Persistence and Metabolism of Fenthion in Orange Fruit

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The persistence and metabolism of fenthion in orange fruit were studied in field conditions. The fenthion was transformed to fenthion sulfoxide and fenthion sulfone. Sunlight photodegradation experiments showed that this transformation is due to the action of sunlight. Residues were found only in the fruit peel. Fenthion showed a rapid degradation rate with a half-life of ca. 6 days. Fenthion sulfoxide was degraded more slowly with a half-life of ca. 14 days and represented the major residue. Fenthion sulfone was present in low quantities.

Keywords: *Fenthion; orange fruit; metabolism; persistence; residues*

The main transformation products of fenthion in plants are due to enzymatic action on the thiono sulfur atom, yielding the three metabolites **IV**, **V**, and **VI**, and to sunlight action on the sulfur atom of the methylmercapto moiety, yielding **II** and **III** (Figure 1; FAO/WHO, 1973). These metabolites show a toxicity that is similar to or higher than that observed for the active ingredient (ai) (Metcalf et al., 1963; Dubois and Kinoshita, 1964). Regarding this fact, the maximum residue limit (MRL) is estimated by considering the sum of the ai and its metabolites **II**−**VI** (FAO/WHO, 1973). This approach has been accepted by many countries including Spain, Denmark, Finland, and Sweden. In other countries, such as Italy, however, the MRL is estimated by considering the ai only. The metabolism of fenthion has been studied mainly on herbaceous crops (e.g., bean, cotton, cabbage, rice; FAO/WHO, 1973). Recently the toxic metabolites of fenthion have been studied on arboreous crops (e.g., olives; Cabras et al., 1993). With regard to citrus fruits, which constitutes another important arboreous crop in Mediterranean countries, there are no known studies on the metabolic fate of fenthion. This is probably due to the commonly used methodology for the determination of fenthion residues, based on the oxidation of the various compounds to sulfone, which is in turn hydrolyzed to the corresponding phenol (Anderson et al., 1966). This paper aims at contributing to the knowledge of the fate of fenthion in orange fruit.

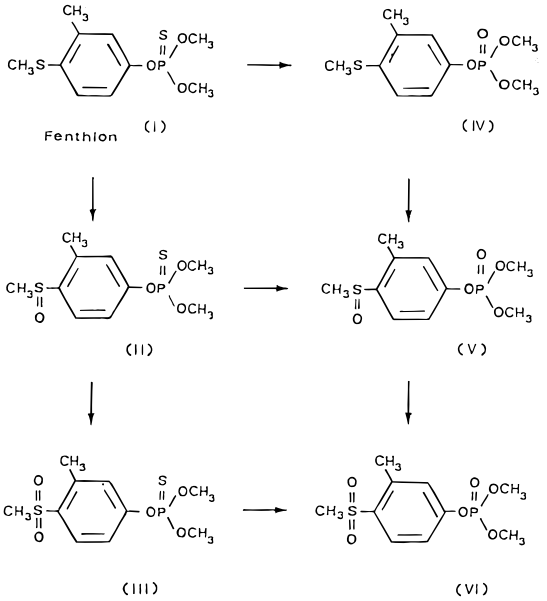

Figure 1. Fenthion (**I**) and its metabolites: fenthion sulfoxide (**II**), fenthion sulfone (**III**), fenoxon (**IV**), fenoxon sulfoxide (**V**), and fenoxon sulfone (**VI**).

EXPERIMENTAL PROCEDURES

Field Trials. The trial was carried out in a citrus grove (cv. Washington Navel) of the Centro Regionale Agrario Sperimentale located at Uta, near Cagliari, Italy. The citrus grove was planted in 1970 with a planting space of 5 × 6 m. A random-block design with four replications was used, and each block contained 16 trees in a single row. Treatments were carried out with an Agrumobar sprayer (Officine Carpi, Modena, Italy). The commercial formulation Lebaycid 25 (24.2% fenthion) was used at the dose recommended for citrus by the manufacturer (320 g of ai/ha) and at double the dose. Fenthion was applied on April 4, 11, and 20, 1995. Samplings started before and after the last treatment on the dry plants and were repeated at 6, 13, 20, and 27 days. Random 10-fruit samples were collected from each block and immediately analyzed for insecticide residues. The weather conditions were continuously recorded with an SM 3800 automatic weather station (SIAP, Bologna, Italy). Rainfall was continuously recorded with an AD-2 automatic weather station (Silimet, Modena, Italy). During the experiments total rainfall was 31.6 mm, with rain events on April 16, 24, 25, and 26 of 5.2, 7.2, 9.2, and 8.8 mm, respectively. Maximum and minimum average temperatures were 21.9 and 5.0 °C in April and 27.4 and 8.3 °C in May, respectively.

