

# Three-year study of fenthion and dimethoate pesticides in olive oil from organic and conventional cultivation

A. M. Tsatsakis\*, I. N. Tsakiris, M. N. Tzatzarakis,  
Z. B. Agourakis, M. Tutudaki and A. K. Alegakis

Laboratory of Toxicology, Department of Medicine, University of  
Crete, Voutes GR-71409, Iraklion, Crete, Greece

(Received 16 July; revised 20 November 2002; accepted  
26 November 2002)

*Residues of fenthion and dimethoate pesticides were determined in organic and conventional olive oils by liquid–liquid and solid-phase extractions with subsequent gas chromatography and mass spectrometric analysis. The olive oil samples were collected from Crete during 1997–99. The average concentrations of fenthion in conventional olive oils were 0.1222, 0.145 and 0.1702 mg kg<sup>-1</sup>, and for dimethoate were 0.0226, 0.0264 and 0.0271 mg kg<sup>-1</sup> for 1997, 1998 and 1999, respectively. The average concentrations of fenthion in organic olive oils were 0.0215, 0.0099 and 0.0035 mg kg<sup>-1</sup> for 1997, 1998 and 1999, while for dimethoate they were 0.0098, 0.0038 and 0.0010 mg kg<sup>-1</sup>, respectively. All the olive oils contained residue levels lower than the maximum residue levels according to the FAO/WHO Codex Alimentarius. The organic olive oil contained significantly lower concentrations of the two pesticides. The levels of fenthion and dimethoate in organic olive oils exhibited a decreasing trend following the implementation of the new cultivation method. We propose procedures that should be established in the organic cultivation in order to maximize its effectiveness.*

**Keywords:** fenthion, dimethoate, olive oil, gas chromatography-mass spectrometry, organic cultivation

## Introduction

Olive oil is of great economic importance for Greece where the olive groves occupy an area of 738 600 ha, according to the Hellenic statistic office. This area represents 21% of the total cultivated area and 75% of the total bosky area. The prefecture of Iraklion with 81 500 ha of olive groves is one of the most important olive oil-producing areas in Greece. The organic olive groves occupy 5845 ha. Up to 1998, 3000 olive oil press factories (the majority processing conventional and organic olive oil together), 200 factories of standardization, 25 refinement factories and 50 seed oil factories were operating in Greece. Olive oil is a very important component of the Greek diet, also known as the Mediterranean diet (Kafatos *et al.* 2000), and a high olive oil content in diet is thought to be cardioprotective.

Certification organizations for organic oil in Greece demand practically zero levels (lower than 0.001 mg kg<sup>-1</sup>) of pesticide residues in the virgin oil. This is not easy to achieve due to possible contamination of the olives during the cultivation processes. Such contamination may not be a result of direct pesticide application. It may originate from contamination from under- and groundwater, drift from neighbouring grounds and from the technological processing during oil production from the olives (contamination from water of the conventional olive processing in same factories). In order to diminish contamination from the last source, factories only processing organic olive oil have been created recently.

Olive oil is the most difficult vegetable oil to analyse compared with sunflower oil, corn oil and soybean oil. This is not only because of the relatively high amount of lipids that elute from the clean-up, but also because of the potential lipid interference at the GC determination stage (Lentza-Rizos and Avramides 1995). The fate of pesticide residues during oil extraction is closely related to the fat-solubility of the compounds under consideration. The transfer

\* To whom correspondence should be addressed.  
e-mail: aris@med.uoc.gr or toxlab@med.uoc.gr

factor (TF) is used as a measure of concentration. It is the quotient of the concentration of the active ingredient in the oil divided by the concentration of the active ingredient in the olives. The TF for dimethoate is 0.03 and for fenthion is 3.3 or 5.20 (Farris *et al.* 1992). When the TF is higher than 1, there is evidence that concentration of the residues is taking place. The most commonly used insecticide in Greece is fenthion. Generally, results obtained in recent years indicate a considerable decrease in residue concentrations (Lentza-Rizos 1994, Lentza-Rizos and Avramides 1995).

