

## Survey of Polychlorinated Dibenzo-*p*-dioxins (PCDDs), Polychlorinated Dibenzo-*p*-furans (PCDFs), Polychlorinated Biphenyls (PCBs), and Mineral Components in Italian Citrus Cold-Pressed Essential Oils

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Investigation of PCDDs, PCDFs, PCBs, Al, As, Pb, Ba, Co, Cu, Cr, Fe, Mn, Cd, Ag, Sn, Zn, and Hg contents in 60 samples of cold-pressed essential oils produced in Calabria and Sicily in 2003–2005 was carried out. PCDDs, PCDFs, and PCBs were analyzed by HRGC-HRMS techniques using U.S. EPA 1613/94 and U.S. EPA 1668/A (1999) analytical methods. Mineral components were determined through GFAAS techniques; Hg content was determined by FI-M/H-AAS. The results of this study showed that essential oil contamination was due to a widespread pollution, typical background of rural areas, with relatively higher concentrations of PCDDs compared to PCDFs and little presence of PeCDF. Congeners OCDD, HpCDF, and OCDF were found at high concentrations. Regarding mineral components, mean values of Cr, Fe, and Ni were in agreement with data reported in the literature. Concentrations of As and Pb were below the maximum limits accepted by the current legislation. Finally, none of the samples analyzed were contaminated with Hg.

**KEYWORDS:** Bergamot cold-pressed oil; orange cold-pressed oil; mandarin cold-pressed oil; lemon cold-pressed oil; dioxins; furans; PCBs; mineral components

### INTRODUCTION

Nowadays the Italian citrus industry is suffering a precarious condition. This is mainly due to the high costs of raw materials and the inadequate dealings between the industries and the cultivators. Furthermore, competition between Italian producers and the other big producers of citrus derivatives on the international market, such as Brazil and Argentine, is continuously increasing. Nevertheless, the Italian citrus industry (mainly located in the Sicilian and Calabrian regions) is composed of a few modern and high-technology companies (about 10) and several small–medium enterprises (about 90), the last of these capable only of extracting essential oil and crude juice from fruits. In Sicily most of the industry tends to extract juices from

pigmented oranges (red orange juice), whereas in Calabria juices are produced from yellow oranges.

With regard to citrus essential oils in Sicily, the production consists of essential oils from lemons, mandarins, and, to a minor extent, bitter oranges. In Calabria, instead, is more active the production of essential oil from bergamot (*Citrus bergamia* Risso et Poit), which grows only in a small area of Reggio Calabria province, situated on the Ionic seacoast.

The citrus industry extracts three distinct products from citrus fruit transformation: “peel oil”, also known as “cold-pressed oil”, is the essential oil in the external part of the fruit (called the flavedo), and the other two byproducts are identified as “essential oil” and “aqueous essence”, respectively, which are recovered during the juice concentration step. This stage occurs by evaporation from citrus juices, and it produces these two byproducts that are useful in the food, flavors, and cosmetic industries.

Therefore, due to the importance of these products for the Calabrian and Sicilian economies, a serious and constant quality control examination is needed, especially regarding pollutants, both inorganic (toxic mineral elements) and organic, such as

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polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs), and polychlorinated biphenyls (PCBs).

The scope of this survey is to check the presence (or absence) of toxic organic pollutant such as PCDDs, PCDFs, and PCBs in essential oils produced in southern Italy with the aim to check the status of the contamination due to those very toxic and ubiquitous chemicals.

PCDDs) and PCDFs, known as "dioxins", are organic environmental contaminants extremely stable and ubiquitous in the environment. Sixty percent of dioxins are produced during the combustion of municipal and industrial waste and various organic compounds (fossil fuels, petroleum refinement, natural combustions).

Combustion sources emit large quantities of dioxins into the atmosphere from where they are dispersed as vapor and particulates, leading to their ubiquitous presence in the environment. Deposition of both particulates and vapor from the air contaminates soil and water, and vegetation provides a significant entry into the terrestrial food chain.

For this reason and considering the absence in the literature of studies of this kind, in early 2003 an introductory work on only a few citrus essential oils was started (1–3). We planned to verify the main levels of organic compounds such as PCDDs, PCDFs, and PCBs. These pollutants are ubiquitous, and some of them are very toxic, stable, and hydrophobic, so they accumulate in fatty animal tissues and therefore in the human food chain (4, 5).

During the present survey we have extended the screening to both inorganic (minerals) and organic (PCDDs, PCDFs, and PCBs) substances on the entire category of essential oils from citrus (bergamot essential oil included) produced from the Italian industry during the past three working campaigns (2003–2005).

## MATERIALS AND METHODS

Sixty cold-pressed citrus essential oil samples respectively extracted from mandarin, lemon, orange, and bergamot (15 samples each) and produced in Calabria and Sicily (southern Italy) during the crop years 2003, 2004, and 2005 were analyzed.

All essential oils were sampled on-line during the extraction process. About 50 mL of sample was collected at regular intervals (2, 4, 6, and 8 h) and then poured into dark glass bottles and stored at 5 °C in the dark until analysis. The determination of PCDD/Fs, PCBs, aluminum (Al), lead (Pb), barium (Ba), cobalt (Co), mercury (Hg), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), cadmium (Cd), antimony (Sb), silver (Ag), tin (Sn), and zinc (Zn) was performed on all samples.

**Chemicals.** Hexane, toluene, dichloromethane, methanol (pesticide grade), active carbon, concentrated ultrapure sulfuric acid (98% p/p), and nitric acid (65% p/p) were purchased from Carlo Erba Reagenti (Milan, Italy).

Nonane ultrapure and basic alumina were purchased from Fluka (Steinheim, Germany).

Silica gel 60, 70–230 mesh, was purchased from Merck (Darmstadt, Germany). Glass wool and glass fiber disks (Whatman GF/D) used for the preparation of carbon/glass fiber columns, were purchased from Whatman (Kent, U.K.).

Anhydrous sodium sulfate, sodium borohydride, sodium hydroxide (pellets), and potassium hydroxide (pellets) analytic grade were purchased from Applichem (Darmstadt, Germany).

Mg(NO<sub>3</sub>)<sub>2</sub> 1% as nitrate (code B0190634), NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 10% (code 9303445), and Pd 1% (code 0190635) were purchased from Perkin-Elmer (Boston, MA) and used to prepare matrix modifier solutions.

Double-distilled deionized water (Milli-Q Millipore 18.2 MΩ-cm resistivity; heavy metal free) was used for all dilutions. All plastic and glassware were cleaned by soaking in diluted HNO<sub>3</sub> (1+9, v/v) and rinsed with distilled water prior to use.

**PCDD, PCDF, and PCB Analysis.** All of the standards were purchased from Cambridge Isotope Laboratory (Andover, MA).

**PCDD and PCDF Standard Solutions:** calibration solution CIL EDF-9999 (CS1–CS5); daily check standard CIL EDF-999-3 (CS3); internal standard (I.S.) stock solution, CIL EDF-8999, containing 15 <sup>13</sup>C<sub>12</sub> 2,3,7,8-PCDD/F congeners at 100 pg/μL and <sup>13</sup>C-OCDD at 200 pg/μL; internal standard working solution, obtained by dilution of I.S. stock solution CIL EDF-8999 containing <sup>13</sup>C<sub>12</sub> PCDD/F at 2 pg/μL and <sup>13</sup>C<sub>12</sub> OCDD at 4 pg/μL; syringe standard (S.S.) stock solution, CIL EDF-5999, containing <sup>13</sup>C<sub>12</sub>1,2,3,4-TCDD and <sup>13</sup>C<sub>12</sub>1,2,3,7,8,9-HxCDD at 200 pg/μL; syringe standard working solution at 4 pg/μL, obtained by dilution from S.S. stock solution CIL EDF-5999.

**PCB Standard Solution:** calibration solution CIL EC-4939 (CS1–CS5); daily check standard CS1; <sup>13</sup>C<sub>12</sub> PCB-202 at 80 μg/mL added to all calibration solutions; I.S. stock solution (<sup>13</sup>C<sub>12</sub> labeled WHO PCB), EC-4937, containing <sup>13</sup>C<sub>12</sub> ortho-PCBs (105, 114, 118, 123, 156, 157, 167, 189) and <sup>13</sup>C<sub>12</sub> non-ortho-PCBs (77, 81, 126, 169) at 1 μg/mL; I.S. working solution, containing <sup>13</sup>C<sub>12</sub> PCBs at 100 pg/μL obtained by dilution from I.S. stock solution EC-4937; S.S. stock solution, EC-1408, containing <sup>13</sup>C<sub>12</sub> PCB202 at 40 μg/mL; S.S. working solution at 20 pg/μL, obtained by dilution from stock solution EC-1408.

**Materials and Chromatographic Columns.** Anhydrous sodium sulfate (~400 g) was washed with dichloromethane (1 L), filtered on a Büchner funnel, and activated in the oven overnight at 130 °C.

**Silica gel 60, 70–230 mesh** (~1000 g), was washed with dichloromethane (6 L), filtered on a Büchner funnel, and activated in the oven overnight at 130 °C.

**Acid-modified silica gel (40/60 w/w)**, 70–230 mesh, already activated (~600 g), was amended with concentrated sulfuric acid (400 g) and mixed for 5 h.

**For base-modified silica gel**, potassium hydroxide (~300 g) was mixed with methanol (800 mL) for 20 min. Then silica gel 60 already activated (~400 g) and methanol (200 mL) were added and mixed for 2 h. The slurry was filtered on a Büchner funnel, washed with dichloromethane (200 mL), and activated in the oven overnight at 130 °C.

**Basic alumina** (~400 g) was activated in a muffle furnace at 400–450 °C overnight and used on the day of activation.

**The fractionation column** was a glass column (650 mm × 55 mm i.d.) packed from the bottom with anhydrous sodium sulfate activated (~10 g), base-modified silica gel (~25 g), acid-modified silica gel 40/60 w/w (~40 g), and anhydrous sodium sulfate activated (~30 g).

