Contamination of Italian Citrus Essential Oils: Presence of Phthalate Esters

Giuseppa Di Bella, Marcello Saitta, Mariacristina Pellegrino, Francesco Salvo, and Giacomo Dugo*,†

Dipartimento Chimica Organica e Biologica, Università di Messina, Salita Sperone 31, 98166 Messina, Italy

Contamination by phthalate esters of Sicilian and Calabrian citrus essential oils, produced in the crop years 1994−1996, was investigated using a GC−MS system with direct injection of the samples. A total of 35 lemon oils, 31 orange oils, and 21 mandarin oils were analyzed. Diisobutyl phthalate and/or bis(2-ethylhexyl) phthalate were found in almost all samples, while di-*n*-butyl phthalate was present in 8. Concentrations up to a maximum of 62 ppm were found for diisobutyl phthalate and up to a maximum of 29.9 ppm for bis(2-ethylhexyl) phthalate.

Keywords: *Citrus oils; phthalate esters; gas chromatography*

INTRODUCTION

In previous studies we have used gas chromatography with NPD, FPD, and ECD detectors to determine the levels of organophosphorus and organochlorine pesticide residues in citrus essential oils (Dugo et al., 1987, 1990, 1992, 1997; Di Bella et al., 1991; Saitta et al., 1995; Della Cassa et al., 1995). More recently we have investigated the contamination of citrus oils by phosphorated plasticizers using HRGC−FPD (Di Bella et al., 1996; Saitta et al., 1997).

In this study we have investigated phthalate residues in Italian citrus oils.

Phthalate compounds represent the biggest group of plasticizing agents, both in terms of production volume and of sales volume. Their toxicity is beyond dispute: phthalates have an extremely wide sphere of activity and may be considered as polytrophic toxins. They are hepatotoxic, cause damage to the gastrointestinal tract, and are carcinogenic (Cagliero and Gaudino, 1987; Gorenkov et al., 1992; Liubchenko et al., 1994, 1995).

MATERIALS AND METHODS

Sampling. A total of 35 lemon oils, 31 orange oils, and 21 mandarin oils were analyzed. All were produced in the crop years 1994−1996 and were commercially available on the Sicilian and Calabrian markets. All samples were collected in small glass bottles and stored at 4 °C under nitrogen atmosphere until analysis.

Standards. Dimethyl phthalate, diethyl phthalate, dipropyl phthalate, diisobutyl phthalate, di-*n*-butyl phthalate, butyl benzyl phthalate, bis(2-ethylhexyl) phthalate, and di-*n*-octyl phthalate (99% purity) were purchased from Aldrich Chemical Co. A solution containing a mixture of all the phthalates (1 μg/mL of each) was prepared in *n*-hexane.

GC Analysis. Samples were directly analyzed without cleanup procedures using a Finnigan MAT GCQ gas chromatograph−mass spectrometer, equipped with a DB-5 MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness).

† Fax: 0039 90 393895.

Table 1. Time Intervals, Selected Ion Monitoring, and Detection Limits for Phthalate Esters under Analysis

phthalate (P)	time (min)	SIM (*m/e*)	detection limits (pg)
dimethyl P	6.5−8	163, 194	40
diethyl P	8−9.4	149, 177	10
dipropyl P	9.4−10.2	149, 191, 209	8
diisobutyl P	10.2−10.9	149, 167, 223	3
di-*n*-butyl P	10.9−13	149,223	4
butyl benzyl P	13−14.2	149, 206	30
bis(2-ethylhexyl) P	14.2−17	149, 167	6
di-*n*-octyl P	14.2−17	149, 167	20

The oven temperature program was as follows: 60−275 °C at 15 °C/min and 275 °C for 14 min. The temperature of the injector was 250 °C. The transfer line was heated to 275 °C. Mass spectra were recorded in the electron impact (EI) mode at 70 eV: full scan range 40−500 D; selected ion monitoring (SIM) according to Table 1. Helium was the carrier gas (40 cm/s). The injection volume was 1 μL.

Reproducibility. The reproducibility of the analyses was assessed by adding diisobutyl phthalate, di-*n*-butyl phthalate, and bis(2-ethylhexyl) phthalate at a concentration of 1 ppm to a distilled lemon essential oil; this sample was analyzed six times on different days. The following results were obtained: diisobutyl phthalate, 1.04 ± 0.23 ppm; di-*n*-butyl phthalate, 0.95 ± 0.18 ppm; bis(2-ethylhexyl) phthalate, 1.11 ± 0.15 ppm.