Formulations of dimethoate and fenthion in the form of emulsifiable concentrates (EC) are commercially available. Up to now 19 different formulations for dimethoate and only one for fenthion are registered in Greece (Gianopolitis 2000). The preharvest interval is 20 and 40 days for dimethoate and fenthion, respectively. Both are extremely effective in controlling the major insects infesting olives, hence they are the most commonly used insecticides. The formulations of dimethoate are classified as dangerous, irritant to humans and harmful to the aquatic organisms. The formulation of fenthion is classified as dangerous for humans, but is not classified for aquatic organisms. Suicide attempts and intoxications during spray applications have been reported using these two pesticides (Tsatsakis *et al.* 1996, 1998, 2002).

Organic cultivation is a system of cultivation used to produce fruits and vegetables by avoiding the harmful effects of pesticides on the environment. Organic agriculture confronts all the factors that determine the amount and the quality of the harvest. This system accepts that in the agro-ecosystem, precisely like all ecosystems, each factor is co-varied and affected by all the other factors. Nowadays, organic cultivation in Greece is being validated by national authorities, which are responsible for the accurate implementation of this system according to Greek law and European directives. Furthermore, the authorities can certify the products, the production procedure, or both.

The aim of this paper is to present fenthion and dimethoate residue data for olive oil samples from organic cultivation (during the initial 3 years following the introduction of this cultivation method) and conventional cultivation on the island of Crete. These results will be used to assess the relationship between the quality of organic and conventional olive oil and the levels of selected pesticide residues. Assessment of

the factors that affect the levels of pesticide residues in olive oil during the cultivation and production procedures is another objective of this paper. Finally, the monitoring of the pesticide residues for the two different types of olive oil during the 3 years (the first 3 years of organic cultivation) is essential in order to establish the effectiveness of the implementation of organic cultivation in Greece (Tzatzarakis *et al.* 2002).

## Material and methods

### Reagents

Fenthion was obtained from Rieble-de Haen (Germany). Dimethoate and dichlorvos were obtained from Cheminova Agro A/S (Lemvig, Denmark). Dimethoate is a grey-white crystalline solid at room temperature with a melting point of 43–45°C, vapour pressure 1.1 mPa (20°C) and a water solubility 25 g L<sup>-1</sup> (21°C). Pure fenthion is a colourless liquid, while technical fenthion is a yellow or brown oily liquid with a weak garlic odour, a melting point of 7.5°C, a vapour pressure of 4 mPa (20°C) and a water solubility of 2 mg L<sup>-1</sup> (20°C) (Kidd and James 1991). Chloroform, *n*-hexane (analytical grade) and methanol (HPLC grade) were obtained from Lab-Scan (Stillorgan Ind. Park Co, Dublin, Ireland). Merck (E. Merck, Darmstadt, Germany) provided acetonitrile. Figure 1 shows the structures of dimethoate and fenthion.

### Extraction procedures

*Liquid-liquid extraction (step 1)*. The liquid-liquid extraction procedure followed was a modified procedure of Lentza-Rizos (1994). A sample of olive oil (15 g) was measured into a 100-ml beaker and mixed with 50 ml *n*-hexane saturated with acetonitrile and

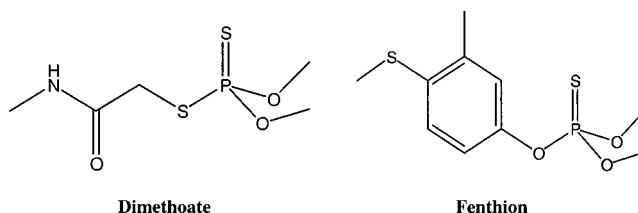


Figure 1. Structure of dimethoate and fenthion.

100 ml acetonitrile saturated with *n*-hexane. Distilled water (1 ml) was added and the mixture was extracted for 5 min and left for 15 min for the phases to separate. The acetonitrile phase was collected and extracted again with a 25-ml portion of *n*-hexane saturated with acetonitrile. The above procedure was repeated once. The acetonitrile phases from all the extractions were collected and evaporated at 30°C in a rotary vacuum evaporator. The solid residue was collected with acetone (5 ml) and was evaporated using a gentle stream of nitrogen. Finally, it was dissolved in water (5 ml).