**The carbon/glass fiber column** was a glass column (90 mm × 15 mm i.d.) packed with carbon/glass fiber mixture prepared as follows: weigh 150 g of approximately 3 mm wide glass fiber filter disks, homogenize with dichloromethane (40 mL) using an Ultra-Turrax at medium speed for 15–30 s, and add 15 mg of active carbon (6). The carbon/glass fiber column was conditioned with toluene (60 mL), methanol (60 mL), toluene (60 mL), and dichloromethane/hexane (30:70 v/v) and was reusable.

**Column 1** was a Pasteur pipet packed from the bottom with anhydrous sodium sulfate activated (0.5 cm), acid-modified silica gel 40/60 (w/w) (6.5–7.5 cm), and anhydrous sodium sulfate activated (0.5 cm) (7).

**Column 2** was a glass column (400 mm × 25 mm i.d.) packed with anhydrous sodium sulfate activated (~2 g), acid-modified silica gel 40/60 (w/w) (~50 g), and anhydrous sodium sulfate activated (~2 g).

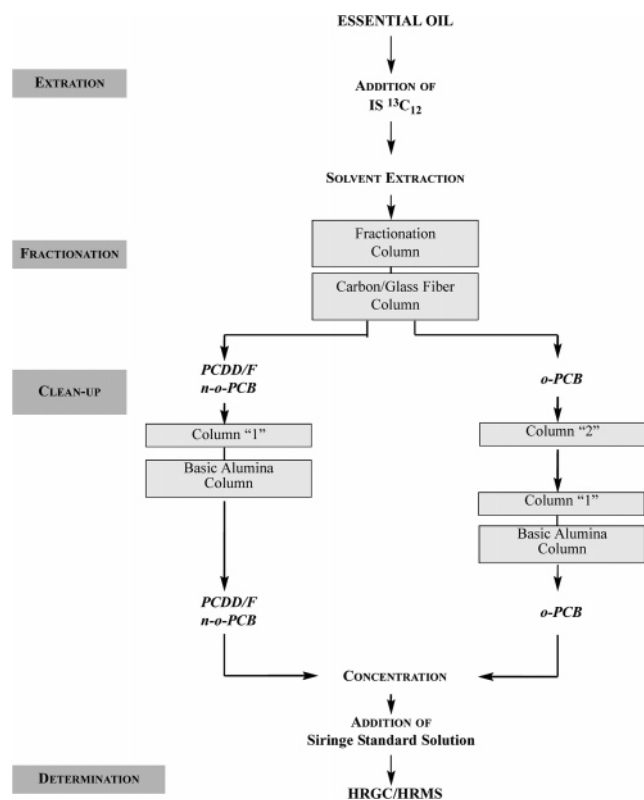
**The basic alumina column** was a Pasteur pipet packed from the bottom with anhydrous sodium sulfate activated (0.5 cm), basic alumina (6.5–7.5 cm), and anhydrous sodium sulfate activated (0.5 cm) (6).

**Column 2** was washed with dichloromethane (50 mL × 3) followed by hexane (100 mL). **Columns 1** and **basic alumina** were washed with dichloromethane (20 mL) followed by hexane (20 mL).

**Extraction and Fractionation of Analytes.** In brief, the analytical method used for PCDD/F and PCB analyses consists of three steps: extraction, cleanup according to the methods of Krokos et al. (7) and Santelli et al. (1), and quantification by HRGC-HRMS [methods U.S. EPA1613/94 (8) and U.S. EPA1668/A (9)].

Each batch of samples (six or more samples) contains one blank that was spiked with the internal standards and followed the same process as the real samples. The method blank ensures the absence of contaminants in the reagents.

**Sample Preparation.** Figure 1 shows a flow chart of the analytical steps adopted for the extraction and cleanup of PCDD/Fs and PCBs in



**Figure 1.** Extraction procedure, clean up, and instrumental analysis of PCDD/Fs and PCBs in cold-pressed citrus essential oil.

cold-pressed citrus essential oils as reported from Santelli et al. in our previous papers (*J*). Briefly, an aliquot of sample (5 g exactly weighed) was spiked with 200  $\mu$ L of PCDD/F I.S. working solution and 25  $\mu$ L of PCB I.S. working solution and mixed with 70 g of acid-modified silica gel 40/60 (w/w) and 200 mL of hexane in a glass bottle.

The mixture was transferred to the top of a chromatographic system, which consisted of the fractionation column connected in series with the carbon/glass fiber column. The analytes were eluted with a mixture of dichloromethane/hexane 30:70 v/v (400 mL) followed by hexane (100 mL).

In these conditions the planar compounds (PCDD/F and non-ortho-PCBs) were immobilized into the carbon column and the nonplanar compounds (ortho-PCBs) were passed through into a reservoir.

**Clean-up of the Planar Fraction (PCDD/Fs and Non-ortho-PCBs).** The carbon column containing PCDD/Fs and non-ortho-PCBs was inverted and eluted with toluene (170 mL). The eluate was concentrated to dryness under a nitrogen stream (Turbovap II, Zymark), rinsed with hexane, and then concentrated to 0.5 mL in hexane.

This extract was transferred to the top of a cleanup chromatographic system constituted of a column 1 connected in series with the basic alumina column.

A first elution with hexane (20 mL) let the purified non-ortho-PCBs pass through the system straight into a reservoir put at the bottom. The alumina column, which contains now PCDD/Fs, was then eluted with dichloromethane (40 mL). Both the eluates were concentrated under a stream of nitrogen (Turbovap II, Zymark) to 0.5 mL and transferred into a vial; 25  $\mu$ L of PCB S.S. working solutions and 25  $\mu$ L of PCDD/Fs S.S. working solutions were added, and then the solution was concentrated to 25  $\mu$ L under a nitrogen steam in a Reactitherm (heating/stirring module, Pierce).

**Clean-up of the Nonplanar Fraction.** One-fifth of the extract containing ortho-PCBs was transferred to the top of a chromatographic system made of a column 2 and eluted with 100 mL of a mixture dichloromethane/hexane 30:70 and hexane (100 mL).

The extract was then concentrated to 0.5 mL under a stream of nitrogen and transferred to the top of a chromatographic system made of column 1 and a basic alumina column joint in series.

A first elution with hexane (10 mL) allowed the elution of PCBs in the basic alumina column, whereas the interfering substances passed through the system straight into a reservoir put at the bottom. The alumina column, which contains now PCDD/Fs, was then eluted with dichloromethane/hexane 30:70 dichloromethane (30 mL). The eluate was concentrated under a stream of nitrogen to 0.5 mL and transferred into a vial.

The fraction obtained was added to 25  $\mu$ L of the PCBs S.S. working solutions (as above) and concentrated to 25  $\mu$ L.

**HRGC/HRMS.** Analysis and quantification of the samples was carried using an Agilent GC (HP6890) interfaced to a Micromass AutoSpec Ultima mass spectrometer. All extracts were chromatographed through a J&W DB-5MS column (60 m; 0.25 mm i.d.; 0.25  $\mu$ m film) with the injector set to splitless mode at 280  $^{\circ}$ C. One microliter of extract was injected using a CTC-PAL autosampler.

The carrier gas used was pure helium. During PCDD/F analysis the GC oven temperature program was 140  $^{\circ}$ C for 4 min, then raised at 10  $^{\circ}$ C/min to 220  $^{\circ}$ C, raised at 28.5  $^{\circ}$ C/min to 260  $^{\circ}$ C, held for 2.9 min, and finally raised at 12.5  $^{\circ}$ C/min to 310  $^{\circ}$ C.

For the analysis of PCBs the GC oven temperature program was 140  $^{\circ}$ C for 1 min, then raised at 20  $^{\circ}$ C/min to 280  $^{\circ}$ C, held for 10 min, then raised at 25  $^{\circ}$ C/min to 300  $^{\circ}$ C, and held for 1.2 min.

The mass spectrometer was set up in electron impact ionization mode with an electron energy of 35 eV, a trap current of 650  $\mu$ A, and a source temperature of 275  $^{\circ}$ C. The resolution was between 10000 and 11000 (10% valley definition).

Multiple-group selected ion monitoring (SIM) was performed (five functions for PCDD/F run and three functions for PCB run); perfluorokerosene (PFK) was used to provide lock masses and to tune the instrument. SIM was employed using the two most intense ions from the molecular ion cluster for each homologue ( $M^+$  and  $M + 2^+$ ).

The mass ratio proves the identity of the peaks, whereas just one peak ( $M^+$ ) is used for quantification. Utilizing a calibration curve, performed using EPA standards CS1-CS5, the relative response (labeled to native) and response factors can be measured as a function of concentration. A standard (CS3 for PCDD/Fs and CS1 for PCBs, as above) is used for calibration verification as reported in U.S. EPA 1613/94 (8) and U.S. EPA 1668/A (9). Using isotopic dilution coupled with analysis by HRGC/HRMS makes the method used in our laboratory highly specific and selective. The technique makes it possible to determine analyte concentrations in parts per quadrillion (ppq,  $10^{-6}$  ppm).

For each sample three runs were performed: the extract containing the planar fraction was run twice, once for the determination of PCDD/Fs and once for the determination of non-ortho-PCB concentration, whereas the nonplanar fraction extract was used to determine the ortho-PCB concentrations.

**Mineral Component Analysis.** All of the standards were purchased from Perkin-Elmer (Boston, MA).

GFAA Mix Standard Stock solution N9300244 in nitric acid 5% (v/v) contains 10  $\mu$ g/mL Ag, 100  $\mu$ g/mL Al, 100  $\mu$ g/mL As, 50  $\mu$ g/mL Ba, 5  $\mu$ g/mL Be, 5  $\mu$ g/mL Cd, 50  $\mu$ g/mL Co, 20  $\mu$ g/mL Cr, 50  $\mu$ g/mL Cu, 20  $\mu$ g/mL Fe, 20  $\mu$ g/mL Mn, 50  $\mu$ g/mL Ni, 100  $\mu$ g/mL Pb, 100  $\mu$ g/mL Sb, 100  $\mu$ g/mL Se, and 100  $\mu$ g/mL Tl.

Pure AS Calibration Standard Stock standard solutions (1000 mg/L) of Zn in 2% HNO<sub>3</sub>; Sn 20% HCl and Hg in 10% HNO<sub>3</sub> (codes N9300168, N9300161, and N9300174).