RESULTS AND DISCUSSION

Figure 1 shows the SIM chromatogram of the standard mixture of phthalate esters, all present at a concentration of 1 ppm.

Quantitative analysis was carried out by comparing the peak areas of samples with the corresponding peaks of the standard mixture.

In Table 1 are reported the detection limits for phthalate esters. The chromatogram of a lemon oil sample containing 0.01 ppm of bis(2-ethylhexyl) phthalate (Figure 2) shows the high sensitivity of the method.

In Figures 3 and 4, the full scan and SIM chromatograms are reported for the same sample of lemon

10.1021/jf980735b CCC: $18.00
Published on Web 02/03/1999

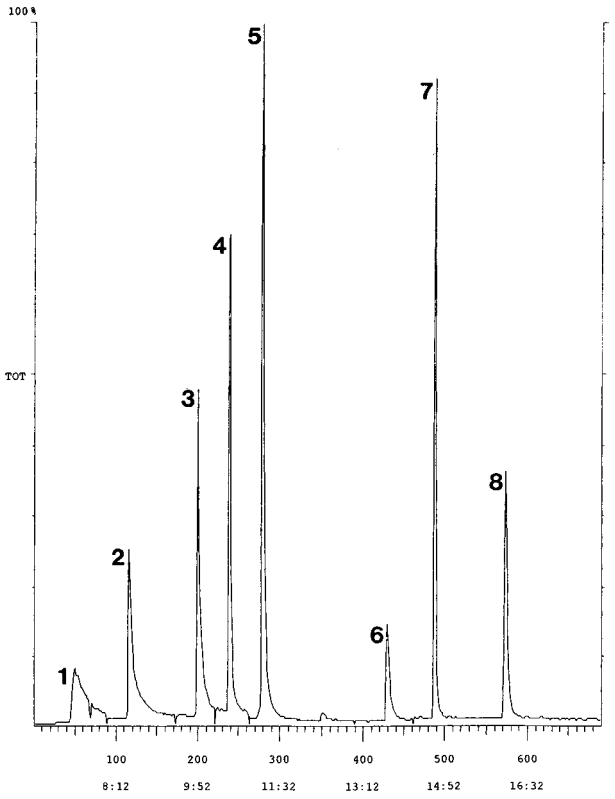

Figure 1. GC−MS SIM chromatogram of the standard phthalate mixture: dimethyl phthalate (1); diethyl phthalate (2); dipropyl phthalate (3); diisobutyl phthalate (4); di-*n*-butyl phthalate (5); butyl benzyl phthalate (6); bis(2-ethylhexyl) phthalate (7); di-*n*-octyl phthalate (8).

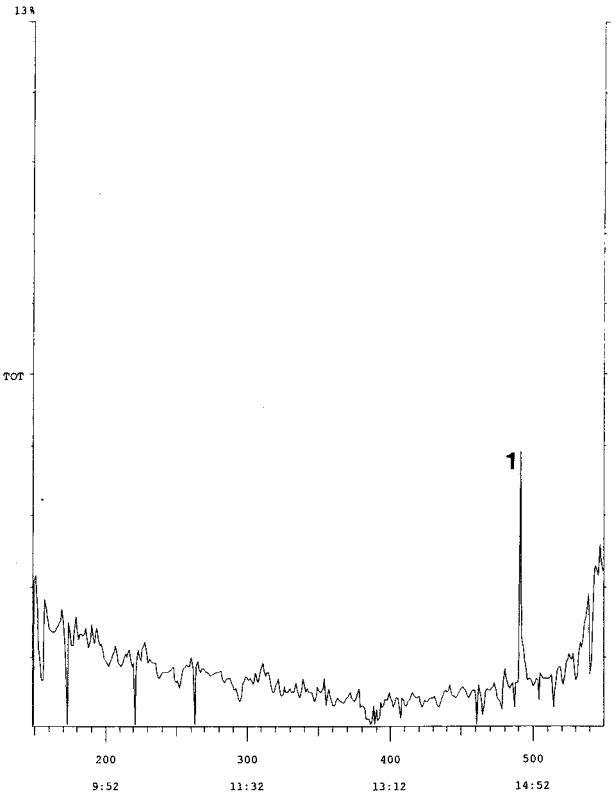

Figure 2. GC−MS SIM chromatogram of a lemon essential oil (sample 6): bis(2-ethylhexyl) phthalate (1).