**Solid-phase extraction (step 2).** The 5-ml aqueous solution obtained in step 1 was re-extracted using a Techelut SPE column, C<sub>18</sub> (200 mg) cartridges. The conditioning of cartridges was done with methanol (2 ml) and distilled water (2 ml). The sample was eluted from the column with a flow rate of 1 ml min<sup>-1</sup>. The elution solvent was chloroform:methanol (9:1). Extraction residues were dissolved in ethyl acetate (25 µl) before injection into GC-NPD and GC-MS, respectively.

#### Preparation of standard curves

Two separate stock solutions of fenthion and dimethoate were prepared in methanol at a concentration of 100 µg ml<sup>-1</sup> and stored at 0°C. Seven new diluted solutions containing the above compounds were prepared from the initial stock solutions and used for the fortified pesticide-free olive oil standards. The final fortified oil standard concentrations were 0, 0.0025, 0.00625, 0.0125, 0.01875, 0.0375 and 0.0750 mg kg<sup>-1</sup>. The internal standard was dichlorvos.

#### Instrumental method

The qualitative determination was performed on a gas chromatograph model Carlo Erba, Vega Series 6000 (Milan, Italy) with nitrogen-phosphorus detector (NPD) connected with Varian 4400 data system. The column used was a DB-5, 30 m × 0.32 mm, 0.25-µm film thickness capillary column. Helium (99.999%) was used as the carrier gas with flow rate of 2.4 ml min<sup>-1</sup>. The injector and detector temperatures were 270°C and 300°C, respectively. The column initial temperature was 180°C for 3 min,

then increased to 230°C at 10°C min<sup>-1</sup> and was held at 230°C for 35 min, it increased to 270°C with steps of 5°C min<sup>-1</sup> and was maintained at 270°C for 5 min. Under these conditions, the retention time of dichlorvos was 7.85 min, of dimethoate was 20.71 min and of fenthion was 37.67 min. Figure 2 shows a GC-NPD chromatogram indicating the peak of each pesticide.

Electron ionization mass spectrometric confirmatory analysis was performed on a Finnigan Mat GCQ system equipped with an AT<sup>TM</sup>-5MS (30 m × 0.25 mm i.d. × 0.25 µm) capillary column (Alltech). Pure helium (99.999%) was used as a carrier gas with a velocity of 20 cm s<sup>-1</sup>. One microlitre of the test solution was injected into the system in the splitless mode. Analysis conditions were as follows. The column temperature programme started from 70°C for 3 min, was raised to 270°C at 10°C min<sup>-1</sup> and held for 10 min and was finally raised to 310°C at 10°C min<sup>-1</sup>, where it remained stable for 2 min. The injector temperature was 270°C. The transfer line temperature was set at 275°C. The mass spectrometer acquisition parameters were: ion source 200°C, electron impact ionization 70 eV and electron multiplier voltage 1200 V. The mass spectrometer was operated at the selected ion monitoring mode and was programmed for the detection of *m/z* 87, 93 and 125 for dimethoate, 245 and 278 for fenthion, and 79, 109

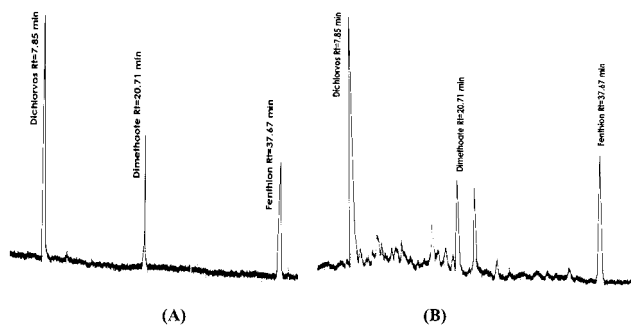


Figure 2. (A) Typical GC-NPD chromatogram of dichlorvos (1.5 µg ml<sup>-1</sup>), dimethoate (1.0 µg ml<sup>-1</sup>) and fenthion (0.5 µg ml<sup>-1</sup>). The retention time of dichlorvos was 7.85 min, of dimethoate was 20.71 min and of fenthion was 37.67 min. (B) GC-NPD chromatogram of the olive oil sample obtained in the first year of organic cultivation. The concentrations of fenthion and dimethoate are 0.0261 and 0.0135 mg kg<sup>-1</sup>, respectively.

