Organochlorine Pesticide Residues in Italian Citrus Essential Oils, 1991−1996

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Organochlorine pesticide contamination in 148 lemon essential oils, 123 sweet orange oils, 121 mandarin oils, and 147 bergamot oils produced in Italy in the years 1991−1996 was studied by HRGC-ECD. Confirmation analyses were carried out by GC-MS. Tetradifon, dicofol and its decomposition product 4,4′-dichlorobenzophenone were found. Over the course of the study dicofol and tetradifon residues steadily decreased; the percentage of contaminated samples reflects this course and decreases considerably from 1991 to 1996.

Keywords: *Essential oils; organochlorine insecticides; gas chromatography*

INTRODUCTION

Pesticide residue analysis in essential oils is very important for their quality and marketing. Citrus essential oils are used in the food, pharmaceutical, and cosmetic industries and in disinfectant formulation, so low levels of contamination are required. The first research about organophosphorus pesticides contamination in Italian citrus essential oils dates back to 1978 (Leoni et al., 1978). Subsequently, from 1987 to date this kind of contamination has been evaluated by analyzing directly essential oils by HRGC-NPD and FPD (Dugo et al., 1987, 1990, 1992, 1994, 1997; Di Bella et al., 1991, 1995). Organochlorine acaricides are used against some citrus parasites, such as *Brevipalpus*, *Panonychus*, and *Tetranychus* species. The presence of these contaminants has been already found in some Italian essential oils: the samples, subjected to previous cleanup, were analyzed by HRGC-ECD (Saitta et al., 1995). In this paper are reported the results of a study of organochlorine pesticide contamination in Italian citrus essential oils from 1991 to 1996.

MATERIALS AND METHODS

Essential Oil Sources. Cold-pressed lemon, orange, mandarin, and bergamot oils were from Italy. All of the samples were commercially available Sicilian and Calabrian oils. They were produced in the years from 1991 to 1996. All samples were kept at 5 °C under nitrogen until analysis.

Standards. Pesticide standards were purchased from Dr. Ehrenstorfer, Augsburg, GmbH. Standard solutions of aldrin, dieldrin, *p*,*p*′-DDE, *o*,*p*′-DDD, endrin, *p*,*p*′-DDD, *o*,*p*′-DDT, *p*,*p*′-DDT, dicofol, and tetradifon were prepared in *n*-hexane and stored at 5 °C in a refrigerator. Bromophos-methyl was used as an internal standard at a concentration of 1 μg/mL for quantification by GC-ECD. Silica gel (70−230 mesh) was obtained from Merck (Darmstadt, Germany) and dichloromethane from Baker (Deventer, Holland).

Cleanup Procedure. A 10 mm i.d. × 500 mm length glass column with a Teflon stopcock was used. Silica gel was activated at 550 °C for 3 h. The chromatographic column was packed with a slurry of silica gel (8 g) in dichloromethane, and the solvent was then drained to just above the top of the silica.

Table 1. Recovery of Pesticides (Six Analyses)

pesticide	%, mean ± SD	pesticide	%, mean ± SD
aldrin	95.2 ± 3.2	*p*,*p*′-DDD	101.6 ± 4.1
dieldrin	97.3 ± 3.8	*o*,*p*′-DDT	102.3 ± 5.6
p,*p*′-DDE	100.6 ± 4.1	*p*,*p*′-DDT	103.1 ± 4.0
o,*p*′-DDD	99.6 ± 4.6	dicofol	105.6 ± 2.9
endrin	94.2 ± 2.9	tetradifon	102.1 ± 1.9

Table 2. Relative Retention Times (rrt) and Detection Limits (dl) of Pesticides

	rrt (RTX −1701)	dl (ppb)	rrt (RTX −5)	dl (ppb)
aldrin	0.869	20	0.916	30
bromophos-methyl	1		1	
p,*p*′-DDE	1.111	40	1.151	30
dieldrin	1.129	30	1.149	20
endrin	1.169	20	1.252	30
o,*p*′-DDD	1.173	30	1.175	30
o,*p*′-DDT	1.196	60	1.241	70
p,*p*′-DDD	1.268	40	1.245	50
p,*p*′-DDT	1.295	70	1.311	70
dicofol	1.400	40	1.438	40
tetradifon	1.521	30	1.472	20

Essential oil (0.2 mL) was introduced into the column, and the elution was performed with 30 mL of dichloromethane: the first fraction (0−12 mL) was discarded, and the second one (12−30 mL) was collected. The solvent was evaporated to 0.5 mL under a mild stream of nitrogen; 0.2 mL of the internal standard solution was added, and the mixture was directly analyzed by gas chromatography (GC).

