Persistence and Fate of Fenthion in Olives and Olive Products

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Two field experiments were carried out—with three and five treatments, respectively—to study the persistence and metabolism of fenthion in olives. Fenthion showed a low degradation rate, with a half-life of ca. 38 days. This accounts for a noteworthy effect of the number of treatments on the residue amounts at harvest time. Besides the active ingredient, five metabolites were detected in olives; among these, fenthion sulfoxide and fenoxon sulfoxide were quantitatively the most important. Olives were also processed into oil, and the partitioning of fenthion among oil, cake, and vegetation water was evaluated. Fenthion residues in oil were ca. 3 times higher than in olives, while its metabolites showed analogous residues in olives and oil or were totally absent.

Fenthion (*O,O*-dimethyl *O*-4-(methylthio)-*m*-tolyl phosphorothioate, **I**; Figure 1) is an organophosphorous insecticide with a persistent action, very efficient in the control of the olive fly. For this reason it is widely employed in olive-growing. The biological activity of fenthion is mainly due to fenthion sulfoxide (**II**), into which the parent compound is rapidly converted (Drabek and Neumann, 1985). The subsequent oxidation of **II** to fenthion sulfone (**III**), whose biological activity is considerably lower, is quite slow (Fest and Schmidt, 1982). The oxidation of the methylthio group has been ascribed to a light-induced reaction. Another oxidative process—caused by the plant enzymes—can involve the other sulfur atom in the fenthion moiety, thus leading to the formation of another three metabolites (fenoxon, *O,O*-dimethyl *O*-4-(methylthio)-*m*-tolyl phosphate, **IV**; fenoxon sulfoxide, **V**; fenoxon sulfone, **VI**) (FAO/WHO, 1973). The metabolites show higher toxicity than the parent compound: as a matter of fact, the lethal dose (LD_{50}) is 220 mg/kg for **I**, 125 mg/kg for **II–IV**, 50 mg/kg for **V**, and 30 mg/kg for **VI**. Hydrolysis of **II–VI** leads to low toxic compounds (FAO/WHO 1973). The metabolism of fenthion in some plants such as bean, cotton, cabbage, and rice has been studied. In all of these cases the main metabolites detected were **II** and **III**, though in variable amounts (FAO/WHO, 1973). Research on the metabolism of fenthion in olives is lacking, since the literature data concern only the active ingredient (AI). Experimental studies carried out in Turkey, Greece, and Italy showed that fenthion residues in olives—1 month after treatment—were 0.39, 0.15, and 0.80 ppm, respectively (FAO/WHO, 1973). Analogous values are reported in other evaluations of fenthion residues in olives (FAO/WHO, 1978a).

This paper aims to contribute to the knowledge of the fate of fenthion in olives, by means of the determination of its metabolites and the evaluation of its persistence after application. The investigation was extended to all of the products obtained from olive processing (oil, cake, and vegetation water) to evaluate the partitioning of fenthion and its metabolites among them.

EXPERIMENTAL PROCEDURES

Materials and Methods. The trial was carried out in two secular olive groves, located near Diano Marina (Imperia, Italy),