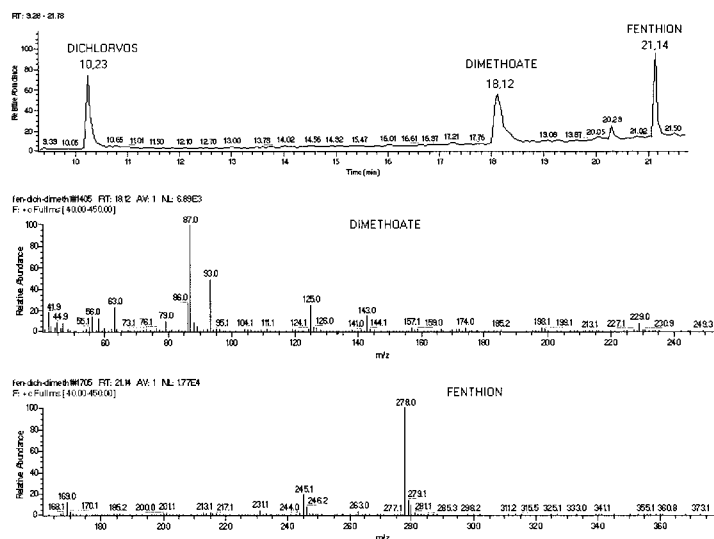


Figure 3. Typical GC-MS chromatogram of dichlorvos ( $20 \mu\text{g ml}^{-1}$ ), dimethoate ( $20 \mu\text{g ml}^{-1}$ ) and fenthion ( $15 \mu\text{g ml}^{-1}$ ). The retention time of dichlorvos was 10.23 min ( $m/z=79, 109, 185$ ), of dimethoate was 18.12 min (87, 93, 125) and of fenthion was 21.14 min (245, 278).

and 185 for dichlorvos. Under these conditions, dichlorvos eluted at 10.23 min, dimethoate at 18.12 min and fenthion at 21.14 min (figure 3).

#### Quantification of olive oil extracts

A seven-point curve was prepared daily by analysis of pesticide-free olive oil fortified with fenthion and dimethoate concentrations of 0, 0.0025, 0.00625, 0.0125, 0.01875, 0.0375 and  $0.0750 \text{ mg kg}^{-1}$ . The calibration curve is shown in figure 4. The recovery rate for fenthion was 78% ( $y = 3\text{E} + 07x - 3949.5$ ,  $r^2 = 0.9946$ ) and for dimethoate was 84% ( $y = 1\text{E} + 07x - 14482$ ,  $r^2 = 0.9971$ ). Detection limits for fenthion and dimethoate were  $0.0011$  and  $0.0018 \text{ mg kg}^{-1}$ , respectively.

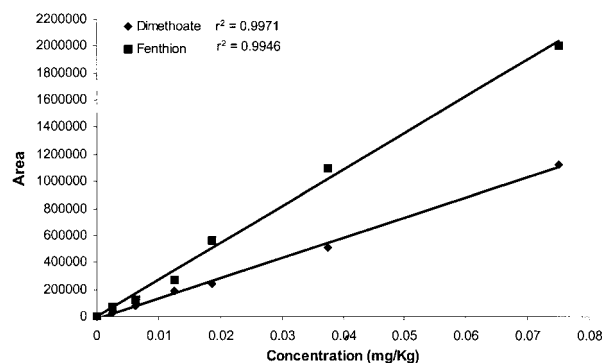


Figure 4. Calibration curve for fenthion and dimethoate in fortified olive oil samples in concentrations of 0, 0.0025, 0.00625, 0.0125, 0.01875, 0.0375 and  $0.0750 \text{ mg kg}^{-1}$ . The recovery rate for fenthion was 78%, and for dimethoate was 84%.