essential oil. A comparison of these chromatograms shows the excellent discrimination of phthalate residues

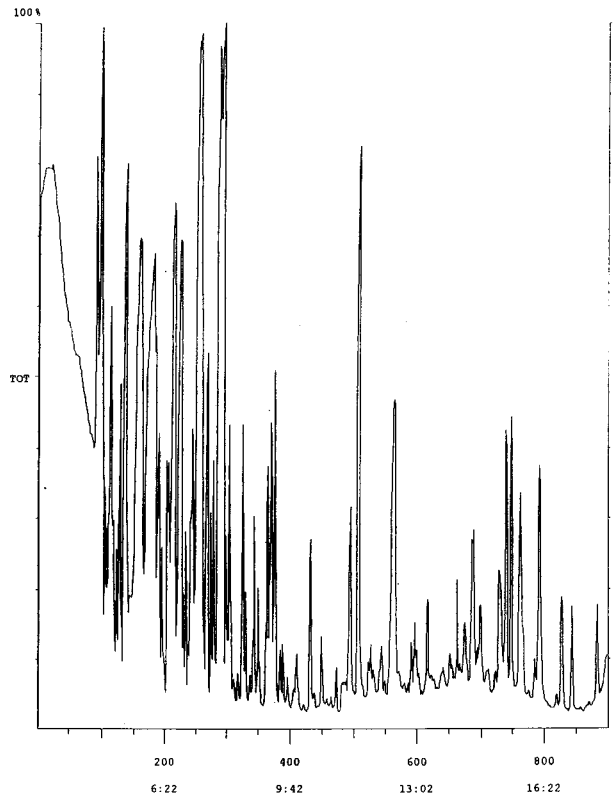

Figure 3. GC−MS full scan chromatogram of a lemon essential oil (sample 7).

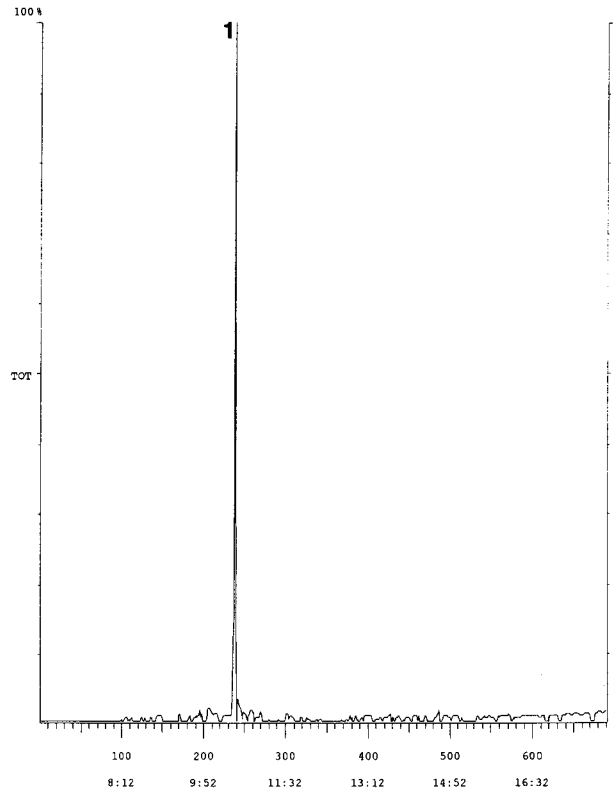

Figure 4. GC−MS SIM chromatogram of a lemon essential oil (sample 7): diisobutyl phthalate (1).

which can be achieved with selected ion monitoring. All the samples were analyzed using the SIM procedure.

The phthalate residues found were diisobutyl phthalate, di-*n*-butyl phthalate, and bis(2-ethylhexyl) phtha-