**Standard Solution Preparation.** The element standard solutions used for five-point calibration curves were prepared by diluting stock solutions with nitric acid 0.2% (v/v). The element concentration ranges were between 20 and 100  $\mu$ g/L for Al, As, Pb, Sb, Tl, and Sn; between 5 and 50  $\mu$ g/L for Ba, Co, Cu, Ni; between 1 and 20  $\mu$ g/L for Cr, Fe, and Mn; and between 2 and 10  $\mu$ g/L for Ag. The concentration range for Be, Cd, Hg, and Zn was between 0.5 and 5  $\mu$ g/L.

**Sample Preparations.** The digestion procedure of citrus essential oils was performed according to the Italian Officials methods for vegetable stuff analysis (MUACV) (10). Briefly, 5 g of sample, exactly weighed, was placed into a 250 mL digestion tube (Velp Scientifica, Milan, Italy). Wet ashing was performed by adding 20 mL of a mixture of concentrated sulfuric/nitric acid (1:5) and 10 mL of deionized water. This mixture was heated in a Digestion Blocks Mineral Twenty (PBI,

Table 1. PCDDi (Picograms per Gram of Whole Weight) and  $\Sigma$ TEQ<sub>(PCDD)<sub>i</sub> in Cold-Pressed Citrus Essential Oil</sub>

sample	2,3,7,8-TCDD (1) <sup>c</sup>	1,2,3,7,8-PeCDD (1) <sup>c</sup>	1,2,3,4,7,8-HxCDD (0.1) <sup>c</sup>	1,2,3,6,7,8-HxCDD (0.1) <sup>c</sup>	1,2,3,7,8,9-HxCDD (0.1) <sup>c</sup>	1,2,3,4,6,7,8-HpCDD (0.01) <sup>c</sup>	OCDD (0.0001) <sup>c</sup>	$\Sigma$ PCDDi <sup>a</sup>	$\Sigma$ TEQ <sub>(PCDD)<sub>i</sub><sup>b</sup></sub>
lemon									
1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
2	<0.01	<0.01	<0.01	2.47	1.43	0.84	2.34	7.11	0.42
3	2.65	6.58	<0.01	<0.01	<0.01	4.84	19.75	33.85	9.30
4	1.34	0.16	<0.01	<0.01	0.22	2.00	6.83	10.57	1.55
5	<0.01	6.31	<0.01	<0.01	<0.01	4.48	19.52	30.35	6.39
6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
7	<0.01	<0.01	<0.01	1.92	1.12	<0.01	<0.01	3.09	0.33
8	3.24	8.33	<0.01	0.56	0.32	5.92	24.45	42.83	11.74
9	0.67	0.08	<0.01	<0.01	0.11	1.23	3.42	5.53	0.78
10	0.80	2.61	<0.01	0.50	0.34	2.43	9.69	16.39	3.54
11	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
12	<0.01	<0.01	<0.01	1.37	0.81	<0.01	<0.01	2.23	0.24
13	1.47	3.08	<0.01	<0.01	<0.01	2.68	10.35	17.61	4.59
14	2.52	3.66	<0.01	1.11	0.84	4.16	16.23	28.53	6.43
15	<0.01	6.31	<0.01	<0.01	<0.01	4.48	19.52	30.35	6.39
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
$\bar{x} \pm SD^d$	0.85 ± 1.14	2.48 ± 3.05	<0.01	0.53 ± 0.81	0.35 ± 0.47	2.21 ± 2.11	8.81 ± 8.92	15.24 ± 14.48	3.45 ± 3.83
max	3.24	8.33	<0.01	2.47	1.43	5.92	24.45	42.83	11.74
recovery % <sup>e</sup>	62–78	67–84	72–75	68–82	62–73	65–78	71–79		
mandarin									
1	0.99	0.70	<0.01	<0.01	0.20	1.47	4.48	7.86	1.73
2	1.85	<0.01	<0.01	<0.01	1.45	<0.01	<0.01	3.35	2.01
3	0.56	<0.01	<0.01	<0.01	<0.01	3.59	39.87	44.06	0.65
4	0.92	<0.01	14.76	<0.01	4.89	1.79	6.57	28.95	2.92
5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
7	0.88	0.72	<0.01	<0.01	0.22	1.23	4.48	7.55	1.64
8	1.73	<0.01	<0.01	<0.01	1.62	<0.01	<0.01	3.4	1.90
9	0.65	<0.01	<0.01	<0.01	<0.01	3.03	39.64	43.36	0.73
10	0.92	<0.01	14.00	<0.01	4.06	1.79	6.57	27.36	2.76
11	1.60	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.66	1.61
12	0.53	<0.01	<0.01	<0.01	2.44	<0.01	<0.01	3.02	0.79
13	0.99	0.81	<0.01	<0.01	0.43	1.47	3.65	7.37	1.86
14	1.95	<0.01	<0.01	<0.01	1.56	<0.01	<0.01	3.56	2.12
15	0.56	<0.01	<0.01	<0.01	<0.01	3.21	30.46	34.27	0.64
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
$\bar{x} \pm SD^d$	0.94 ± 0.61	0.16 ± 0.30	1.93 ± 5.06	<0.01	1.13 ± 1.57	1.18 ± 1.31	9.05 ± 14.63	14.39 ± 16.25	1.43 ± 0.91
max	1.95	0.81	14.76	<0.01	4.89	3.59	39.87	44.06	2.92
recovery % <sup>e</sup>	63–72	65–81	68–81	69–85	63–77	69–73	71–82		
orange									
1	1.66	0.58	2.05	2.15	0.58	1.71	2.88	11.61	2.74
2	0.48	0.55	<0.01	3.01	1.88	2.47	19.49	27.89	1.56
3	0.57	1.07	0.94	2.25	1.03	1.87	3.75	11.48	2.08
4	0.49	0.59	<0.01	<0.01	1.84	1.17	4.18	8.29	1.28
5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
6	4.00	<0.01	<0.01	<0.01	<0.01	3.90	7.88	15.82	4.06
7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
8	1.31	1.27	1.36	2.94	1.45	2.52	7.20	18.06	3.19
9	<0.01	0.39	<0.01	<0.01	1.42	0.52	0.73	3.08	0.55
10	1.11	0.39	1.37	1.43	0.39	1.14	1.92	7.74	1.83
11	6.52	<0.01	<0.01	<0.01	<0.01	6.50	8.85	21.91	6.61
12	<0.01	0.15	<0.01	1.63	1.04	1.17	12.58	16.58	0.45
13	<0.01	0.67	<0.01	<0.01	<0.01	0.57	<0.01	1.29	0.68
14	1.97	0.99	0.85	1.39	2.68	2.47	11.09	21.44	3.49
15	1.47	0.86	0.57	0.93	2.40	2.04	8.79	17.06	0.17
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	0.02
$\bar{x} \pm SD^d$	1.31 ± 1.80	0.50 ± 0.42	0.48 ± 0.67	1.05 ± 1.14	0.98 ± 0.93	1.87 ± 1.66	5.96 ± 5.62	12.16 ± 8.66	2.01 ± 1.79
max	6.52	1.27	2.05	3.01	2.68	6.50	19.49	27.89	6.61
recovery % <sup>e</sup>	64–81	63–79	71–84	65–81	65–74	66–72	67–79		
bergamot									
1	<0.01	<0.01	0.24	0.08	0.12	0.10	1.47	2.01	0.03
2	<0.01	<0.01	0.43	0.62	0.42	0.25	0.24	1.96	0.11
3	0.21	<0.01	<0.01	<0.01	<0.01	0.54	0.59	1.34	0.21
4	<0.01	3.50	<0.01	<0.01	<0.01	3.96	15.52	22.98	0.35
5	1.66	0.58	1.96	1.85	0.58	<0.01	2.87	9.49	2.10
6	1.20	<0.01	<0.01	0.98	1.33	<0.01	<0.01	3.51	1.31
7	0.48	0.60	1.20	2.96	1.58	2.54	18.86	25.68	0.98
8	0.36	1.03	0.94	2.54	1.03	1.87	3.65	11.42	0.82
9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
10	0.50	0.59	6.25	<0.01	1.84	1.17	4.12	14.48	1.20
11	<0.01	<0.01	<0.01	1.58	<0.01	<0.01	<0.01	1.58	0.16
12	0.56	<0.01	<0.01	<0.01	<0.01	3.56	25.32	29.44	0.56

Table 1 (Continued)

sample	2,3,7,8-TCDD (1) <sup>c</sup>	1,2,3,7,8-PeCDD (1) <sup>c</sup>	1,2,3,4,7,8-HxCDD (0.1) <sup>c</sup>	1,2,3,6,7,8-HxCDD (0.1) <sup>c</sup>	1,2,3,7,8,9-HxCDD (0.1) <sup>c</sup>	1,2,3,4,6,7,8-HpCDD (0.01) <sup>c</sup>	OCDD (0.0001) <sup>c</sup>	ΣPCDDi <sup>a</sup>	ΣTEQ <sub>(PCDDi)</sub> <sup>b</sup>
begarmot (continued)									
13	0.92	<0.01	11.46	<0.01	4.56	<0.01	6.20	23.14	2.11
14	<0.01	<0.01	<0.01	1.26	<0.01	<0.01	<0.01	1.26	0.13
15	3.25	<0.01	<0.01	<0.01	<0.01	3.89	5.30	12.45	3.25
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
$\bar{x} \pm SD^d$	0.61 ± 0.89	0.42 ± 0.91	1.50 ± 3.20	0.79 ± 1.02	0.76 ± 1.23	1.10 ± 1.57	5.61 ± 7.90	10.72 ± 10.27	0.89 ± 0.96
max	3.25	3.50	11.46	2.96	4.56	3.96	25.32	29.44	3.25
recovery % <sup>e</sup>	66–81	63–77	65–82	63–81	67–76	64–79	69–83		

<sup>a</sup> Sum of PCDDi concentrations (pg/g of sample). <sup>b</sup> ΣTEQ<sub>(PCDDi)</sub> = Σ(PCDDi × TEFi) (pg of TEQ/g). <sup>c</sup> Toxic equivalency factors (WHO TEF). <sup>d</sup> Mean ± standard deviation. <sup>e</sup> <sup>13</sup>C<sub>12</sub> PCDDs internal standard recovery ranges (%).