## Results and discussion

#### Statistical analysis

Data were subject to statistical analysis to define if there was a statistically significant difference between the values of the analysed pesticide residues in conventional and organic olive oils. An independent sample two-tailed *t*-test was used. Differences at  $p < 0.001$  were considered as significant.

To assess the significance of the pesticide residues in olive oil, consumption figures were necessary. Household budget surveys conducted in 1981–82 and 1987–88 by the Greek National Statistical Service (Trichopoulou and Vassilakou 1995) showed that the olive oil consumption in Greece was indeed high, especially in rural areas compared with olive oil consumption in the rest of the European Union.

Table 1. Statistical analysis of dimethoate and fenthion levels in olive oils samples from organic and conventional cultivation in Crete.

Year		Mean (mg kg <sup>-1</sup> ) ± SD	Relative SD	N	Minimum (mg kg <sup>-1</sup> )	Maximum (mg kg <sup>-1</sup> )	p
Dimethoate							
1997	SOC	0.0098 ± 0.0027	27.551	53	0.0045	0.0135	< 0.001
	SCC	0.0226 ± 0.0108	47.7876	50	0.0089	0.0486	
1998	SOC	0.0038 ± 0.0025	65.7895	53	0	0.0090	< 0.001
	SCC	0.0264 ± 0.0107	40.5303	50	0.0088	0.0485	
1999	SOC	0.0010 ± 0.0003	30.0000	53	0	0.0045	< 0.001
	SCC	0.0271 ± 0.0106	39.1144	50	0.0090	0.0501	
Fenthion							
1997	SOC	0.0215 ± 0.0060	27.9070	53	0.0108	0.0351	< 0.001
	SCC	0.1222 ± 0.0728	59.5745	50	0.0162	0.2952	
1998	SOC	0.0099 ± 0.0031	31.3131	53	0.0027	0.0162	< 0.001
	SCC	0.1457 ± 0.0727	49.8970	50	0.0288	0.3105	
1999	SOC	0.0035 ± 0.0024	68.5714	53	0	0.0081	< 0.001
	SCC	0.1702 ± 0.0746	43.8308	50	0.0315	0.2952	

N, number of samples; SD, standard deviation; SOC, samples of olive oil from organic cultivation; SCC, samples of olive oil from conventional cultivation.

The results from the statistical analysis of dimethoate and fenthion levels from organic and conventional olive oils samples are shown in table 1.

According to these results, there was a statistically significant difference between the following:

- Value of fenthion residues in organic and conventional olive oil samples in the same year.

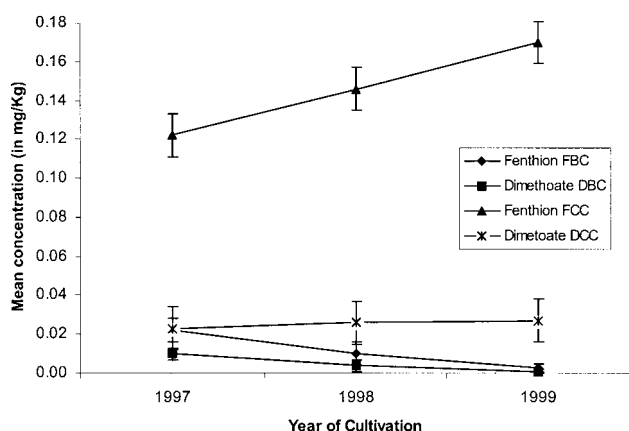


Figure 5. Average concentrations (mg kg<sup>-1</sup>) of fenthion and dimethoate in olive oil samples from organic and conventional cultivation over 3 years. Fenthion FCC and dimethoate DCC are fenthion and dimethoate average concentrations in olive oil samples from conventional cultivation, and Fenthion FOC and dimethoate DOC are fenthion and dimethoate average concentrations in olive oil samples from organic cultivation.

- Values of dimethoate residues in organic and conventional olive oil samples in the same year.
- Values of fenthion residues in organic olive oil samples in sequential years.
- The values of dimethoate residues in organic olive oil samples in sequential years.