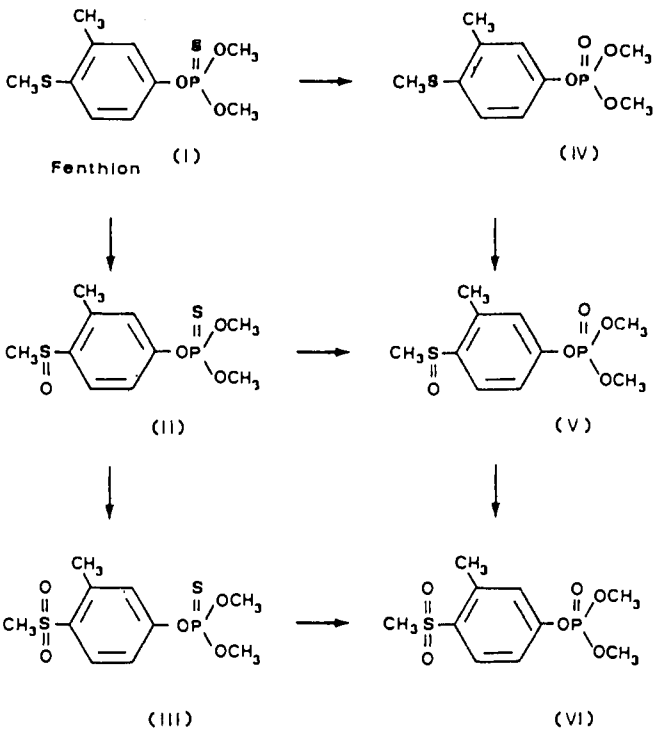

Figure 1. Fenthion (**I**) and its main metabolites: fenthion sulfoxide (**II**), fenthion sulfone (**III**), fenoxon (**IV**), fenoxon sulfoxide (**V**), and fenoxon sulfone (**VI**).

which had been subjected to a rejuvenating pruning in 1985; the cultivar was Taggiasca. The olive grove surfaces were 2000 and 3500 m^2, and the plant densities per hectare were 150 and 200, respectively. A random-block scheme was used, with three replications, and each block contained four plants. The treatments were carried out with the poisoned bait method, using Lebaycid 25, containing 25% of AI, and Buminal (protein bait) applied with a K-90 low-volume sprayer (Turbine, Pavia, Italy). The insecticide mixture was prepared at the dose of 0.8 kg/hL of Lebaycid, and ca. 250 mL—corresponding to ca. 500 mg of fenthion—was sprayed on each plant. Three treatments were carried out in one of the olive groves (on September 8 and 16 and October 24, 1991) and five treatments in the other (August 14, September 7 and 16, and October 1 and 24, 1991). Samplings started immediately after the last treatment (ca. 1 h after spraying) and were repeated on November 4, 13, and 27 and December 16 which was the harvest day. Random 2–3-kg samples, consisting of ca. 1000 drupes (200–300 per plant), were collected from each block and stored at −20 °C until analysis.

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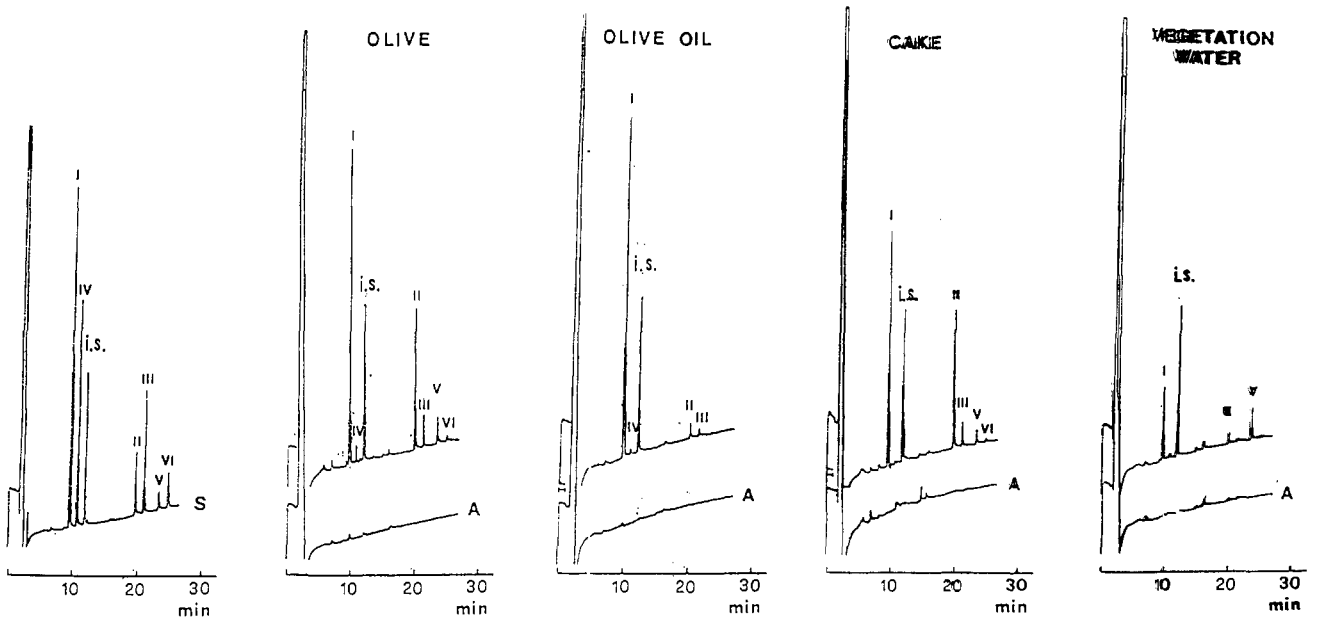