Milan, Italy) with temperatures maintained between 100 and 350 °C. The temperature program was performed according to the method of Cautela et al. (2, 3): 100 °C for 60 min, then 180 °C for 80 min, 240 °C for 60 min, then 350 °C, held for 60 min. After digestion was complete, the samples were cooled and quantitatively transferred in Brand volumetric flasks and then filled to a volume of 50 mL with water.

For mercury analysis, 2.5 g of essential oil was diluted to 25 mL with methanol and analyzed by flow injection analysis system AAS.

**Mineral Component Instrumental Analysis.** Aluminum (Al), arsenic (As), lead (Pb), barium (Ba), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), cadmium (Cd), antimony (Sb), silver (Ag), tin (Sn), and zinc (Zn) determinations were performed by graphite furnace atomic absorption spectrometry (GFAAS).

Analysis and quantification of the samples were carried using an AAnalyst 600 spectrometer (Perkin-Elmer) interfaced to an AS800 autosampler, whereas mercury (Hg) was detected by flow injection analysis system mercury hydride system atomic spectrometry (FI-M/H-AAS), using a flow injection system FIAS100 (Perkin-Elmer) interfaced to the AAnalyst 600 spectrometer.

Argon was used as the inert gas for graphite furnace measurements and for the flow injection system. Pyrolytic-coated graphite tubes with a platform were used for determinations. The wavelength range selection, sample volume, ramp and hold times for drying, ashing, and atomization and cleaning temperatures were optimized prior to analysis to obtain the maximum absorbance with minimum background in accordance with recommended analytical conditions reported in the Perkin-Elmer technical procedures books (11, 12).

Spectrometer, furnace, and flow injection system control, signal acquisition, and data reprocessing were managed using WinLab 32 software.

For each element determination samples were analyzed in triplicate, and matrix spike recovery tests were done in accordance with Cautela et al. (2, 3) by spiking each digested sample with a known amount of standard solution of the analytes.

The detection limit (LOD) was defined as the concentration corresponding to 3 times the standard deviation of 20 calibration blanks (nitric acids 0.2% v/v).

Each batch of samples (15 samples) contained one digestion blank to ensure the absence of contaminants in the reagents and a standard reference material NCD DC 73348 (bush branches and leaves) purchased from LabService (Anzola Emilia, Italy) to ensure quality control, accuracy, and quality checks of the procedures.

## RESULTS AND DISCUSSION

The toxicity and carcinogenicity of 17 PCDD and PCDF congeners and 12 PCB “dioxin-like” compounds (4, 5) are well documented. The complex nature of PCDD, PCDF, and PCB mixtures complicates the evaluation of the toxicity of these compounds in foods and feedstuffs. For this purpose the concept of toxic equivalency factors (TEFs) has been developed and introduced by the World Health Organization (WHO) to facilitate the risk assessment and regulatory control of exposure

to these mixtures (13, 14). As is well-known, this calculation system is based on the following points:

- Only PCDD and PCDF 2,3,7,8-chlorine-substituted congeners are toxic.
- Toxicity is related to the affinity of each congener to Ah cytoplasmic receptor protein. Better affinity gives higher toxicity.
- The most toxic congener is 2,3,7,8-TCDD as it has the best affinity with the cellular receptors. 2,3,7,8-TCDD has been given a WHO TEF of 1. The toxicity of the other congeners (16 2,3,7,8-PCDDs/PCDFs and 12 PCBs dioxin-like) is related to that of 2,3,7,8-TCDD, and their WHO TEFs are fractions of 1.

The analytical results for each of the 17 toxic PCDD/F congeners and 12 toxic PCB congeners are expressed as a concentration of the total equivalent toxicity of TCDD (TEQ). The sum of all the TEQ values gives the total TEF for a sample, following the system specified by WHO (8, 9):

$$\text{WHO-TEQ} = \sum(\text{PCDDi} \times \text{TEFi}) + \sum(\text{PCDFi} \times \text{TEFi}) + \sum(\text{PCBi} \times \text{TEFi}) \quad (1)$$

**Tables 1–3** report contamination levels of each congener of PCDDs, PCDFs, and PCBs found in the various essential oil samples analyzed, expressed as picograms per gram of whole weight.

In **Tables 1–3** are also reported the internal standard recoveries (percent). These recoveries were used to check that the analytical procedures were satisfactory. The recoveries of the individual <sup>13</sup>C<sub>12</sub> PCDD/Fs labeled internal standard are in the range of 61–89% in accordance with EPA method 1613 and Annex II of the Commission Directive 2002/70/EC. The recoveries of the <sup>13</sup>C<sub>12</sub> labeled dioxin-like PCB congeners are in the range of 60–89% and met the criteria specified in U.S. EPA method 1668A.

In **Figure 2** are reported mean percent contributions of PCDDs, PCDFs, and PCBs TEQs to the TEQ in the samples analyzed. WHO TEQ values were calculated using an upper-bound concentration (15) assuming that all of the results under the LOD would be equal to that limit. All of the concentrations are expressed on a whole weight basis.

The PCDD data in **Table 1** demonstrate that the contamination found in the various types of essential oils analyzed is approximately the same. The mean values of sums of PCDD congeners concentrations (ΣPCDDi) for each type of oil are very similar, with a minimum found in bergamot oils (10.72 pg/g) and a little higher in orange (12.16 pg/g), mandarin (14.39 pg/g), and lemon oils (15.24 pg/g).

The values found for the most toxic TCDD (WHO TEF = 1) are between the LOD 0.01 and 6.52 pg/g of whole weight (sample 11 orange oil). A mean of 0.85 pg/g was found in lemon oil samples, 0.94 pg/g in mandarin oils, 1.31 pg/g in orange oils, and about 0.61 pg/g in bergamot oil samples. 1,2,3,7,8-

PeCDD (WHO TEF = 1) was found in 73% of the orange essential oil samples analyzed, 60% of the lemon oil samples, 33.3% of the bergamot samples, and 20% of the mandarin essential oil samples. Concentrations found vary between the LOD of 0.01 and 8.33 pg/g of whole weight (sample 8 lemon oil).

**Table 2.** PCDF<sub>i</sub> (Picograms per Gram of Sample) and ΣTEQ<sub>(PCDF)<sub>i</sub></sub> in Cold-Pressed Citrus Essential Oil