It should also be noted that there was no statistical difference between: the levels of fenthion residues in conventional olive oil samples in sequential years; and the levels of dimethoate residues in conventional olive oil samples in sequential years.

The average concentration of fenthion and dimethoate in olive oil samples from organic and conventional cultivation for each year is shown in figure 5. The percentage of residue variation between sequential years is shown in table 2.

The maximum residue levels (MRLs) for olive oil according to FAO/WHO (1998) are 1 mg kg<sup>-1</sup> for fenthion and 0.05 mg kg<sup>-1</sup> for dimethoate. According

Table 2. Percentage of residue variation between sequential years.

	Samples of organic olive oils			Samples of conventional olive oils		
	1997	1998	1999	1997	1998	1999
Dimethoate	-	-61.22	-89.79	-	16.81	19.91
Fenthion	-	-53.95	-83.72	-	19.23	39.28

to the results of the 3-year monitoring, only in one sample of conventional olive oil (in 1999) was the level of dimethoate residue found above the FAO/WHO *Codex Alimentarius* MRLs. Moreover, higher residues of the analysed pesticides were detected in the conventional olive oil samples compared with organic olive oil samples. The first olive oil sample with no detectable residues of dimethoate was found in 1998, a year after the implementation of organic cultivation, while the first sample with no detectable residue of fenthion was found in 1999. More specifically, in 1998, eight samples with no detectable amount of dimethoate were found and none of fenthion. In 1999, 30 samples with no detectable amount of dimethoate and 10 samples with no detectable amount of fenthion were found.

It is obvious that the levels of pesticide residues in organic olive oil samples decreased from year to year. This decrease may be attributed to the switch from conventional to organic cultivation. The levels of fenthion residues were higher than the levels of dimethoate residues. This is probably due to the higher water solubility of dimethoate. Because of this, a large fraction of the active ingredient draws away with the water phase during the olive oil production. Furthermore, dimethoate degrades faster than fenthion, which is fat soluble. In conventional olive oil samples, the percentage of residue increment of fenthion was higher than dimethoate, and in organic olive oil samples the percentage of residue reduction of fenthion was lower than dimethoate. According to this, we conclude that probably in conventional cultivation fenthion was preferred for the plant protection.

Some contamination in organic olive oil samples probably originated from oil factories. Residues of pesticides remain in the machines in oil factories during the processing of conventional olive oil and contaminate the organic olive oil during its processing. To prevent the oil contamination by residues, it is suggested that the olives from organic cultivation be processed in certified factories that produce only organic olive oil.

Dimethoate has low persistence in the soil environment. A representative soil half-life is 20 days. Dimethoate is highly soluble in water ( $25 \text{ g l}^{-1}$ ) and it adsorbs only very weakly to soil particles. It is degraded by hydrolysis, especially in alkaline soils, and evaporates from the soil surface. Fenthion has a moderate persistence in soil with an average half-life 34 days. As it absorbs fairly strongly to soil particles,

it is not likely to move or leach through the soil. Thus, the contamination of olives with fenthion and dimethoate from soils and underground water is unlikely.

Drift is always a potential danger for the organic cultivation. During the 3-year follow-up, no crop dusting was performed. In addition, protection measures were always taken to avoid drift from neighbouring fields. Despite this, however, it was practically impossible to eliminate the affect of this factor. Consequently, the hypothesis that the residues in organic olive oils arise from the contamination of the olives because of drift is true but of low importance.

## Conclusion

The results reported here indicate that organic cultivation has been applied successfully. There were observable and measurable parameters that allowed control and evaluation of the efficacy of the specific cultivation system. Three years after the beginning of the organic cultivation, the percentage of the samples with no detectable residues was 18.8% for fenthion and 56.6% for dimethoate. During this period, there were significant differences in the levels of fenthion and dimethoate residues measured in organic oils in sequential years.

The system of organic cultivation should be established by law, which will allow full traceability. This will help one easily detect and isolate the problematic fields and the responsible farmers. Compared with conventional cultivation, organic cultivation has the advantage of production of olive oils free from pesticide residues and the minimization of environmental pollution.