GC. The levels of organochlorine insecticides were determined with a double-channel Shimadzu GC-17A gas chromatograph fitted with two electron capture detectors and Restek RTX-5 (30 m × 0.32 mm, 0.25 μm film thickness) and Restek RTX-1701 (30 m × 0.32 mm, 0.25 μm film thickness) columns operating in the splitless mode; helium was used as carrier gas at a constant flow rate of 36 cm/s. The injector temperature was maintained at 230 °C. The column oven was temperature programmed from an initial value of 50 °C (2 min hold) to 150 °C at a rate of 25 °C/min and then to 270 °C at 4 °C/min (20 min hold). The temperature of the detectors was 280 °C.

Gas Chromatography−Mass Spectrometry (GC-MS). Confirmation of residues was carried out by GC-MS using a Finnigan GCQ system fitted with a DB-5MS (30 m × 0.25 mm, 0.25 μm film thickness) column operating in the splitless mode;

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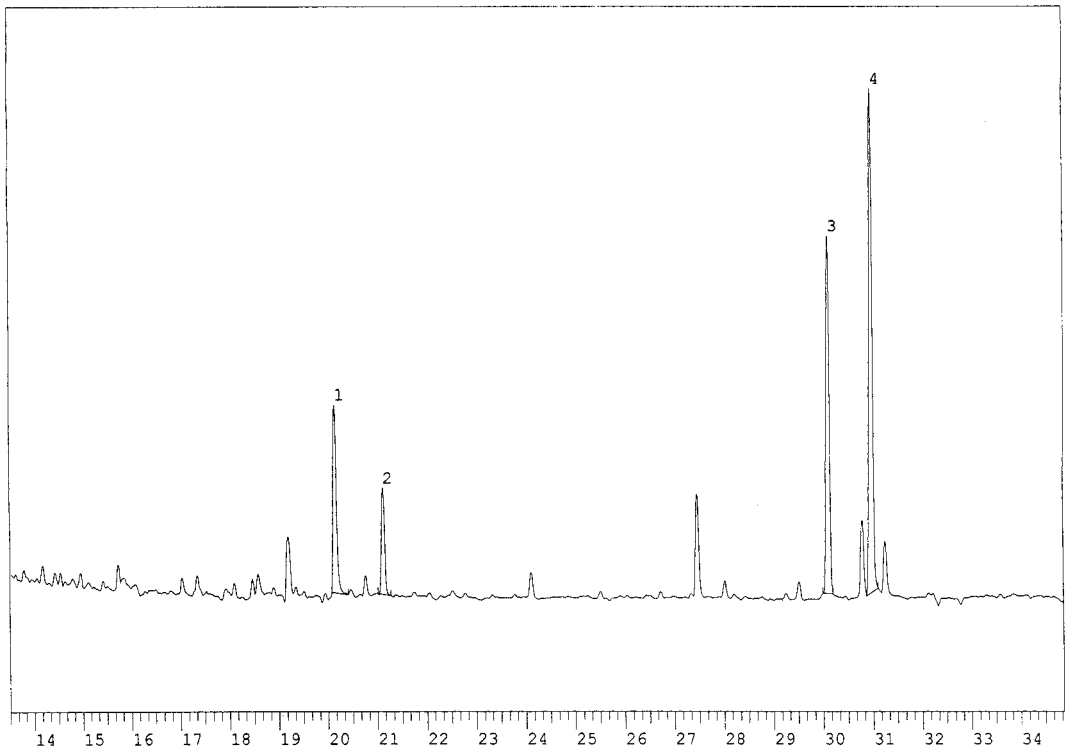

Figure 1. ECD chromatogram of lemon oil (RTX-1701 column): (peak 1) 4,4′-dichlorobenzophenone; (peak 2) bromophos-methyl; (peak 3) dicofol; (peak 4) tetradifon.