Figure 2. Chromatography of fenthion (I) and its main metabolites (II–VI): standard solution (S; concentration range 0.10–0.13 ppm); olive, oil, cake, and vegetation water samples with the respective controls (A). The operating conditions are reported under Experimental Procedures.

After harvest, a 10-kg portion of olives was processed into oil by means of an experimental oil press, using a discontinuous process (crushing, kneading, pressing, and centrifugation). Also the samples obtained from olive processing (oil, cake, and vegetation water) were stored in the same way.

Extraction Procedure. A hundred olives were weighed to establish the average weight of the fruits (ca. 2 g). The entire sample was ground in a stainless steel mixer. A portion of the ground sample together with 12 stones to reach 25 g (which would represent the weight of 12 olives) was weighed in a 250-mL screw-capped flask; 100 mL of chloroform was added, and the flask was shaken in a flask shaker (Stuart Scientific) for 30 min. The organic layer was filtered through a Whatman No. 42 filter containing 5 g of anhydrous sodium sulfate. Fifty microliters of the internal standard (i.s.) solution of parathion was added to 5 mL of the filtered organic layer before gas chromatographic analysis. The same procedure was used for cake and vegetation water. Olive oil was only diluted with chloroform (1 g in 10 mL) before injection.

Recovery Assays. Untreated olive samples were fortified with I–VI and processed according to the above-described procedure. Recovery assays, carried out at 0.02, 0.50, and 2.00 ppm, showed values obtained from four replicates ranging between 88% and 103%, with a maximum coefficient of variation (CV) of 9. Analogous values were obtained from recovery assays carried out on cake and vegetation water with the same procedure as for olives.

Apparatus and Chromatography. A gas chromatograph Carlo Erba (Carlo Erba, Milano, Italy) Mega 5160 was employed, equipped with an NPD 40 nitrogen–phosphorus detector, an AS 550 autosampler (Carlo Erba), and a split–splitless injector, connected to an HP 3396-II reporting integrator (Hewlett-Packard, Avondale, PA). A Durabond fused silica column (30 m × 0.32 mm i.d.) (J&W Scientific, Folsom, CA) was employed, with DB 210 (50% trifluoropropylsilicone, 50% methylsilicone) liquid phase (film thickness 0.25 μm). The injector and the detector were operated at 250 and 260 °C, respectively. The glass liner of the injector was treated with dimethylchlorosilane (5% in toluene) before use. The sample (2 μL) was injected splitless (60 s), and the oven temperature was programmed as follows: 150 °C for 1 min, raised to 250 °C (10 °C/min), and held for 20 min. Helium was the carrier gas at a pressure of 65 kPa. Calibration curves for fenthion and its metabolites were constructed with the internal standard method by measuring peak heights vs concentrations. Good linearity was achieved in the range 0–1 ppm with correlation coefficients between 0.9970 and 0.9994. Under these conditions, the limit of determination for fenthion and its metabolites (Thier and Zeumer, 1987) ranged between 0.002 and 0.01 ppm.