Both in organic and conventional agriculture, the scientist should pay attention to the cultivation technique in order to eliminate the factors that contribute to the creation of conditions that favour insect infestations. Nitrogenous fertilization and irrigation should be well balanced and great care should be taken during hot and moist years where insect infestation is very likely to occur.

The results point out the necessity of monitoring not only in olive oil, but also in the great majority of the agricultural products in order to define residue levels. For the conventional olive oil samples collected around the area of Crete during 1997–99, the average concentrations of fenthion ranged from 0.1222 to

0.1702 mg kg<sup>-1</sup> and for dimethoate from 0.0226 to 0.0271 mg kg<sup>-1</sup>. For olive oil from organic cultivation, fenthion average concentration ranged from 0.0215 to 0.0035 mg kg<sup>-1</sup>, while for dimethoate it was from 0.0098 to 0.0010 mg kg<sup>-1</sup>. It is easy to see that pesticide residue levels in conventional oils remained stable during the monitored period while those in organic olive oils decreased. This emphasizes the need for new oil factories that will process only organic olive oil. Despite the difference in the residue levels, it has to be pointed out that all the analysed olive oils were absolutely safe for the consumers according to FAO/WHO (1998) MRLs.

## References

- FAO/WHO, 1998, *Residues of Pesticides in Foods and Animals Feeds*. Codex Alimentarius CX/PR 98/6 (FAO/WHO).
- FARRIS, G. A., CABRAS, P., and SPANEDDA, L., 1992, Pesticide residues in food processing. *Italian Journal of Food Science*, **3**, 149–170.
- GIANOPOLITIS, K., 2000, Insecticides. *Index 2000* (Athens: Agrotipos SA).
- KAFATOS, A., VERHAGEN, H., MOSCHANDREAS, J., APOSTOLAKI, I., and VAN WESTEROP, J. J., 2000, Mediterranean diet of Crete: foods and nutrient content. *Journal of American Dietetic Association*, **100**, 1487–1493.
- KIDD, H., and JAMES, D. R. (eds), 1991, *The Agrochemicals Handbook*, 3rd edn (Cambridge: Royal Society of Chemistry Information Services), pp. 5–14.
- LENTZA-RIZOS, CH., 1994, Monitoring of pesticide residues in olive products: Organophosphorus insecticides in olives and oil. *Journal of AOAC International*, **77**, 5.
- LENTZA-RIZOS, CH., and AVRAMIDES, E. J., 1995, Pesticides residues in olive oil. *Reviews of Environmental Contamination and Toxicology*, **144**, 111–134.
- TRICHOPOULOU, A., and VASSILAKOU, T., 1995, *Food Availability in Greece per Capita 1981–1982 & 1987–1988* (Athens: Greek National Statistical Service).
- TSATSAKIS, A. M., AGURIDAKIS, P., MICHALODIMITRAKIS, M. N., TSAKALOV, A. K., ALEGAKIS, A. K., KOUMANTAKIS, E., and TROULAKIS, G., 1996, Experience with acute organophosphate poisonings in Crete. *Veterinary and Human Toxicology*, **38**, 101–107.
- TSATSAKIS, A. M., BERTSIAS, G. K., LIAKOU, V., MAMMAS, I. N., STIAKAKIS, I., and TZANAKAKIS, G. N., 2002, Severe fenthion intoxications due to ingestion and inhalation with survival outcome. *Human and Experimental Toxicology*, **21**, 49–54.
- TSATSAKIS, A. M., MANOUSAKIS, A., ANASTASAKI, M., TZATZARAKIS, M., KATSANOULAS, K., DELAKI, C., and AGOURIDAKIS, P., 1998, Clinical and toxicological data in fenthion and omethoate acute poisoning. *Journal of Environmental Science and Health*, **B33**, 657–670.
- TZATZARAKIS, M. N., TSATSAKIS, A. M., TSAKIRIS, I. N., AGOURAKIS, Z. B., TUTUDAKI, M., and ALEGAKIS, A., 2002, Fenthion and dimethoate residue levels in olive oil samples from biological and conventional cultivations in Crete. *Proceedings of the International Conference on Rural Health in Mediterranean and Balkan Countries*, Bari, Italy, p. 138.