parathion was 30.60 min. Total ion chromatogram (TIC) was used for quantification. The external standard method was used for calibration.

The calibration curve was established at 5 concentration levels i.e. 1, 0.5, 0.1, 0.05 and 0.025 ppm with five replicates per level. The equation describing the curve was $y = (449331.9\pm13612.0)x + (16484\pm740.1)$, $R=0.997$ and CL = 95%. The values for limit of detection (LOD) and limit of quantification (LOQ) were 0.003 ppm and 0.009 ppm respectively. The recovery of methyl parathion from fruits was studied at three fortification levels i.e. 0.5, 1, 2 ppm. The results are shown in Table 2.

RESULTS AND DISCUSSION

The concentration of the active ingredient on the fruit decreased due to volatilization, plant growth, metabolism in the plant tissues and the effects of climatic factors. Temperature affects the volatilization from the plant surface, the speed of penetration in to plant tissue and also the movement and the metabolism in the plant. Solar radiation is responsible for the photolytic degradation. There are many factors, such as the meteorology, which should be considered, in order to harvest fruits without pesticide residues. The quantity of the solar radiation that abuts on the foliage depends on the latitude and the percentage of cloudiness. Also very important is the geographic locality of the area. Fields with southerly locality accept greater amounts of solar radiation compared to northward fields. Finally, the shape of the tree affects the amount of direct and diffusive solar radiation (Sfakiotakis 1993).

The density of the foliage affects the intensity of the solar radiation at different parts of the tree. The apple trees accept most solar radiation at the outer parts of the foliage, about 70 – 100% (Looney 1968). The temperature of the tree was not always the same at all parts of the tree. The insolation, the percentage of

Table 3. Concentration of methyl parathion (mg/Kg) in fruits samples of upper and lower parts of the foliage versus time

Days after last application	0	2	4	6	8	10	12	14
Fruits samples of the upper parts of the foliage	2.65	1.06	0.56	0.27	0.17	0.09	0.04	Nd
Fruits samples of the lower parts of the foliage	3.12	1.95	0.80	0.41	0.24	0.1	0.08	Nd

shadow from other leaves and the air temperature affect the microclimate of foliage. Generally the leaves at the outer part of the foliage with meridian insolation have higher temperatures than the northward ones. The leaves of the internal parts of the foliage have smaller temperature variations.

Table 3 shows the concentration of methyl parathion (mg/ Kg) in fruit samples from the upper and lower parts of the foliage versus time. According to the statistical analysis (t test) there was no significant difference between these values. Based on these results we calculated the average concentration of the active ingredient for each day. Figure 1 shows the concentration of methyl parathion (mg/ Kg) in apples versus time. The fact that there was no statistical significant difference between the initial levels of residues from the two different parts of the foliage indicates three things. The first is that there was no spottiness during the application. To be more specific the applicator spent the same time during the application at all the parts of the foliage. This is very important because usually applicators spend more time at the parts of the foliage with higher concentration in fruits. The second is that the levels of the active ingredient which remain initially on the fruits where independent from the part of the foliage. This is true only for homogeneous application. The third is that the height of the apple trees, approximately 3 m., was not sufficiently critical for compartmentation of the foliage in to two different degradation areas.

Figure 1 shows the results of the concentration of Methyl Parathion in apples versus time. The residue levels were lower than MRLs after the 8^{th} day post application. Based on these results the half-life of the detectable residues was between 2 and 3 days. According to the bibliography the percentage of residue reduction during the first days was more than 60%. In our trial the corresponding percentage was 47.7% during the first 2 days following application. The main reason for this was the increase of volatilization speed of