sample	2,3,4,7,8-PeCDF (0.5) <sup>c</sup>	2,3,7,8-TCDF (0.1) <sup>c</sup>	1,2,3,4,7,8-HxCDF (0.1) <sup>c</sup>	1,2,3,6,7,8-HxCDF (0.1) <sup>c</sup>	2,3,4,6,7,8-HxCDF (0.1) <sup>c</sup>	1,2,3,7,8,9-HxCDF (0.1) <sup>c</sup>	1,2,3,7,8-PeCDF (0.05) <sup>c</sup>	1,2,3,4,6,7,8-HpCDF (0.01) <sup>c</sup>	1,2,3,4,7,8,9-HpCDF (0.01) <sup>c</sup>	OCDF (0.0001) <sup>c</sup>	ΣPCDF <sub>i</sub> <sup>a</sup>	ΣTEQ <sub>(PCDF)<sub>i</sub></sub> <sup>b</sup>
lemon												
1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
2	0.29	0.70	0.29	0.35	0.36	0.45	0.25	3.49	<0.01	2.29	8.48	0.41
3	<0.01	4.79	<0.01	<0.01	<0.01	<0.01	2.03	<0.01	<0.01	7.68	14.57	0.59
4	0.20	1.67	<0.01	<0.01	0.58	<0.01	1.57	<0.01	<0.01	3.70	7.77	0.41
5	5.85	<0.01	2.93	2.89	3.96	2.86	<0.01	19.71	<0.01	10.95	49.18	4.39
6	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.19	0.01
8	1.29	5.79	0.65	0.64	0.85	0.63	2.51	4.29	<0.01	9.87	26.52	1.67
9	<0.01	0.23	<0.01	<0.01	<0.01	<0.01	0.80	<0.01	<0.01	<0.01	1.11	0.07
10	0.20	1.67	<0.01	<0.01	0.58	<0.01	1.57	<0.01	<0.01	3.70	7.77	0.41
11	4.58	<0.01	2.28	2.24	2.98	2.19	<0.01	15.16	<0.01	6.02	35.48	3.41
12	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
13	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
14	3.85	7.78	1.93	1.90	2.53	1.87	3.46	12.84	<0.01	14.24	50.42	3.83
15	<0.01	0.95	<0.01	<0.01	0.09	<0.01	1.18	<0.01	<0.01	1.24	3.52	0.17
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
$\bar{x} \pm SD^d$	1.09 ± 1.96	1.58 ± 2.49	0.55 ± 0.98	0.54 ± 0.97	0.80 ± 1.28	0.54 ± 0.95	0.90 ± 1.11	3.71 ± 6.59	<0.01	3.99 ± 4.71	13.69 ± 17.99	1.03 ± 1.54
max	5.85	7.78	2.93	2.89	3.96	2.86	3.46	19.71	<0.01	14.24	50.42	4.39
recovery % <sup>e</sup>	68–74	63–79	61–84	69–78	66–83	64–82	67–81	71–83	69–84	74–81		
mandarin												
1	1.43	2.35	1.51	1.41	1.67	0.55	1.40	0.57	0.49	4.89	16.27	1.43
2	0.26	3.20	<0.01	<0.01	<0.01	<0.01	<0.01	32.18	<0.01	5.59	41.29	0.26
3	1.15	1.47	<0.01	<0.01	<0.01	<0.01	0.93	<0.01	<0.01	<0.01	3.62	1.15
4	1.12	0.61	0.71	<0.01	<0.01	<0.01	0.26	14.35	<0.01	1.36	18.45	1.12
5	0.21	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.30	0.21
6	<0.01	0.70	1.10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.88	0.01
7	1.34	1.75	1.18	1.12	1.33	0.44	1.10	<0.01	0.39	3.60	12.28	1.34
8	0.35	3.80	0.34	0.30	0.35	0.12	0.31	38.78	0.11	6.88	51.33	0.35
9	<0.01	0.08	<0.01	<0.01	<0.01	<0.01	0.49	<0.01	<0.01	<0.01	0.65	0.01
10	0.29	<0.01	0.06	<0.01	<0.01	<0.01	<0.01	1.15	<0.01	<0.01	1.57	0.29
11	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
12	0.18	1.90	1.75	0.58	0.69	0.23	0.60	13.21	0.21	2.60	21.95	0.18
13	<0.01	2.54	<0.01	<0.01	<0.01	<0.01	0.43	<0.01	<0.01	<0.01	3.05	0.01
14	<0.01	<0.01	0.67	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.76	0.01
15	0.12	1.50	1.53	0.39	0.46	0.16	0.40	8.81	0.14	1.74	15.26	0.12
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
$\bar{x} \pm SD^d$	0.43 ± 0.53	1.33 ± 1.26	0.59 ± 0.66	0.26 ± 0.45	0.31 ± 0.53	0.11 ± 0.17	0.40 ± 0.44	7.28 ± 12.55	0.10 ± 0.15	1.78 ± 2.38	12.58 ± 15.73	0.57 ± 0.49
max	1.43	3.80	1.75	1.41	1.67	0.55	1.40	38.78	0.49	6.88	51.33	1.55
recovery % <sup>e</sup>	62–81	68–84	71–87	65–81	70–85	69–82	62–89	69–82	73–81	70–83		
orange												
1	1.31	2.35	1.09	1.07	1.46	1.51	1.25	3.87	<0.01	1.41	15.33	1.50
2	<0.01	0.99	0.48	0.65	0.43	0.49	<0.01	4.71	1.56	2.12	11.45	0.37
3	1.09	1.41	1.34	1.42	1.35	0.72	1.40	3.69	2.34	3.03	17.79	1.30
4	<0.01	1.16	<0.01	<0.01	0.74	<0.01	0.30	<0.01	<0.01	0.65	2.91	0.21
5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
6	2.28	6.94	<0.01	<0.01	<0.01	<0.01	2.13	<0.01	<0.01	2.41	13.82	1.94
7	0.84	1.12	0.79	0.76	1.14	1.21	0.81	<0.01	<0.01	0.84	7.54	0.96
8	0.48	2.22	0.78	0.96	0.75	0.79	0.45	5.84	2.08	2.69	17.03	0.89
9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.55	<0.01	<0.01	<0.01	0.64	0.04
10	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
11	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
12	3.22	5.42	0.61	0.63	0.65	0.61	3.00	2.27	1.04	3.54	20.99	2.58
13	<0.01	2.54	<0.01	<0.01	<0.01	<0.01	0.43	<0.01	<0.01	<0.01	3.05	0.28
14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
15	2.91	8.58	0.41	0.42	0.43	0.41	2.71	1.52	0.70	3.17	21.26	2.64
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
$\bar{x} \pm SD^d$	0.81 ± 1.13	2.19 ± 2.70	0.37 ± 0.46	0.40 ± 0.48	0.47 ± 0.53	0.39 ± 0.49	0.87 ± 1.02	1.47 ± 2.07	0.52 ± 0.83	1.33 ± 1.36	8.81 ± 8.31	0.85 ± 0.94
max	3.22	8.58	1.34	1.42	1.46	1.51	3.00	5.84	2.34	3.54	21.26	2.64
recovery % <sup>e</sup>	67–84	65–81	64–82	67–80	66–81	70–81	67–81	67–89	64–79	67–84		
bergamot												
1	<0.01	0.06	<0.01	<0.01	<0.01	<0.01	2.66	0.69	0.75	1.39	5.60	0.16
2	<0.01	0.09	0.08	0.06	0.54	0.49	<0.01	0.75	1.27	0.08	3.38	0.15
3	<0.01	<0.01	0.22	0.11	0.11	<0.01	<0.01	1.12	0.56	0.26	2.42	0.07
4	<0.01	<0.01	2.90	2.60	3.96	2.86	<0.01	12.20	<0.01	5.20	29.76	1.36
5	<0.01	2.35	1.06	1.06	1.46	1.51	<0.01	3.87	<0.01	1.41	12.76	0.79
6	<0.01	1.30	<0.01	<0.01	<0.01	1.20	<0.01	15.20	1.23	5.20	24.18	0.42
7	<0.01	0.99	0.50	0.65	0.43	0.30	<0.01	4.71	1.56	2.12	11.28	0.36
8	1.01	0.09	1.40	1.42	1.35	0.72	0.95	3.69	2.33	3.03	16.01	1.11
9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
10	<0.01	1.12	<0.01	<0.01	0.74	<0.01	0.30	<0.01	0.80	0.65	3.66	0.22
11	<0.01	<0.01	<0.01	<0.01	<0.01	0.25	<0.01	<0.01	<0.01	<0.01	0.34	0.03

Table 2. (Continued)

sample	2,3,4,7,8-PeCDF (0.5) <sup>c</sup>	2,3,7,8-TCDF (0.1) <sup>c</sup>	1,2,3,4,7,8-HxCDF (0.1) <sup>c</sup>	1,2,3,6,7,8-HxCDF (0.1) <sup>c</sup>	2,3,4,6,7,8-HxCDF (0.1) <sup>c</sup>	1,2,3,7,8,9-HxCDF (0.1) <sup>c</sup>	1,2,3,7,8-PeCDF (0.05) <sup>c</sup>	1,2,3,4,6,7,8-HpCDF (0.01) <sup>c</sup>	1,2,3,4,7,8,9-HpCDF (0.01) <sup>c</sup>	OCDF (0.0001) <sup>c</sup>	ΣPCDFi <sup>a</sup>	ΣTEQ <sub>(PCDFi)</sub> <sup>b</sup>
bergamot (continued)												
12	<0.01	2.01	<0.01	<0.01	<0.01	<0.01	0.93	<0.01	0.96	<0.01	3.97	0.27
13	<0.01	0.45	0.60	<0.01	0.26	<0.01	0.26	11.00	<0.01	1.36	13.97	0.26
14	<0.01	<0.01	0.70	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.79	0.08
15	2.10	<0.01	<0.01	<0.01	<0.01	<0.01	2.13	<0.01	<0.01	2.41	6.71	1.16
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10	0.01
$\bar{x} \pm SD^d$	0.22 ± 0.58	0.57 ± 0.80	0.50 ± 0.80	0.40 ± 0.75	0.59 ± 1.05	0.49 ± 0.81	0.49 ± 0.84	3.55 ± 5.10	0.64 ± 0.73	1.54 ± 1.78	8.99 ± 8.95	0.43 ± 0.45
max	2.10	2.35	2.90	2.60	3.96	2.86	2.66	15.20	2.33	5.20	29.76	1.36
recovery % <sup>e</sup>	65–78	63–81	62–87	70–83	64–79	68–87	62–79	64–84	62–81	64–83		

<sup>a</sup> Sum of PCDFi concentrations (pg/g of sample). <sup>b</sup> ΣTEQ<sub>(PCDFi)</sub> = Σ (PCDFi × TEFi) (pg TEQ/g). <sup>c</sup> Toxic equivalency factors (WHO TEF). <sup>d</sup> Mean ± standard deviation. <sup>e</sup> <sup>13</sup>C<sub>12</sub> PCDFs internal standard recovery ranges (%).

Data in **Table 1** regarding hexachlorinated dioxin congeners 1,2,3,4,7,8-HxCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD (WHO TEFs = 0.1) show little contamination due to these analytes.

Seventy-five percent of the samples analyzed do not contain 1,2,3,4,7,8-HxCDD, 73% are free of 1,2,3,4,7,8-HxCDD, and 42% of the samples analyzed are free of 1,2,3,7,8,9-HxCDD. The maximum concentration of 1,2,3,4,7,8-HxCDD (14 pg/g) was found in two mandarin oil samples, whereas none was found in lemon oil samples. No contamination of 1,2,3,6,7,8-HxCDD was found in mandarin oil samples; elsewhere its concentration varied between the LOD and 2.0 pg/g.

Thirty percent of samples analyzed showed no contamination due to the less toxic congeners 1,2,3,4,6,7,8-HpCDD (WHO TEF = 0.01) and OCDD (WHO TEF = 0.001). On the other hand, the remaining samples often contain higher concentrations than the ones found for the other dioxin congeners. Peaks of 30 pg/g were found, for example, in mandarin samples.

These results are similar to the ones found in a previous survey on lemon, mandarin, and orange essential oil samples produced in Calabria and Sicily in 2003–2004 (1).

**Table 2** shows data regarding 10 toxic congeners of PCDFs found in the various types of essential oils analyzed. PCDFs have lower toxicity than PCDDs, with WHO TEF values between 0.5 and 0.0001.

The results demonstrate that, as for PCDDs, the contamination levels found in the four types of essential oils are very similar.

The maximum concentration of the most toxic congener, 2,3,4,7,8-PeCDF (WHO TEF = 0.5), was 5.85 pg/g, found in a lemon oil sample. The mean values of this congener were between 0.22 and 2.19 pg/g. The mean concentrations found for congeners with WHO TEF = 0.1 (2,3,7,8-TCDF; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF) were found between a minimum of 0.11 pg/g of 1,2,3,7,8,9-HxCDF in bergamot oil samples and a maximum of 1.58 pg/g of 2,3,7,8-TCDF in lemon oils.

2,3,4,7,8,9-HpCDF (WHO TEQ = 0.01) was found in about 30% of all the samples analyzed, whereas 1,2,3,4,7,8,9-HpCDF (WHO TEF = 0.01) was found in nearly half of the samples. The highest concentrations were found for 1,2,3,4,7,8,9-HpCDF, with a mean of 7.30 pg/g in mandarin essential oil samples, whereas OCDF (WHO TEF = 0.0001) was found at its highest mean concentration (3.99 pg/g) in lemon oil samples. The high concentrations of the congeners containing the most chlorine atoms do not affect greatly the total TEQ calculated for PCDFs, as these congeners are also the least toxic.