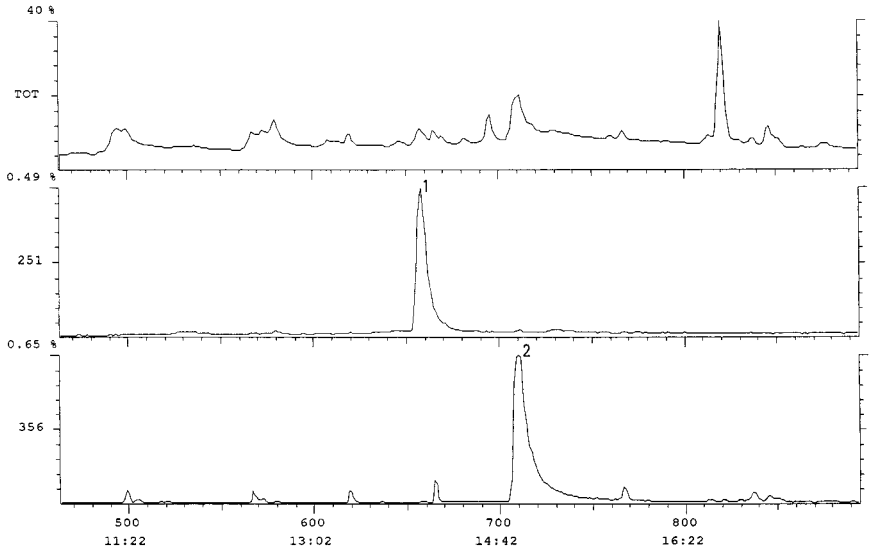

Figure 2. GC-MS chromatogram of orange oil: (peak 1) dicofol; (peak 2) tetradifon.

Table 3. Organochlorine Pesticide Residues in Lemon Oil

production year	1991	1992	1993	1994	1995	1996
no. of samples	25	24	24	24	26	25
% contaminated samples	92.0	91.6	83.3	75.0	73.1	64.0
4,4′-DCBP, concn range (ppm)	nd[a]−5.16	nd−3.18	nd−2.95	nd−1.95	nd−1.24	nd−0.94
mean value (ppm)	1.72	1.29	1.08	0.66	0.38	0.16
dicofol, concn range (ppm)	nd−6.94	nd−5.24	nd−3.24	nd−2.37	nd−1.24	nd−0.88
mean value (ppm)	2.26	1.75	1.01	0.76	0.43	0.23
tetradifon, concn range (ppm)	nd−2.22	nd−1.93	nd−1.93	nd−0.78	nd−0.62	nd−0.32
mean value (ppm)	0.86	0.75	0.45	0.20	0.16	0.08

[a] nd, not detectable.

helium was used as carrier gas at a constant flow rate of 40 cm/s. The injector was maintained at 250 °C and the transfer line at 275 °C. The mass spectrometer was used in electron impact mode, full scan from 40 to 500 D. The column oven was temperature programmed from 60 to 275 °C at a rate of 15 °C/min (14 min hold).

Recovery of Pesticides from Distilled Essential Oil Fortified at 1 ppm with 10 Insecticides. A distilled lemon essential oil was used to calculate the recoveries by adding a solution of the organochlorine insecticides so that the pesticide concentrations were 1 ppm. After the cleanup procedure, the internal standard was added and the solution was then