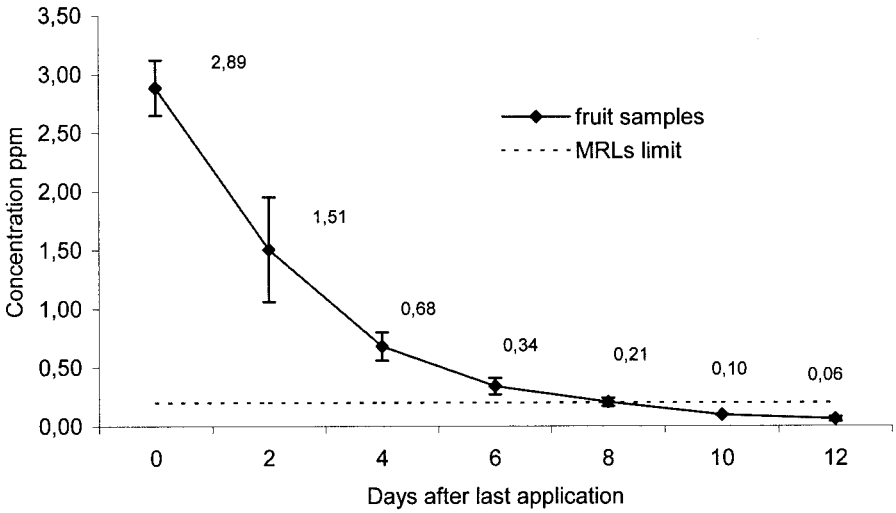

Figure 1. Methyl parathion concentration (mg/Kg) in apples versus time

the active ingredient due to high temperatures and the photolytic degradation. Methyl parathion is a non-systemic substance; hence the active ingredient was not able to penetrate the epidermis of fruits. According to this there was not metabolism in plant tissues. Furthermore during the trial period there was no rain so there was no run off of active ingredient from the fruit surface. Moreover maturation did not affect the degradation progress. As we know the variation in the size of the fruit as a result of the ripening process affects the quantity of residues, due to the increase both of the weight and the surface of the fruit. In our trial there was no such effect because the fruits had already reached the final size. Figure 2 shows the duration of solar radiation, the maximum and the minimum temperatures of each day during the trial. There was full sun during the whole period of the study and the rest of the climatic parameters were obtained from the closest national meteorological station.

Concerning the part of integrated crop management the responsible scientist should compose specific directives for homogeneous application of the product to the foliage. An uneven distribution of the pesticide solution might cause two potential problems. The first is that the initial levels of active ingredient on the fruits may be higher than expected and the levels of residues during harvesting will be found above or close to MRLs. The second problem is that the insects and the pathogens are not only at the fruits but also on the leaves, which should also be spayed in order to achieve satisfactory protection and avoid the qualitative debasement of fruits. Moreover cooperation with local weather stations should be established in order to create a database, which would be useful to predict the best harvest time. Provided this database is enhanced with the results of pesticide residue analysis, the scientist may be able to predict for specific dose, how many days after the last application the fruits are free of

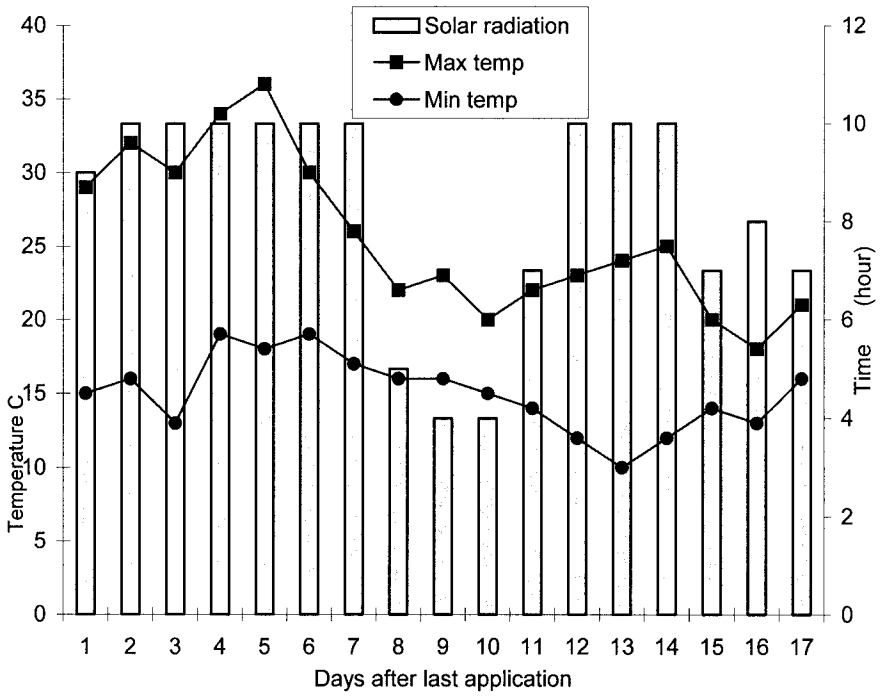

Figure 2. Maximum, minimum temperatures and duration of solar radiation

pesticide residues under local climatic conditions.