Data regarding 12 PCB dioxin-like compounds analyzed (**Table 3**) clearly demonstrate that contamination is widely spread [in accordance with Santelli et al. (1)]. Most of the

samples were found to be contaminated with PCB-105, -114, and -118, showing mean values between 112 pg/g of PCB-118 and 174 pg/g of PCB-118. These congeners exhibit the highest concentrations but the lowest toxicities (WHO TEF values between 0.0001 and 0.0005); therefore, they do not have a major effect on total TEQ.

Forty-two percent of the samples analyzed were positive to PCB-126, the most toxic PCB congener (WHO TEF = 0.1). The mean concentrations were between 0.76 pg/g in mandarin samples and 36.6 pg/g in orange samples, with peaks of 182 pg/g in bergamot sample 15, 108 pg/g in orange sample 15, 179 pg/g in orange sample 6, and 252 pg/g in orange sample 12. Congeners PCB-169 (WHO TEF = 0.01) and PCB-189 (WHO TEF = 0.0001) were nearly absent in every sample, and PCB-157 (WHO TEF = 0.0005) was not found in orange samples.

From a comparison of the data shown in **Tables 1–3**, with regard to the total concentrations of the three groups of analytes under examination the results are clear that PCB contamination is predominant compared with levels of PCDDs and PCDFs found. From a toxicological point of view (as shown in **Figure 2**) total TEQs of the samples are more influenced by PCDDs than PCBs, because of the low WHO TEF values given to PCBs compared to the PCDDs and PCDFs. By applying eq 1 to the data shown in **Tables 1–3**, the total toxic equivalents TEQs for the samples analyzed can be calculated (**Table 4**). The results obtained during this survey are not comparable with any legal maximum limit permitted, as for the time being essential oils do not fall in any of the official regulations regarding maximum limits of dioxins and PCB contamination in foods, feedstuffs, or environmental samples.

It is probably more interesting, at this moment in time, to try to understand which is the emission source of the contamination found via the analysis of the congener profile. It is well-known that different emission sources produce characteristic congener profiles of PCDD/Fs and PCBs, a fingerprint of the contamination type. Therefore, it is possible to define the causes of the contamination (16).

The results obtained in this survey demonstrate that the contamination found is a background contamination typical of rural areas. They are also in accordance with a survey made in 2002 by the Italian Agency for Protection of Environment and Technical Services (APAT) on dioxin and PCB analysis in environmental samples in Campania (southern Italy) after dioxin contamination was found in dairy products (17).

The contamination found in essential oil samples was characterized by (a) concentration of PCDDs higher than PCDFs (18–21); (b) low levels of PeCDFs and generally low levels of all the congeners containing a low number of chlorine atoms

(19, 22); (c) relatively high concentrations of congeners OCDD, HpCDF, and OCDF with predominance of OCDD (18, 23, 24); and (d) relatively high concentrations of congeners PCB-118, PCB-105, and PCB-126 (17, 21).

**Table 3.** PCB<sub>i</sub> (Picograms per Gram of Sample) and  $\Sigma$ TEQ<sub>(PCB<sub>i</sub>)</sub> in Cold-Pressed Citrus Essential Oil

sample	PCB-126 (0.1) <sup>c</sup>	PCB-169 (0.01) <sup>c</sup>	PCB-114 (0.0005) <sup>c</sup>	PCB-156 (0.0005) <sup>c</sup>	PCB-157 (0.0005) <sup>c</sup>	PCB-81 (0.0001) <sup>c</sup>	PCB-77 (0.0001) <sup>c</sup>	PCB-123 (0.0001) <sup>c</sup>	PCB-118 (0.0001) <sup>c</sup>	PCB-105 (0.0001) <sup>c</sup>	PCB-189 (0.0001) <sup>c</sup>	PCB-167 (0.0001) <sup>c</sup>	$\Sigma$ PCB <sub>i</sub> <sup>a</sup>	$\Sigma$ TEQ <sub>(PCB<sub>i</sub>)</sub> <sup>b</sup>
<b>lemon</b>														
1	<0.01	<0.01	<0.01	<0.01	<0.01	0.77	16.40	<0.01	<0.01	<0.01	<0.01	<0.01	17.27	0.01
2	<0.01	<0.01	116.59	9.95	1.04	<0.01	20.24	61.91	329.04	<0.01	<0.01	5.00	543.82	0.11
3	<0.01	<0.01	<0.01	3.32	<0.01	3.00	51.58	<0.01	57.00	73.32	<0.01	2.71	190.99	0.02
4	14.65	<0.01	28.97	7.83	1.71	0.94	17.48	<0.01	<0.01	<0.01	<0.01	<0.01	71.64	1.49
5	<0.01	<0.01	<0.01	5.20	<0.01	<0.01	<0.01	8.33	126.54	104.09	<0.01	4.18	248.41	0.03
6	12.69	<0.01	9.56	4.32	1.34	0.42	3.39	<0.01	<0.01	<0.01	<0.01	<0.01	31.78	1.28
7	<0.01	<0.01	<0.01	<0.01	<0.01	0.16	7.01	<0.01	<0.01	345.43	<0.01	<0.01	352.69	0.04
8	3.28	<0.01	141.83	11.89	1.43	0.62	29.63	75.41	397.49	24.90	<0.01	6.16	692.66	0.46
9	<0.01	<0.01	28.96	5.19	1.09	0.93	17.47	<0.01	<0.01	<0.01	<0.01	<0.01	53.72	0.02
10	<0.01	<0.01	<0.01	0.59	<0.01	3.00	47.18	<0.01	<0.01	73.32	<0.01	1.31	125.45	0.01
11	1.48	0.02	14.57	7.83	0.29	0.48	10.58	15.36	177.80	121.83	0.02	5.37	355.62	0.19
12	6.14	<0.01	<0.01	0.45	0.56	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	7.24	0.62
13	<0.01	<0.01	<0.01	3.96	0.93	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	4.98	0.01
14	<0.01	<0.01	106.88	8.20	0.85	<0.01	13.19	57.23	294.87	<0.01	<0.01	4.21	485.47	0.10
15	7.06	<0.01	33.66	6.90	1.86	1.24	15.90	18.01	91.28	33.20	<0.01	<0.01	209.14	0.74
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	4.98	0.01
$\bar{x} \pm SD^d$	3.03 ± 4.92	<0.01	32.07 ± 48.42	5.04 ± 3.74	0.74 ± 0.67	0.77 ± 0.99	16.67 ± 15.77	15.76 ± 26.32	98.27 ± 138.24	51.74 ± 91.54	0.01	1.93 ± 2.39	226.06 ± 217.55	0.34 ± 0.49
max	14.65	0.02	141.83	11.89	1.86	3.00	51.58	75.41	397.49	345.43	0.02	6.16	692.66	1.49
recovery % <sup>e</sup>	64–88	73–84	70–85	69–84	72–89	78–85	74–91	71–87	70–84	69–85	81–87	68–89		
<b>mandarin</b>														
1	1.16	<0.01	<0.01	<0.01	<0.01	2.37	15.81	<0.01	16.83	388.69	<0.01	3.96	428.88	0.16
2	<0.01	<0.01	<0.01	9.00	0.75	0.65	6.27	<0.01	74.40	42.41	<0.01	<0.01	133.54	0.02
3	<0.01	<0.01	<0.01	3.03	<0.01	2.43	6.04	48.80	32.00	137.38	<0.01	1.59	231.32	0.03
4	2.46	<0.01	196.74	0.69	13.35	4.00	6.67	61.29	148.97	186.00	<0.01	<0.01	620.20	0.39
5	<0.01	<0.01	179.92	6.90	9.36	2.33	5.30	15.99	122.46	358.05	<0.01	0.99	701.33	0.15
6	0.68	<0.01	<0.01	7.74	<0.01	2.36	4.44	<0.01	214.86	112.16	<0.01	<0.01	342.30	0.11
7	0.67	<0.01	<0.01	<0.01	<0.01	1.84	13.72	<0.01	<0.01	345.43	<0.01	3.19	364.92	0.10
8	0.50	<0.01	48.71	10.91	3.70	1.18	8.36	13.69	112.00	112.11	<0.01	0.78	311.97	0.11
9	1.47	<0.01	130.51	0.68	8.39	2.04	5.01	38.67	99.79	154.93	<0.01	<0.01	441.50	0.25
10	<0.01	<0.01	99.35	<0.01	<0.01	<0.01	<0.01	33.93	<0.01	<0.01	<0.01	<0.01	133.38	0.05
11	<0.01	<0.01	<0.01	9.18	<0.01	3.51	9.01	26.50	173.25	460.11	0.02	1.54	683.15	0.07
12	1.65	<0.01	97.40	11.57	5.92	3.42	8.22	27.37	290.06	281.00	<0.01	1.56	728.60	0.28
13	1.49	<0.01	99.35	<0.01	7.44	2.94	2.49	33.93	73.77	46.61	<0.01	<0.01	268.05	0.22
14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.12	0.01
15	1.33	<0.01	244.85	10.29	3.95	3.07	7.23	18.25	264.99	205.09	<0.01	1.04	760.11	0.31
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.12	<0.01
$\bar{x} \pm SD^d$	0.76 ± 0.79	<0.01	73.13 ± 84.28	4.67 ± 4.74	3.53 ± 4.39	2.14 ± 1.22	6.60 ± 4.33	21.23 ± 19.64	108.23 ± 95.17	188.67 ± 147.36	0.01	0.98 ± 1.24	409.96 ± 241.77	0.15 ± 0.12
max	2.46	<0.01	244.85	11.57	13.35	4.00	15.81	61.29	290.06	460.11	0.02	3.96	760.11	0.39
recovery % <sup>e</sup>	65–84	78–89	72–87	75–82	73–87	69–81	70–87	67–82	72–87	77–81	71–84	69–87		
<b>orange</b>														
1	2.00	<0.01	<0.01	1.91	<0.01	0.51	18.12	<0.01	<0.01	139.42	<0.01	2.27	164.29	0.22
2	4.46	<0.01	169.60	4.44	<0.01	0.94	20.41	<0.01	<0.01	168.93	<0.01	0.13	368.96	0.55
3	<0.01	<0.01	<0.01	9.79	<0.01	<0.01	60.42	<0.01	97.99	68.74	<0.01	0.55	237.56	0.03
4	2.34	<0.01	386.41	11.22	<0.01	0.65	25.36	21.92	<0.01	419.83	<0.01	6.56	874.33	0.48
5	<0.01	<0.01	25.32	<0.01	<0.01	0.83	65.28	<0.01	<0.01	61.68	<0.01	<0.01	153.19	0.03
6	179.46	<0.01	<0.01	1.67	<0.01	1.77	19.51	50.34	8.50	<0.01	<0.01	14.37	275.67	17.95
7	<0.01	<0.01	<0.01	<0.01	<0.01	0.22	7.18	<0.01	<0.01	345.43	<0.01	<0.01	352.92	0.04
8	<0.01	<0.01	91.37	2.12	<0.01	0.65	9.47	<0.01	<0.01	94.80	<0.01	<0.01	198.49	0.06
9	<0.01	<0.01	241.07	3.96	<0.01	<0.01	<0.01	3.85	<0.01	205.18	<0.01	0.59	454.71	0.14
10	<0.01	<0.01	229.96	<0.01	<0.01	<0.01	<0.01	1.22	<0.01	<0.01	<0.01	<0.01	231.27	0.12
11	<0.01	<0.01	<0.01	2.43	<0.01	1.22	82.71	6.04	8.89	140.29	<0.01	2.00	243.63	0.03
12	252.02	<0.01	156.46	6.30	<0.01	2.35	41.39	71.04	47.95	281.00	<0.01	20.02	878.58	25.33
13	<0.01	<0.01	229.96	<0.01	<0.01	0.07	3.48	1.22	<0.01	271.58	<0.01	<0.01	506.37	0.14
14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.12	0.00
15	108.85	<0.01	260.00	6.96	<0.01	1.70	98.10	31.07	59.19	284.06	<0.01	8.49	858.45	11.07
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.12	0.00
$\bar{x} \pm SD^d$	36.62 ± 79.05	<0.01	119.35 ± 128.00	3.39 ± 3.68	<0.01	0.73 ± 0.75	30.10 ± 32.10	12.45 ± 21.97	14.84 ± 29.58	165.40 ± 132.53	0.01	3.67 ± 6.16	386.57 ± 279.12	3.75 ± 7.91
max	252.02	<0.01	386.41	11.22	<0.01	2.35	98.10	71.04	97.99	419.83	<0.01	20.02	878.58	25.33
recovery % <sup>e</sup>	62–81	73–84	77–86	70–87	74–86	70–82	71–85	69–84	70–88	73–84	71–84	64–81		
<b>bergamot</b>														
1	<0.01	<0.01	<0.01	<0.01	1.37	2.10	13.20	<0.01	<0.01	250.23	<0.01	3.96	270.93	0.03
2	<0.01	<0.01	<0.01	3.32	<0.01	2.33	50.20	<0.01	45.00	62.20	<0.01	2.71	165.82	0.02
3	14.65	<0.01	25.03	7.83	1.71	1.30	16.38	<0.01	<0.01	<0.01	<0.01	<0.01	66.96	1.48
4	<0.01	<0.01	<0.01	5.20	<0.01	0.95	0.85	8.33	126.54	75.33	<0.01	4.18	221.43	0.03
5	2.00	<0.01	<0.01	<0.01	<0.01	0.45	18.02	<0.01	<0.01	154.32	<0.01	2.27	177.12	0.22
6	<0.01	<0.01	<0.01	7.37	<0.01	0.68	6.12	<0.01	70.25	25.27	<0.01	1.26	111.01	0.02
7	4.46	<0.01	169.60	4.44	<0.01	<0.01	15.33	<0.01	<0.01	168.93	<0.01	<0.01	362.83	0.55
8	<0.01	<0.01	<0.01	8.33	0.10	<0.01	62.26	<0.01	98.02	68.33	<0.01	0.56	237.65	0.03
9	<0.01	<0.01	<0.01	<0.01	<0.01	0.58	16.40	<0.01	<0.01	0.66	<0.01	0.90	18.61	0.01
10	<0.01	<0.01	268.55	10.26	<0.01	1.35	23.55	15.27	<0.01	152.33	<0.01	2.66	474.01	0.16
11	<0.01	<0.01	22.55	0.95	<0.01	0.96	72.66	<0.01	<0.01	76.15	<0.01	<0.01	173.34	0.03
12	<0.01	0.56	<0.01	3.26	<0.01	2.24	6.25	50.48	25.25	140.13	<0.01	<0.01	228.21	0.03