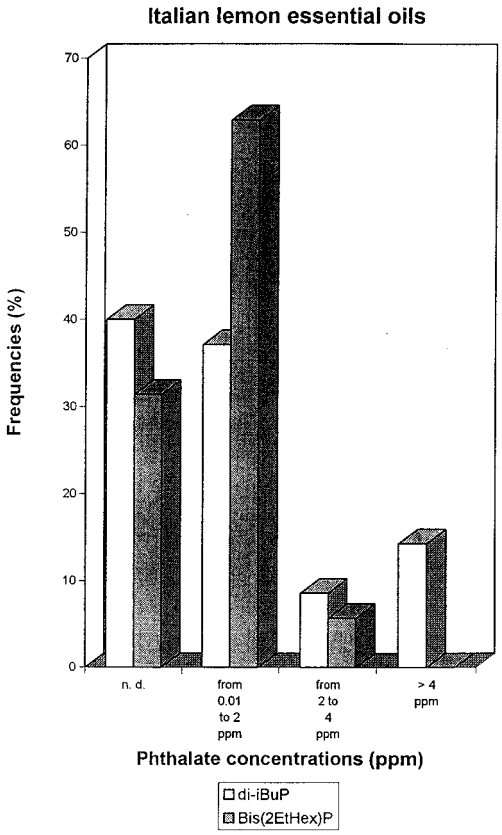

Figure 5. Lemon essential oil: frequencies of the phthalate levels.

Table 2. Phthalate Residues in Lemon Essential Oils (35) Produced in the Crop Years 1994−1996

phthalate (P)	mean value (mg/kg)	concn range (mg/kg) (min−max)	no. of contaminated samples
diisobutyl P	1.70	0.13−16.5	21
di-*n*-butyl P		0.54	1
bis(2-ethylhexyl) P	0.59	0.19−3.02	24

late. The latter was the compound most frequently identified in the samples analyzed.

Lemon Essential Oils. In the 35 lemon essential oil samples analyzed, diisobutyl phthalate and bis(2-ethylhexyl) phthalate were very frequently found, while di-*n*-butyl phthalate was occasionally found (Table 2).

The graph in Figure 5 shows the frequencies of the levels of diisobutyl phthalate and bis(2-ethylhexyl) phthalate. In 40% of samples the concentration of diisobutyl phthalate was below the detection limit, but in 13% it exceeded 4 ppm and reached a maximum of 16.5 ppm. In 30% of the samples no bis(2-ethylhexyl) phthalate residues were detected, while in 60% the concentrations found ranged from 0.01 to 2 ppm. Bis-(2-ethylhexyl) phthalate residues never were greater than 4 ppm. Di-*n*-butyl phthalate residues were detected only in one sample.

Orange Essential Oils. The concentration of diisobutyl phthalate and bis(2-ethylhexyl) phthalate residues were below the detection limit only in 6% and 3% of orange essential oil samples (Table 3). The average concentrations of diisobutyl phthalate and bis(2-ethylhexyl) phthalate found were 1.38 and 3.30 ppm, respectively. The maximum concentrations of diisobutyl phthalate and bis(2-ethylhexyl) phthalate detected were 26 and 29.9 ppm, respectively. Di-*n*-butyl phthalate

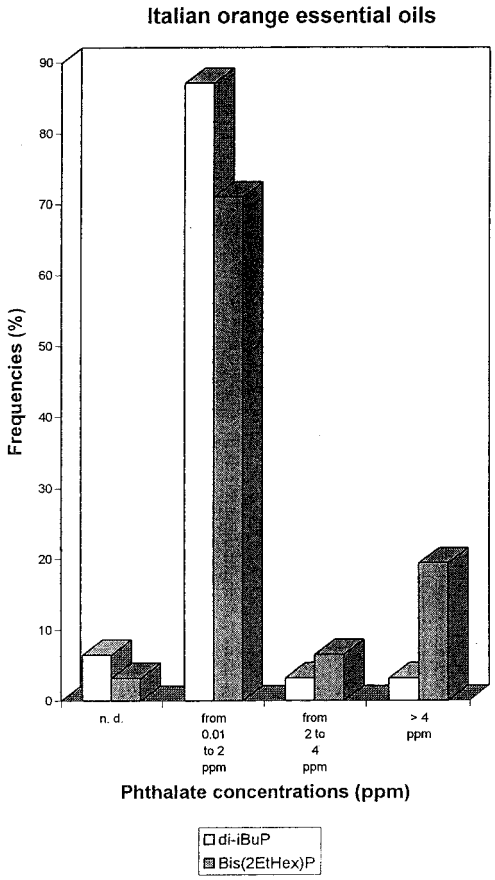

Figure 6. Orange essential oil: frequencies of the phthalate levels.