Studies with human volunteers have found that of 1 to 22 mg/person/day have no effects on cholinesterase activity. In a 4-week study of volunteers given 22, 24, 26, 28 or 30 mg/person/day, mild cholinesterase inhibition appeared in some individuals in the 24, 26 and 28 mg dosage groups (Gallo and Lawryk, 1991). In the 30 mg/person/day (about 0.43 mg/kg/day) group, red blood cholinesterase activity was depressed by 37%.

On the 10^{th} day post-application the residues of methyl parathion on the apples were 0.1 mg/kg. According to this a person should consume 240 Kg of apples for 4 weeks in order to suffer a mild cholinesterase inhibition. Under normal circumstances it is impossible for a human to consume such a large quantity of apples. One parameter that we always should consider is that is impossible to isolate the exposure in groups such as the farmers or the applicators. Implementation of precaution measures is suggested for the most prone to exposure groups, like the farmers and applicators. Monitoring of cholinesterase activity might be useful to assess exposure. Furthermore we suggest establishing communication between the responsible scientists and local hospitals or poison units during the application season, in order to get responsible advice about the formulations and the active ingredient that will be

used. Finally the applicators should always follow the directive of the application in which all the information about the application is included (formulation, active ingredient, dosage etc). These measures may help reduce the number of acute pesticide intoxications.

REFERENCES

Anonymous (1997) Guidelines for the generation of data concerning residues as provided in Annex II, part A, section 6 and Annex III, part A section 8 of Directive 91/414/EEC, concerning the placing of plant protection products on the market .- working document 1607/VI/97 rev. 1 of the Commission of the European Communities

Chaput D (1988) Simplified multiresidue method for liquid chromatographic determination of N-methyl carbamate insecticides in fruits and vegetables. J Assoc Off Anal Chem 71:542-546

Clifton M (1996) Pesticide laboratory training manual. AOAC International, Maryland.

Fillion J, Hindle R, Lacroix M, Selwyn J (1995) Multiresidue determination of pesticides in fruits and vegetables by gas chromatography mass-selective detection and liquid chromatography with fluorescence detection. J Assoc Off Anal Chem 78:1252-1266

Gallo, M. A. and Lawryk, N. J.(1991) Organic phosphorus pesticides. In Handbook of Pesticide Toxicology. Academic Press, New York, NY.5-3

Gianopolitis K (2000) Index 2000 Agrotipos SA, Athens

Kaphalia BS, Takroo R, Mehrotra S, Nigam U, Seth TD (1990) Organochlorine pesticide residues in different Indian cereals, pulses, spices, vegetables, fruits, milk, butter, deshi ghee, and edible oils. J Assoc Off Anal Chem 73: 509-12

Kidd H, James DR (1991) The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK

Looney NE (1968) Light regimes within standard-size apple trees as determined spectrophotometrically. Proc American Soc Hort Sci 93:1-6

Sfakiotakis ME (1993) General Arborization ed. 3, typo MAN, Thessaloniki

Danis TG, Stratis A (2000) Pesticides residues analysis in fresh and canned fruits. In: Procced. 1st European Conference on Pesticide and Related Organic Micro-Pollutants in the Environment, Ioannina, p 79

Tsakiris I, Niklis N, Litras K, Miliadis G, Tsatsakis AM (1998) Monitoring for pesticide residues, problems of applicators and the environment. In: Procced. of 1st Hellenic Congress of Legal Medicine and Toxicology, Thesaloniki, p 67-68

Tsatsakis AM (1998) More fatal methomyl poisonings in Crete. Sci Justice 38:282-3

Tsatsakis AM, Bertsias GK, Mammas IN, Stiakakis I, Georgopoulos DB (2001) Acute fatal poisoning by methomyl caused by inhalation and transdermal absorption. Bull Environ Contam Toxicol 66:415-420