Table 3. (Continued)

sample	PCB-126 (0.1) <sup>c</sup>	PCB-169 (0.01) <sup>c</sup>	PCB-114 (0.0005) <sup>c</sup>	PCB-156 (0.0005) <sup>c</sup>	PCB-157 (0.0005) <sup>c</sup>	PCB-81 (0.0001) <sup>c</sup>	PCB-77 (0.0001) <sup>c</sup>	PCB-123 (0.0001) <sup>c</sup>	PCB-118 (0.0001) <sup>c</sup>	PCB-105 (0.0001) <sup>c</sup>	PCB-189 (0.0001) <sup>c</sup>	PCB-167 (0.0001) <sup>c</sup>	ΣPCBi <sup>a</sup>	ΣTEQ <sub>(PCBi)</sub> <sup>b</sup>
13	<0.01	<0.01	<0.01	0.89	12.33	3.20	6.24	61.29	125.12	158.52	<0.01	<0.01	367.64	0.04
14	<0.01	<0.01	175.12	6.90	9.24	2.36	5.21	14.23	120.27	265.52	<0.01	<0.01	598.88	0.14
15	182.25	<0.01	<0.01	1.68	<0.01	1.60	18.15	45.37	<0.01	<0.01	<0.01	16.58	265.70	18.23
min	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.85	<0.01	<0.01	<0.01	<0.01	<0.01	18.61	<0.01
$\bar{x} \pm SD^d$	13.56 ± 46.82	0.05 ± 0.14	44.06 ± 86.01	4.03 ± 3.45	1.66 ± 3.79	1.34 ± 0.95	22.05 ± 21.84	13.00 ± 21.26	40.70 ± 52.43	106.53 ± 86.13	<0.01	2.34 ± 4.21	249.34 ± 152.41	1.40 ± 4.67
max	182.25	0.56	268.55	10.26	12.33	3.20	72.66	61.29	126.54	265.52	<0.01	16.58	598.88	18.23
recovery % <sup>e</sup>	65–87	72–81	70–85	67–81	74–87	71–86	75–83	69–81	67–82	75–87	70–83	69–87		

<sup>a</sup> Sum of PCBi concentrations (pg/g of sample). <sup>b</sup> ΣTEQ<sub>(PCBi)</sub> = Σ (PCBi × TEFi) (pg of TEQ/g). <sup>c</sup> Toxic equivalency factors (WHO TEF). <sup>d</sup> Mean ± standard deviation. <sup>e</sup> <sup>13</sup>C<sub>12</sub> PCBs internal standard recovery ranges (%).

Table 4. Total Toxic Equivalents TEQs in Cold-Pressed Citrus Essential Oil

sample	ΣTEQ <sub>(PCDD)</sub> <sup>a</sup>	ΣTEQ <sub>(PCDF)</sub> <sup>b</sup>	ΣTEQ <sub>(PCBi)</sub> <sup>c</sup>	ΣTEQ <sup>c</sup>	sample	ΣTEQ <sub>(PCDD)</sub> <sup>a</sup>	ΣTEQ <sub>(PCDF)</sub> <sup>b</sup>	ΣTEQ <sub>(PCBi)</sub> <sup>c</sup>	ΣTEQ <sup>c</sup>
lemon					orange				
1	0.02	<0.01	<0.01	0.04	1	2.74	1.50	0.22	4.46
2	0.42	0.41	0.11	0.94	2	1.56	0.37	0.55	2.49
3	9.30	0.59	0.02	9.91	3	2.08	1.30	0.03	3.41
4	1.55	0.41	1.49	3.44	4	1.28	0.21	0.48	1.97
5	6.39	4.39	0.03	10.80	5	0.02	<0.01	0.03	0.06
6	0.02	<0.01	1.28	1.31	6	4.06	1.94	17.95	23.96
7	0.33	<0.01	0.04	0.37	7	0.02	0.96	0.04	1.02
8	11.74	1.67	0.46	13.87	8	3.19	0.89	0.06	4.14
9	0.78	0.07	0.02	0.87	9	0.55	0.04	0.14	0.73
10	3.54	0.41	<0.01	3.96	10	1.83	<0.01	0.12	1.95
11	0.02	3.41	0.19	3.63	11	6.61	<0.01	0.03	6.65
12	0.24	<0.01	0.62	0.86	12	0.45	2.58	25.33	28.36
13	4.59	<0.01	<0.01	4.60	13	0.68	0.28	0.14	1.11
14	6.43	3.83	0.10	10.36	14	3.49	<0.01	0.00	3.50
15	6.39	0.17	0.74	7.30	15	0.17	2.64	11.07	16.46
min	0.02	<0.01	<0.01	0.04	min	0.02	<0.01	<0.01	0.06
$\bar{x} \pm SD^e$	3.45 ± 3.83	1.03 ± 1.54	0.34 ± 0.49	4.82 ± 4.52	$\bar{x} \pm SD^e$	2.01 ± 1.79	0.85 ± 0.94	3.75 ± 7.91	6.68 ± 8.87
max	11.74	4.39	1.49	13.87	max	6.61	2.64	25.33	28.36
mandarin					bergamot				
1	1.73	1.43	0.16	3.32	1	0.03	0.16	0.03	0.23
2	2.01	0.26	0.02	2.29	2	0.11	0.15	0.02	0.28
3	0.65	1.15	0.03	1.83	3	0.21	0.07	1.48	1.76
4	2.92	1.12	0.39	4.43	4	0.35	1.36	0.03	1.74
5	0.02	0.21	0.15	0.38	5	2.10	0.79	0.22	3.11
6	0.02	<0.01	0.11	0.14	6	1.31	0.42	0.02	1.75
7	1.64	1.34	0.10	3.08	7	0.98	0.36	0.55	1.88
8	1.90	0.35	0.11	2.36	8	0.82	1.11	0.03	1.96
9	0.73	<0.01	0.25	0.99	9	<0.01	<0.01	<0.01	0.00
10	2.76	0.29	0.05	3.10	10	1.20	0.22	0.16	1.58
11	1.61	<0.01	0.07	1.69	11	0.16	0.03	0.03	0.22
12	0.79	0.18	0.28	1.25	12	0.56	0.27	0.03	0.86
13	1.86	<0.01	0.22	2.09	13	2.11	0.26	0.04	2.41
14	2.12	<0.01	<0.01	2.14	14	0.13	0.08	0.14	0.34
15	0.64	0.12	0.31	1.07	15	3.25	1.16	18.23	22.65
min	0.02	<0.01	<0.01	0.14	min	<0.01	<0.01	<0.01	<0.01
$\bar{x} \pm SD^e$	1.43 ± 0.91	0.57 ± 0.49	0.15 ± 0.12	2.01 ± 1.16	$\bar{x} \pm SD^e$	0.89 ± 0.96	0.43 ± 0.45	1.50 ± 4.83	2.72 ± 5.59
max	2.92	1.55	0.39	4.43	max	3.25	1.36	18.23	22.65