Table 3. Phthalate Residues in Orange Essential Oils (31) Produced in the Crop Years 1994−1996

phthalate (P)	mean value (mg/kg)	concn range (mg/kg) (min−max)	no. of contaminate samples
diisobutyl P	1.38	0.03−26	29
di-*n*-butyl P	0.07	0.14−0.53	7
bis(2-ethylhexyl) P	3.30	0.12−29.9	30

Table 4. Phthalate Residues in Mandarin Essential Oils (21) Produced in the Crop Years 1994−1996[a]

phthalate (P)	mean value (mg/kg)	concn range (mg/kg) (min−max)	no. of contaminated samples
diisobutyl P	4.89	0.09−62	20
di-*n*-butyl P		nd	0
bis(2-ethylhexyl) P	1.72	0.06−7.24	21

[a] nd = not detectable.

residues were found in 7 samples. In Figure 6 are reported the frequencies of the levels of diisobutyl phthalate and bis(2-ethylhexyl) phthalate. This shows that the levels of diisobutyl phthalate are in the range 0.01−2 ppm for almost 90% of samples, while 70% of samples show levels of bis(2-ethylhexyl) phthalate in the same range.

Mandarin Essential Oils. Mandarin essential oils show levels of contamination similar to those of orange essential oils: diisobutyl phthalate and bis(2-ethylhexyl) phthalate residues were not detected only in a small percentage of samples (Table 4). The average concentrations at which these two phthalates were detected were 4.89 and 1.72 ppm, respectively. The maximum level

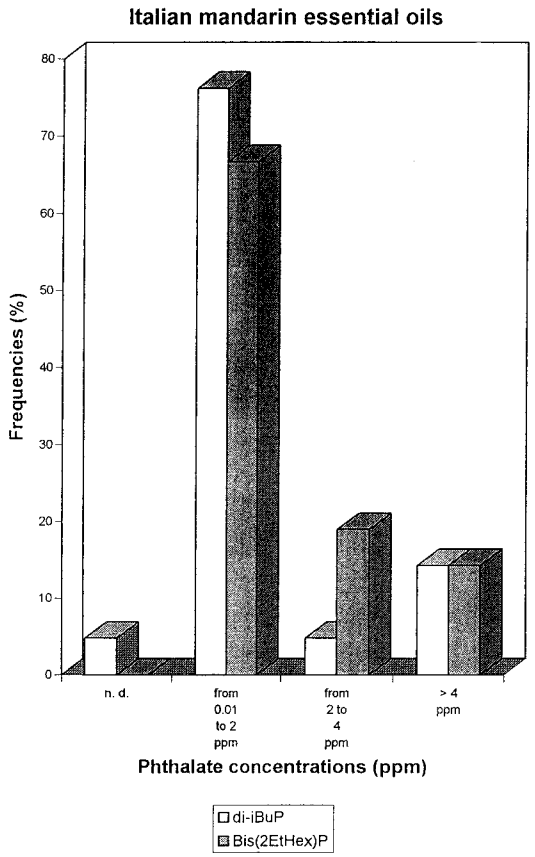

Figure 7. Mandarin essential oil: frequencies of the phthalate levels.

detected for diisobutyl phthalate was 62 ppm, while that for bis(2-ethylhexyl) phthalate was 7.24 ppm.

In Figure 7 are reported the frequencies of the levels of diisobutyl phthalate and bis(2-ethylhexyl) phthalate. This shows that the levels of diisobutyl phthalate are in the range 0.01−2 ppm for 75% of samples, while 68% of samples show levels of bis(2-ethylhexyl) phthalate in the same range and 15% show levels greater than 4 ppm. No detectable levels of di-*n*-butyl phthalate were found.

CONCLUSIONS

These results indicate that contamination of citrus essential oils by phthalate esters is very widespread, although in most instances the levels of contamination are within acceptable limits.

Since essential oils are not directly destined for alimentary use, these levels of contamination do not give cause for concern.

The levels of phthalate contamination detected here in essential oils must however be set alongside the levels of contamination by organophosphorus and organochlorine pesticides, as well as those from phosphorated plasticizers, which have already been reported. Therefore, it is clear that to evaluate quality and commercial use of the essential oils it is necessary to consider phthalate residues too.

LITERATURE CITED

Cagliero, S.; Gaudino, A. Analisi di ftalati nel pulviscolo atmosferico (Analysis of phthalates in the air particulate). *Boll. Chim. Igien.* **1987**, *38*, 613−624.

Della Cassa, E.; Lorenzo, D.; Moyna, P.; Dugo, Giacomo; Saitta, M. Contaminazione di oli essenziali agrumari: residui di pesticidi organofosforici e organoclorici negli oli di limone dell'Uruguay (Contamination of citrus essential oils: organophosphorus and organochlorine pesticide residues in Uruguayan lemon oils). *Abstracts of Papers*; Atti II Congresso Nazionale di Chimica degli Alimenti, Giardini Naxos, Italy, May 24−27, 1995; pp 955−960.