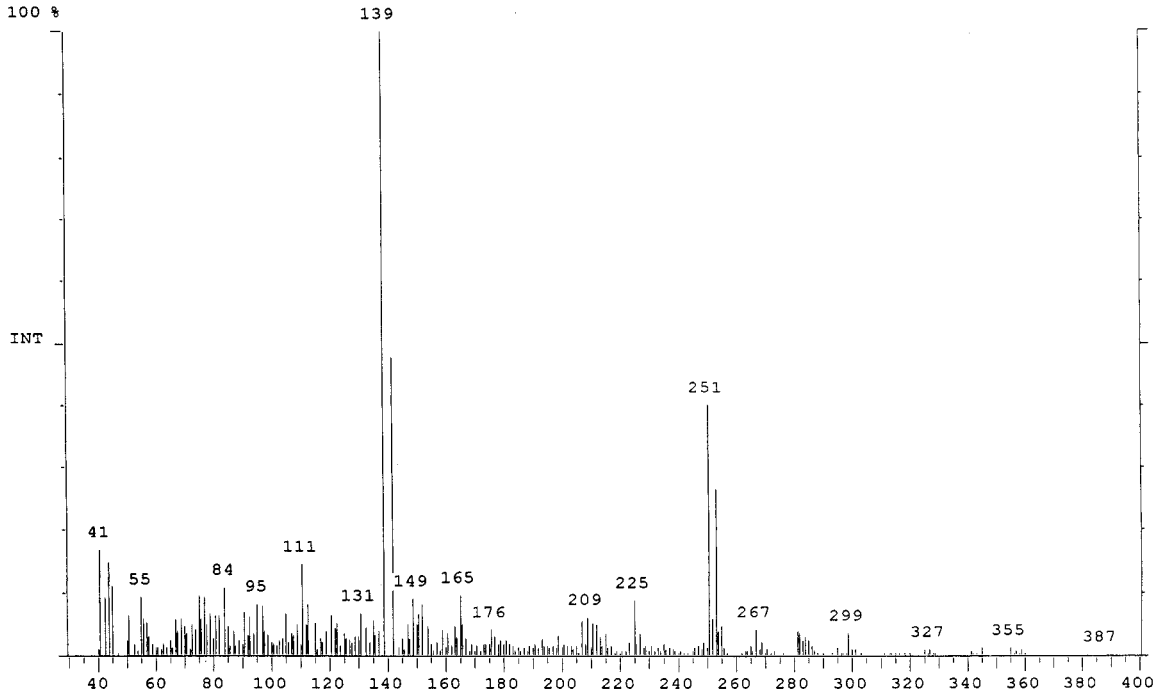

Figure 3. Mass spectrum of dicofol.

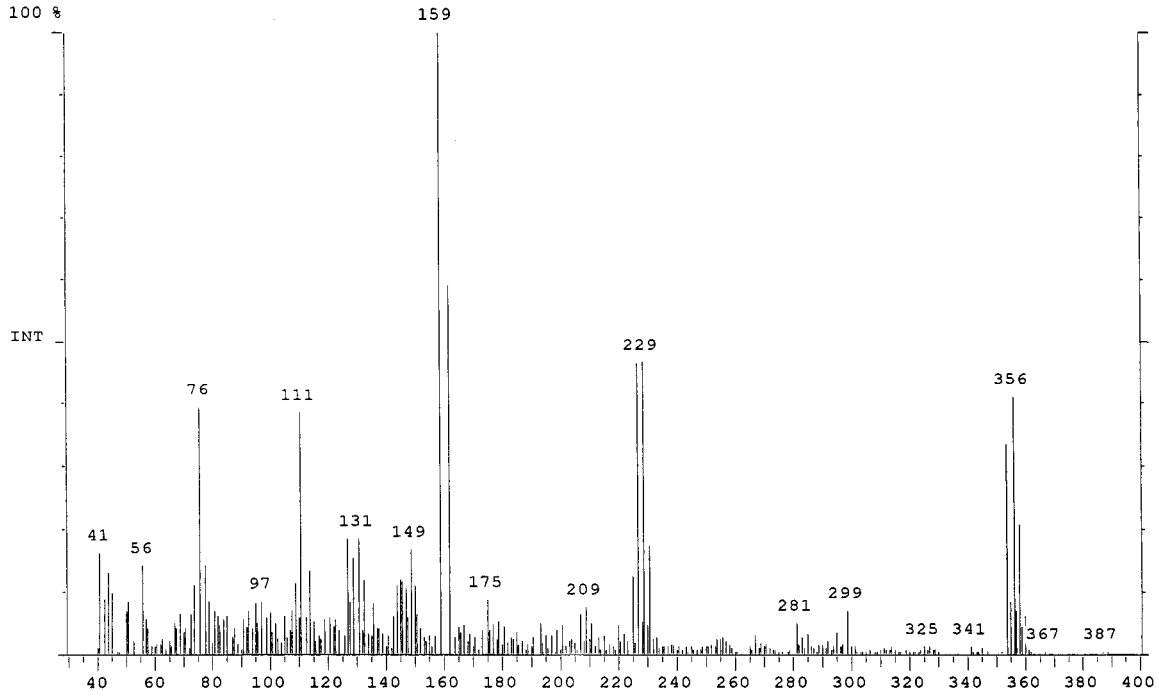

Figure 4. Mass spectrum of tetradifon.

analyzed by GC-ECD. The procedure was repeated six times; the results of the recovery are reported in Table 1. Relative retention times and detection limits of tested pesticides are reported in Table 2.

RESULTS AND DISCUSSION

Tetradifon, dicofol, and its decomposition product, 4,4′-dichlorobenzophenone, were found in many essential oils.

Lemon Oils. A lemon oil chromatogram obtained by ECD (column RTX-1701) is shown in Figure 1. It is possible to observe tetradifon, dicofol, 4,4′-dichlorobenzophenone, and bromophos-methyl (internal standard). Table 3 reports the pesticide concentration range, the percentage of contaminated samples and the average value of contamination during the years of production. The contamination percentage of lemon oils decreases gradually over the years from 92 to 64%. Even the maximum residue levels decreases progressively from 1991 to 1996. Dicofol and tetradifon average values (2.26 and 0.86 ppm, respectively, in 1991) become 0.23 and 0.08 ppm, respectively, in 1996.