Chemicals. Fenthion was an analytical standard 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). Triphenyl phosphate (99%) was used as the internal standard (i.s.) and was of analytical grade (Janssen, Geel, Belgium). Acetone and ethanol were of HPLC grade, while petroleum ether was a special reagent for pesticide determi-

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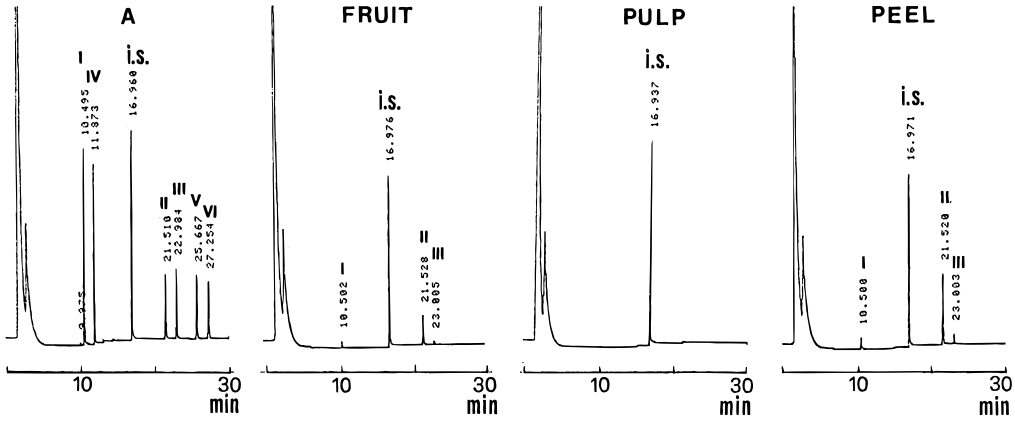

Figure 2. Chromatography of fenthion and its metabolites (**II**−**VI**): standard solutions (A; concentration range 0.2−0.3 ppm); fruit, pulp, and peel samples. The operating conditions are reported under Experimental Procedures.

nation (Carlo Erba, Milan, Italy); anhydrous sodium sulfate and sodium chloride were of analytical grade (Carlo Erba). Stock standard solutions of fenthion and its metabolites (ca. 500 ppm each) were prepared in ethanol. Working standard solutions, containing the i.s. at 0.3 ppm, were obtained by dilution with the extract from untreated oranges.

Apparatus and Chromatography. An HRGC Mega 5160 gas chromatograph (Carlo Erba) was employed. It was fitted 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 × 0.25 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 detector were operated at 250 and 260 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 90 °C for 1 min, raised to 190 °C (30 °C/min) and then to 270 °C (5 °C/min), held for 5 min, raised to 290 °C (20 °C/min), and held for 11 min. Helium was the carrier and make-up gas at 100 and 130 kPa, respectively. Calibration graphs for the fenthion and its metabolites were constructed with the i.s. method by measuring peak heights vs concentrations. Good linearities were achieved in the range 0−2.5 ppm, with correlation coefficients between 0.9992 and 0.9995. Under the above operating conditions the limit of determination (Thier and Zeumer, 1987) was 0.01−0.02 ppm for each of the compounds studied.

Sample Preparation. The oranges were counted and weighed to determine the average weight. They were then cut with a knife into two equal parts and divided into two batches. The orange halves from one batch were directly ground and homogenized, while those from the other batch were weighed and peeled. The peels were weighed to calculate their percentage contribution to the fruit weight and, subsequently, peels and pulp were ground and homogenized separately. In this way it was possible to obtain three different samples: whole fruit, peel, and pulp.

Extraction Procedure. A 10-g aliquot of homogenized sample was weighed in a 100-mL screw-capped flask; 4 g of sodium chloride and 20 mL of an acetone/petroleum ether mixture (1:1 v/v), containing triphenyl phosphate as the i.s., were added, and the flask was agitated in a flask shaker (Stuart Scientific, Great Britain) for 15 min. The phases were allowed to separate, and the organic layer was poured into another flask containing 2 g of anhydrous sodium sulfate and then injected for gas chromatographic analysis.

Recovery Assays. Untreated fruit, peel, and pulp samples were fortified with 0.01−0.02, 0.10, 0.50, and 2.50 ppm of fenthion and its metabolites and processed according to the above procedure. Recoveries from four replicates showed values ranging from 87% to 115% with the exception of fenoxon sulfoxide, which showed values ranging from 58% to 72%.