Di Bella, G.; Dugo, Giacomo; Saitta, M.; Salvo, F.; Ziino, M. Sulla genuinità delle essenze agrumarie. Nota XXXII. Sulla presenza di residui di pesticidi organofosforici in oli essenziali di arancia dolce prodotti in Sicilia e Calabria (Citrus oils genuineness. Note 32. Presence of organophosphorus pesticide residues in sweet orange oils from Sicily and Calabria). *Exedra* **1991**, *5*, 5−8.

Di Bella, G.; Saitta, M.; Dugo, P.; Messina, M.; Dugo, Giacomo. La presenza di plastificanti negli oli essenziali agrumari (Plasticizers in citrus essential oils). *Abstracts of Papers*; Convegno Regionale della Società Chimica Italiana, Catania, Italy, Dec 19−20, 1996.

Dugo, Giacomo; Salvo, F.; Alfa, M.; Dugo, Giovanni. Sulla genuinità delle essenze agrumarie. Nota XVIII. Dosaggio rapido di pesticidi organofosforici negli oli essenziali agrumari (Citrus oils genuineness. Note 18. Quick analysis of organophosphorus pesticides in citrus essential oils). *Essenze Deriv. Agrum.* **1987**, *4*, 610−619.

Dugo, Giacomo; Salvo, F.; Saitta, M.; Di Bella, G. Sulla presenza di residui di pesticidi organofosforici negli oli essenziali di limone estratti con diverse tecnologie industriali (Presence of organophosphorus pesticide residues in lemon essential oils extracted by different industrial technology). *Essenze Deriv. Agrum.* **1990**, *60*, 428−451.

Dugo, Giacomo; Famà, G.; Saitta, M.; Stagno d'Alcontres, I. Sulla genuinità delle essenze agrumarie. Nota XLII. Determinazione di pesticidi organofosforici con rivelatore a fiamma fotometrica (Citrus oils genuineness. Note 42. Organophosphorus pesticides analysis by HRGC and a flame photometric detector). *Essenze Deriv. Agrum.* **1992**, *62*, 127−146.

Dugo, Giacomo; Di Bella, G.; Saitta, M.; Salvo, F. Sulla genuinità delle essenze agrumarie. Nota XLV. Dosaggio rapido di pesticidi organofosforici negli oli essenziali di bergamotto (Citrus oils genuineness. Note 45. Quick analysis of organophosphorus pesticides in bergamot oils). *Essenze Deriv. Agrum.* **1994**, *64*, 234−247.

Dugo, Giacomo; Saitta, M.; Di Bella, G.; Dugo, P. Organophosphorus and organochlorine pesticide residues in Italian citrus oils. *Perfum. Flavor.* **1997**, *22*, 33−44.

Gorenkov, R. V.; Liubchenko, P. N.; Bendikov, E. A.; Ul'ianova, V. V. Antipiryne metabolism and rat liver ultrastructure in single and combined action of trichlorodiphenyl and dioctylphthalate. *Byull. Eksp. Biol. Med.* **1992**, *113*, 285−288.

Liubchenko, P. N.; Gorenkov, R. V.; Plaksina, G. V.; Kholod, O. L.; Salganikova, V. S. Work environment and health status in workers of decorative poly(vinyl chloride) film production. *Gig. Sanit.* **1994**, *4*, 29−31.

Liubchenko, P. N.; Gorenkov, R. V.; Bendikov, E. A.; Petrakov A. V. Functional state of the liver in workers exposed to multicomponent chemical mixture in the production of plastic construction materials. *Med. Tr. Ekol.* **1995**, *6*, 4−7.

Saitta, M.; Dugo, Giacomo; Di Bella, G.; Salvo, F.; Previti, P. Contaminazione di oli essenziali agrumari: determinazione di pesticidi organoclorici (Contamination of citrus essential oils: organochlorine pesticides analysis). *Abstracts of Papers*; Atti II Congresso Nazionale di Chimica degli Alimenti, Giardini Naxos, Italy, May 24−27, 1995; pp 947−953.

Saitta, M.; Di Bella, G.; Bonaccorsi, I.; Dugo, Giacomo. Contamination of citrus essential olis: the presence of phosphorated plasticizers. *J. Essent. Oil Res.* **1997**, *9*, 613−618.

Received for review July 8, 1998. Revised manuscript received November 30, 1998. Accepted December 2, 1998.

JF980735B