Orange Oils. In Figure 2 is shown the GC-MS chromatogram of an orange oil. The total ion chromatogram shows several peaks, but the currents at *m*/*e* 251 and 356 show the presence of dicofol and tetradifon; in

Table 4. Organochlorine Pesticide Residues in Orange Oil

production year	1991	1992	1993	1994	1995	1996
no. of samples	19	18	23	19	22	22
% contaminated samples	94.7	77.8	78.3	68.4	68.2	54.5
4,4′-DCBP, concn range (ppm)	nd[a]−5.48	nd−3.97	nd−3.94	nd−3.12	nd−1.22	nd−1.49
mean value (ppm)	2.41	1.77	1.05	0.85	0.58	0.32
dicofol, concn range (ppm)	nd−5.02	nd−4.11	nd−4.01	nd−2.16	nd−1.12	nd−1.05
mean value (ppm)	1.74	1.36	0.91	0.76	0.44	0.25
tetradifon, concn range (ppm)	nd−2.15	nd−1.92	nd−1.15	nd−1.35	nd−0.62	nd−0.92
mean value (ppm)	0.66	0.48	0.41	0.36	0.20	0.16

[a] nd, not detectable.

Table 5. Organochlorine Pesticide Residues in Mandarin Oil

production year	1991	1992	1993	1994	1995	1996
no. of samples	19	20	20	22	20	20
% contaminated samples	94.7	90.0	80.0	77.3	75.0	50.0
4,4'-DCBP, concn range (ppm)	nd[a]−4.17	nd−2.58	nd−2.04	nd−4.18	nd−2.24	nd−1.43
mean value (ppm)	1.55	1.10	1.00	1.04	0.56	0.28
dicofol, concn range (ppm)	nd−5.95	nd−2.10	nd−2.16	nd−3.15	nd−3.18	nd−1.24
mean value (ppm)	1.96	1.11	1.21	0.92	0.63	0.36
tetradifon, concn range (ppm)	nd−3.12	nd−1.24	nd−1.54	nd−1.01	nd−2.24	nd−0.92
mean value (ppm)	0.95	0.63	0.72	0.43	0.34	0.10

[a] nd, not detectable.

Table 6. Organochlorine Pesticide Residues in Bergamot Oil

production year	1991	1992	1993	1994	1995	1996
no. of samples	20	19	19	20	20	19
% contaminated samples	50.0	52.6	31.6	35.0	40.0	26.3
4,4′-DCBP, concn range (ppm)	nd[a]−0.92	nd−0.72	nd−0.52	nd−0.15	nd−0.92	nd−0.32
mean value (ppm)	0.17	0.22	0.10	0.02	0.10	0.05
dicofol, concn range (ppm)	nd−0.81	nd−0.96	nd−0.56	nd−0.24	nd−1.18	nd−0.42
mean value (ppm)	0.20	0.24	0.10	0.04	0.11	0.05
tetradifon, concn range (ppm)	nd−0.52	nd−0.57	nd−0.31	nd−0.12	nd−0.12	nd−0.32
mean value (ppm)	0.08	0.09	0.04	0.01	0.01	0.03

[a] nd, not detectable.

Figure 3 and 4 the spectra of dicofol and tetradifon, obtained from the orange oil sample, are shown. Table 4 reports the levels of organochlorine pesticide found in the orange oil samples. Oils produced in recent years show lower levels of contamination compared with the samples of 1991, which confirms the trend observed for lemon oils. Average values of 1.74 and 0.66 ppm for dicofol and tetradifon, respectively, decrease to 0.25 and 0.16 ppm in 1996. We also observed that 4,4′-dichlorobenzophenone residues were higher than dicofol; it is possible that dicofol used on the plants was partially decomposed.

Mandarin Oils. Table 5 shows the concentration range of pesticides found in mandarin essential oils, the percentage of contaminated samples, and the average level of contamination. Dicofol residues decrease from 1.96 ppm in 1991 to 0.63 ppm in 1996, and tetradifon levels decrease from 0.95 to 0.34 ppm.

Bergamot Oils. Bergamot essential oils have the lowest levels of contamination. In 1991 50% of analyzed samples were not contaminated; Table 6 shows that the dicofol average value decreases from 0.20 to 0.05 ppm in 1996. These data are probably related to bergamot's high resistance to pest attack and so to lesser use of pesticides compared to other citrus.

Conclusions. This paper shows that organochlorine insecticide residues steadily decreased during the production years. In previous research the organophosphorus pesticide residues found in Italian citrus essential oils produced from 1983 to 1992 had been studied: it had been observed that levels of organophosphorus pesticides decrease dramatically from 1988 to 1992 (Dugo et al., 1997). The data show that farmers are tending to reduce and razionalize the use of organophosphorus and organochlorine pesticides, compared to previous excessive use.

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