Sunlight Photodegradation Test. One-milliliter portions of 2.03 ppm fenthion solutions in CH_2Cl_2 were poured into vials (∅ 1 cm) and evaporated under nitrogen stream at ambient temperature. The same was done for the metabolites **II**, **III**, **V**, and **VI**. The screw-capped vials were then exposed to direct sunlight. At predetermined intervals the vials were removed from the sunlight and stored at −25 °C until analyzed. The residue contained in the vial was dissolved with 1 mL of extraction solvent containing triphenyl phosphate as the internal standard and injected for analysis. The test was carried out in duplicate; the ambient temperature ranged between 22 and 35 °C. Control samples were stored in the dark at room temperature and analyzed at the beginning and at the end of the test. Chromatographic analyses of control samples showed no degradation of fenthion or its metabolites.

RESULTS AND DISCUSSION

During the experiment, the average weight of the fruits was constant (238 ± 13 g), and the peel was ca. one-third (33 ± 1%) of the total weight. Therefore, residue concentrations were not affected by fruit growth.

The analytical method (Cabras et al., 1993) allowed the simultaneous determination of fenthion and five of its metabolites (Figure 2). Considering the data obtained, no residues were observed in the pulp, but only in the peel. Since the MRL was established for whole fruit, in Table 1 we report the data relating to residues in fruit and peel.

The discussion will consider only peel samples, since residues are present only here.

Residues of the active ingredient and two metabolites, fenthion sulfoxide and fenthion sulfone, were detected. The average residue of fenthion before the last treatment was 0.26 ppm and represented 20% of the total residue, while fenthion sulfoxide (1.02 ppm) and fenthion sulfone (0.04 ppm) represented 77% and 3%, respectively. After the last treatment, fenthion residue increased by ca. 0.2 ppm in the single dose and ca. twice this value in the double dose. Subsequently fenthion showed a rapid degradation rate with pseudo-first-order kinetics and a half-life ($t_{1/2}$) of 6 days ($R^2 = 0.96$) for both experiments. The main product of the fenthion oxidative process was fenthion sulfoxide, which showed a lower decay rate than the active ingredient. In fact, the half-life of fenthion sulfoxide, calculated using only the last three values in Table 1 (because these values are unaffected by fenthion degradation) was ca. 14 days. The residue of fenthion sulfone changed very slightly during the experiment. This indicates that its formation and degradation rates are of the same order of magnitude.

Photodegradation. To check the decay rate of fenthion due to sunlight, an experiment was performed

Table 1. Residues (Parts per Million ± SD) of Fenthion and Its Metabolites II and III in Fruit and Peel after Treatment

days after treatment	fruit				peel			
	fenthion	**II**	**III**	sum	fenthion	**II**	**III**	sum
−0[a]	0.12 ± 0.07	0.48 ± 0.24	<0.01	0.60	0.26 ± 0.19	1.02 ± 0.53	0.04 ± 0.02	1.32
			Single Dose					
0	0.26 ± 0.07	0.40 ± 016	<0.01	0.66	0.48 ± 0.09	0.95 ± 0.36	0.02 ± 0.02	1.45
6	0.05 ± 0.03	0.24 ± 0.11	<0.01	0.29	0.15 ± 0.11	0.64 ± 0.31	0.02 ± 0.01	0.81
13	0.02 ± 0.00	0.20 ± 0.12	<0.01	0.22	0.06 ± 0.02	0.72 ± 0.26	0.03 ± 0.01	0.81
20	0.01 ± 0.00	0.17 ± 0.07	<0.01	0.18	0.03 ± 0.01	0.67 ± 0.38	0.03 ± 0.02	0.73
27	0.01 ± 0.01	0.12 ± 0.04	<0.01	0.13	0.02 ± 0.00	0.36 ± 0.08	0.03 ± 0.01	0.41
			Double Dose					
0	0.46 ± 0.20	0.46 ± 0.19	0.03 ± 0.00	0.95	0.59 ± 0.12	1.41 ± 0.41	0.05 ± 0.01	2.05
6	0.10 ± 0.07	0.67 ± 0.22	0.03 ± 0.01	0.80	0.24 ± 0.19	1.69 ± 0.54	0.07 ± 0.03	2.00
13	0.04 ± 0.03	0.54 ± 0.24	0.02 ± 0.01	0.60	0.09 ± 0.08	1.65 ± 0.73	0.07 ± 0.03	1.81
20	0.02 ± 0.00	0.38 ± 0.08	0.02 ± 0.01	0.42	0.04 ± 0.01	1.27 ± 0.39	0.08 ± 0.03	1.39
27	0.01 ± 0.01	0.35 ± 0.15	0.02 ± 0.01	0.38	0.03 ± 0.02	0.79 ± 0.26	0.07 ± 0.02	0.89

[a] −0, before last treatment.