Table I. Residues (Parts per Million ± SD) of Fenthion and Its Metabolites (II–VI) in Olives after Three Treatments (Experiment 1)

	days after last treatment				
compd	0	11	20	34	54
fenthion	0.96 ± 0.28	0.64 ± 0.32	0.51 ± 0.16	0.45 ± 0.18	0.34 ± 0.15
II	0.66 ± 0.24	0.23 ± 0.08	0.21 ± 0.05	0.20 ± 0.04	0.19 ± 0.08
III	0.02 ± 0.00	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.01	0.04 ± 0.02
IV	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
V	0.29 ± 0.14	0.25 ± 0.05	0.33 ± 0.06	0.24 ± 0.04	0.07 ± 0.03
VI	0.03 ± 0.02	0.04 ± 0.00	0.05 ± 0.02	0.03 ± 0.01	nd[a]

[a] nd, not detectable.

Chemicals. Acetonitrile and chloroform were HPLC grade solvents (Carlo Erba); anhydrous sodium sulfate was of analytical grade (Carlo Erba). Fenthion and parathion were analytical standards purchased from Ehrenstorfer (Augsburg, Germany); fenoxon was an analytical standard kindly donated by Bayer (Leverkusen, Germany). Metabolites II, III, V, and VI were analytical standards synthesized according to the method of Cabras et al. (1991). Stock standard solutions of I–VI (ca. 100 ppm each) were prepared in acetonitrile and stored at 4 °C (stable for over 1 year under these conditions). Working standard solutions containing the i.s. at 0.04 ppm were prepared weekly by dilution with chloroform. A stock standard solution of parathion (4 ppm) was prepared in chloroform.

RESULTS AND DISCUSSION

Chromatographic separation of fenthion and its metabolites was first obtained by adapting the method described by Bowman and Beroza (1970). No cleanup was needed after extraction with chloroform, as shown by the chromatograms in Figure 2 (see A, control). It was only necessary to replace the glass liner and clean the injector every 50 injections. Residues of fenthion and its metabolites in olives after three and five treatments (experiments 1 and 2, respectively) are reported in Tables I and II. Fenthion residues were considerably higher in experiment 2 (five treatments) than in experiment 1 (three treatments). This could be ascribed to its low degradation rate. In fact, the half-life of fenthion in the two experiments, 38.4 and 37.4 days (calculated as pseudo-first-

Table II. Residues (Parts per Million ± SD) of Fenthion and Its Metabolites (II–VI) in Olives after Five Treatments (Experiment 2)

	days after last treatment				
compd	0	11	20	34	54
fenthion	1.93 ± 0.71	1.43 ± 0.30	1.34 ± 0.31	0.87 ± 0.34	0.72 ± 0.18
II	0.76 ± 0.22	0.58 ± 0.21	0.56 ± 0.32	0.58 ± 0.26	0.51 ± 0.27
III	0.08 ± 0.04	0.15 ± 0.04	0.17 ± 0.06	0.15 ± 0.05	0.12 ± 0.02
IV	0.04 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.03 ± 0.01
V	0.34 ± 0.11	0.49 ± 0.06	0.62 ± 0.19	0.80 ± 0.15	0.35 ± 0.09
VI	0.08 ± 0.05	0.15 ± 0.02	0.18 ± 0.04	0.09 ± 0.02	0.05 ± 0.01

Table III. Residues (Parts per Million) of Fenthion and Its Metabolites (II–VI) in Olives and Products from Olive Processing

	experiment 1				experiment 2			
compd	olives	oil	cake	veget water	olives	oil	cake	veget water
fenthion	0.34	1.01	0.15	0.09	0.72	2.29	0.28	0.11
II	0.19	0.25	0.45	0.04	0.51	0.27	0.62	0.04
III	0.04	0.05	0.07	0.02	0.12	0.09	0.10	0.02
IV	0.02	0.02	0.01	0.02	0.03	0.06	0.01	0.02
V	0.07	nd[a]	0.21	0.20	0.30	nd	0.28	0.32
VI	nd	nd	0.04	nd	0.06	nd	0.06	0.03

[a] nd, not detectable.

order kinetics), indicates that the AI can be only partly degraded between one treatment and the next, which can lead to its accumulation. The presence of metabolites II–VI was detected in both experiments, while II and V showed the highest concentration. Moreover, II showed high persistence, with almost unchanged residues from day 11 to day 54. The other metabolites (III, IV, and VI) were detected in considerably lower amounts than II and V; they tended to increase up to day 20 and then progressively decreased. V showed analogous behavior. At harvest time, the sum of metabolites II–VI, expressed as fenthion, was 0.31 ppm in experiment 1 and 1.02 ppm in experiment 2, corresponding to 91% and 141% of fenthion residues, respectively. Hence, different evaluations of fenthion residues—with respect to legal limits—can be made when one considers AI alone or the total sum of AI and its metabolites. In Italy, where the maximum residue limit (MRL) is 0.8 ppm for AI alone, all of the analyzed samples were within the legal limit. On the contrary, on the basis of the FAO indications (FAO/WHO, 1978b), which consider the residues as the sum of fenthion and its metabolites, the MRL (1 ppm) was exceeded in experiment 2. Since the metabolites are more toxic than AI, both AI and its metabolites—which represent a relevant percentage—should be considered as a whole in residue evaluations. The products obtained from olive processing (oil, 20%; cake, 40%; vegetation water, 40%) were analyzed. The results are reported in Table III, where they are compared to the residues in the olives at harvest time. From these data it can be seen that for fenthion the weighted mean of the residues in the three fractions agrees with the residue in the olives (0.30 vs 0.34 ppm in experiment 1; 0.61 vs 0.72 ppm in experiment 2). The fenthion residue in the oil was almost 3 times higher than in the olives. Taking into account that on average 5 kg of olives is needed to obtain 1 L of oil, it can be calculated that ca. 70% of fenthion is transferred from the olives to the oil. The remaining part is split between cake (20%) and vegetation water (10%). When one considers that on average 20 kg of cake is needed to obtain 1 L of oil, since cake contains ca. 5% oil, the presence of fenthion suggests that the oil obtained by extraction with nonpolar solvents will have higher residues than that obtained by pressure. Therefore, it would be interesting to verify if and in what way the rectification process can affect the amount of fenthion residues in the oil; research on this problem is lacking. At harvest time, the metabolites in the olives, as a whole, were of the same magnitude order as the AI; in the oil some were absent (V and VI), while the sum of the others was one-fourth that of the fenthion residue. On the other hand, in the cake fenthion sulfoxide (II) and fenoxon sulfoxide (V) were present in the highest amounts and AI represented 20% of total residues. Low amounts of residues were detected in the vegetation water, fenoxon sulfoxide showing the highest concentration and the fenthion residue being ca. half that of fenoxon sulfoxide.

CONCLUSIONS

Fenthion showed a low degradation rate in olives, with a half-life of ca. 38 days. This indicates that the number of treatments considerably affected the amount of residues at harvest time. Besides the AI, five metabolites were detected in the olives, sulfoxides II and V being quantitatively the most important. The residues in the oil were ca. 3 times higher than in the olives, while the concentrations of the other metabolites were analogous (II, III, and IV) or absent (V and VI). The presence of fenthion residues in the oil in a higher amount than in the olives was easily predictable on the basis of the nonpolar nature of the fenthion moiety. Therefore, in the light of our experimental data, it would be more appropriate to establish different MRL values for olives and oil.

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