Table 2. Residues (Parts per Million) of Fenthion(I) and Its Metabolites II, III, V, and VI after Exposure (Hours) to Direct Sunlight in Screw-Capped Vials

	fenthion			
h	**I**	**II**	**III**	sum
0	2.03	0.02	0	**2.05**
1	1.53	0.42	0	**1.95**
2	0.97	0.67	0.02	**1.66**
3	0.87	0.88	0.02	**1.77**
5	0.66	1.15	0.05	**1.86**
8	0.10	1.28	0.07	**1.45**

	fenthion sulfoxide		
h	**II**	**III**	sum
0	0.92	0.01	**0.93**
6	0.72	0.03	**0.75**
12	0.61	0.04	**0.65**
20	0.59	0.04	**0.63**
30	0.57	0.04	**0.61**
60	0.36	0.04	**0.40**
90	0.27	0.05	**0.32**

	fenthion sulfone		
h	**III**	**VI**	sum
0	1.05	0	**1.05**
30	0.88	0.01	**0.89**
30	0.88	0.01	**0.89**
60	0.70	0.11	**0.81**
90	0.61	0.10	**0.71**
120	0.47	0.10	**0.57**
150	0.26	0.13	**0.39**

	fenoxon sulfoxide		
h	**V**	**VI**	sum
0	1.69	0	**1.69**
14	1.37	0.09	**1.46**
14	1.37	0.09	**1.46**
28	1.25	0.14	**1.39**
42	1.26	0.13	**1.39**
56	0.95	0.19	**1.14**
70	0.78	0.24	**1.02**

h	fenoxon sulfone **VI**	h	fenoxon sulfone **VI**
0	1.66	56	1.21
14	1.36	70	1.23
28	1.42	84	1.08
42	1.21		

exposing compounds **I**, **II**, **III**, **V**, and **VI** to sunlight separately. Table 2 shows the kinetic results and the transformation products obtained. Fenthion showed a rapid decay rate with pseudo-first-order kinetics and a half-life of 2.0 h ($r = -0.963$). Sunlight-irradiated fenthion yielded fenthion sulfoxide and sulfone, showing

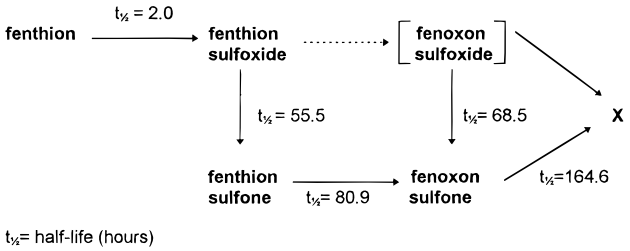

Figure 3. Transformation of fenthion upon exposure to sunlight.

behavior analogous to that observed in orange fruit. The decay rate of fenthion sulfoxide was considerably slower than that of fenthion, showing a $t_{1/2} = 55.5$ h ($r = -0.980$) and fenthion sulfone as the degradation product. The subsequent oxidation of fenthion sulfone in fenoxon sulfone was slower, with $t_{1/2} = 80.9$ h ($r = -0.965$). The decay rate of fenoxon sulfone is the slowest , $t_{1/2} = 164.6$ h ($r = -0.911$). Since the decay rate of fenoxon sulfone is so slow, this compound was not detected during the degradation experiment of fenthion, which was followed for 8 h only. Fenoxon sulfone was also obtained from the oxidative process of fenoxon sulfoxide with a $t_{1/2} = 68.5$ h. As shown in Figure 1, fenoxon sulfoxide could be obtained from fenthion sulfoxide transformation, but during our experiments it was not detected. This could be ascribed to its having formation and decay rates similar to those of fenoxon sulfone. It should be noted that an undetermined peak with the same retention time was observed in the chromatograms relating to the photodegradation of fenoxon sulfoxide and sulfone. This indicates that the molecules of both metabolites degrade to yield the same compound (Figure 3). The difference between the decay rates of fenthion and fenthion sulfoxide observed in the sunlight photodegradation experiment and in the field trials may be related to their fat-soluble properties. After treatment, fenthion and fenthion sulfoxide could be adsorbed onto the surface wax of the fruit, which could act as a sun filter and reduce photokinetic activity.

Conclusions. Fenthion in orange fruit degrades by the action of sunlight, yielding fenthion sulfoxide and fenthion sulfone. These compounds were not detected in the pulp, but only in the peel. The fruit residue therefore depends on the peel percentage. In our case, as the peel represents one-third of the fruit, the residues in the fruit will be ca. one-third of that detected in the peel. From the residues determined, fenthion sulfone was present in low quantities and was the most stable,

while fenthion sulfoxide showed the highest concentrations. Fenthion in orange fruit showed a rapid decay rate ($t_{1/2}$ = 6 days). For this reason not even after repeated treatments did it lead to significative residue accumulation. The same behavior was not expected from fenthion sulfoxide, since it showed a considerably lower decay rate, and the residue increased with the number of treatments. In light of these results, it does not seem appropriate to consider only the active ingredient in establishing the MRL, as in some countries.

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