<sup>a</sup> ΣTEQ<sub>(PCDD)</sub> = Σ (PCDDi × TEFi) (pg of TEQ/g). <sup>b</sup> ΣTEQ<sub>(PCDF)</sub> = Σ (PCDFi × TEFi) (pg of TEQ/g). <sup>c</sup> ΣTEQ<sub>(PCBi)</sub> = Σ (PCBi × TEFi) (pg of TEQ/g). <sup>d</sup> ΣTEQ = ΣTEQ<sub>(PCDD)</sub> + ΣTEQ<sub>(PCDF)</sub> + ΣTEQ<sub>(PCBi)</sub> (pg of TEQ/g). <sup>e</sup> Mean ± standard deviation.

From a comparison of total mean concentrations of PCDDs and PCDFs (Tables 1 and 2) found in the four different types of essential oils analyzed, the results are clear that PCDD concentration was higher than PCDF contamination. These data indicate, as is well documented in the literature, background contamination of rural areas (17–24, 26).

PeCDFs and all furan congeners with low levels of chlorination are produced by waste incinerators and industrial plants (19, 26). The absence of PeCDFs in bergamot samples and the generally low levels of low-chlorinated furan congeners is due to the fact that bergamot essential oils are produced in only a small area of the Reggio Calabria province (southern tip of Italy) where industrial plants are basically absent (25). The amount of PeCDFs found in bergamot essential oil samples was lower than

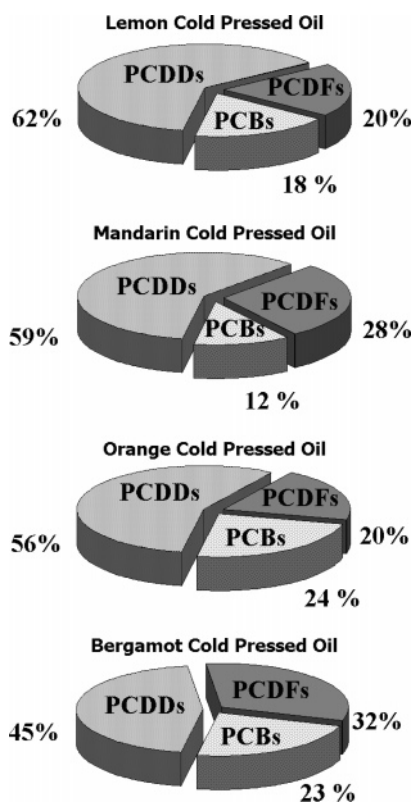
the other PCDF congeners but was also lower than the amount of PeCDFs found in the other types of essential oils. The mean values of the congener 2,3,4,7,8-PeCDF are similar in bergamot and mandarin oil samples, but 4 or 5 times lower than the ones found in orange and lemon samples. The mean values found for the congener 1,2,3,7,8-PeCDF in orange and lemon oil samples are twice the values found in bergamot oil samples (Table 2).

As was reported by Isamu Ogura et al. (18) and more recently by Santelli et al. (23), a typical contamination in rural areas is characterized by high concentrations of OCDD, HpCDFs, and OCDF. These congeners are often produced in rural areas by uncontrolled fires (16).

In the present survey the highest mean concentration values were found for congeners 1,2,3,4,6,7,8-HpCDF (39 pg/g of

**Table 5.** Range and Mean (Micrograms per Kilogram) of Mineral Components in Cold-Pressed Citrus Essential Oil

element	lemon			mandarin			orange			bergamot			recovery %
	min	mean $\pm$ SD <sup>a</sup>	max	min	mean $\pm$ SD <sup>a</sup>	max	min	mean $\pm$ SD <sup>a</sup>	max	min	mean $\pm$ SD <sup>a</sup>	max	
Ag	<0.1	7.2 $\pm$ 10.4	39.3	<0.1	0.3 $\pm$ 0.2	0.7	<0.1	7.3 $\pm$ 17.1	57	<0.1	4.1 $\pm$ 2.3	8.2	98.2
Al	21.3	5064.1 $\pm$ 334	5439.4	<0.5	1.4 $\pm$ 0.9	2.7	<0.5	824.6 $\pm$ 351.1	2923	499.4	955.7 $\pm$ 179.8	1498.2	96.6
As	<0.6	7.0 $\pm$ 11.1	33.7	<0.6	5.2 $\pm$ 9.0	33.7	<0.6	3.1 $\pm$ 8.2	32.3	<0.6	4.3 $\pm$ 6.0	18.8	98.3
Ba	4	36 $\pm$ 70	181	<1	24 $\pm$ 11	59	<1	20 $\pm$ 22	87	<1	104 $\pm$ 52	362	95.2
Be	1.05	4.12 $\pm$ 6.18	20.46	2.23	4.37 $\pm$ 1.64	6.24	<0.04	20.48 $\pm$ 26.98	101.12	0.04	0.83 $\pm$ 0.67	2.21	92.7
Cd	<0.02	1.96 $\pm$ 3.57	15.61	<0.02	0.59 $\pm$ 0.77	2.13	<0.02	0.62 $\pm$ 0.84	2.49	<0.02	4.42 $\pm$ 2.11	8.37	97.5
Co	0.04	42.3 $\pm$ 45.8	109.1	0.4	3.1 $\pm$ 2.8	7.3	2.41	89.1 $\pm$ 115.4	425.3	<0.4	5.3 $\pm$ 3.5	9.7	104.1
Cr	<0.1	182.1 $\pm$ 81.1	248.8	<0.1	2.2 $\pm$ 1.9	5.5	<0.1	21.0 $\pm$ 19.7	103	<0.1	14.1 $\pm$ 7.1	23.4	98.4
Cu	<0.3	1043.5 $\pm$ 345.1	369.4	<0.3	2.1 $\pm$ 2.2	6.7	<0.3	1371.4 $\pm$ 112.2	2164	12.1	29.3 $\pm$ 5.2	42.8	94.9
Fe	151.1	2336.6 $\pm$ 315.9	2877.4	1.1	98.6 $\pm$ 120.3	282.1	<0.3	1433.1 $\pm$ 291.4	1823	163	269 $\pm$ 149	374	98.3
Hg	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	97.4
Mn	<0.1	189.9 $\pm$ 42.4	451.4	<0.1	13.8 $\pm$ 9.2	19.3	<0.1	308.7 $\pm$ 91.3	612.1	<0.1	88.2 $\pm$ 19.5	429.5	101.5
Ni	<0.8	194.2 $\pm$ 39.2	514.9	2.9	182.3 $\pm$ 41.4	254.2	<0.8	294.4 $\pm$ 39.8	652	<0.8	17.2 $\pm$ 11	33	90.8
Pb	<0.2	5.9 $\pm$ 6.4	19.8	<0.2	12.2 $\pm$ 11.6	34.1	<0.2	23.2 $\pm$ 70.7	323.1	<0.2	46.1 $\pm$ 19	160	94.1
Sb	<0.5	4.1 $\pm$ 5.86	14.4	11.1	147.4 $\pm$ 204.3	513.2	<0.5	59.2 $\pm$ 96.3	291.3	<0.5	63.3 $\pm$ 72	187	92.1
Sn	<2	1178 $\pm$ 610	2432	<2	3489 $\pm$ 643	3728	<2	3632 $\pm$ 693	4528	<2	582 $\pm$ 539	1520	97.7
Zn	<0.3	664.6 $\pm$ 421.1	1537.4	3.1	142.7 $\pm$ 51.2	273.3	<0.3	822.3 $\pm$ 189.1	967.2	<0.3	418.4 $\pm$ 434.6	1140.3	103.2

<sup>a</sup> Standard deviation.**Figure 2.** Relative contribution of PCDDs, PCDFs, and PCBs to TEQ in cold-pressed citrus essential oil.

mandarin essential oil), OCDF (14 pg/g of lemon essential oil), OCDD (9.05 pg/g of mandarin essential oil).

Additional analyses have been completed on mineral components. Trace levels of elements naturally present in citrus essential oils and heavy metals such as Hg, As, Cd, Pb, and Ni, which are known to be toxic inorganic pollutants, were quantified.

The use of essential oils as food additives is regulated in Italy by D.L. n. 107, 25/01/1992, which conforms with Council Directives 88/388/CEE and 91/71/CEE regarding the use of aromas in foods and materials for food preparation (27).

Even if the present legislation sets maximum levels accepted for some heavy metals (mercury, arsenic, and lead) in aromas

used in food and pharmaceutical preparations, the most widely used extracting techniques for citrus essential oils cannot avoid contact between the oils and metal surfaces, washing waters, and metal containers. Therefore, contamination with other metals such as Cr, Fe, and Ni is quite possible (28).

**Table 5** shows results obtained from the analysis of the mineral component of the four types of citrus essential oils. In all of the samples analyzed the mean contents of Cr, Fe, and Ni were found to be in accordance with the data reported in the literature (1–3, 29, 30).

Recoveries (percent) on standard reference materials assessed in the range of 92.1 and 104.1% for Sb and Co, respectively (**Table 5**).

With regard to arsenic and lead, in all of the samples analyzed the mean concentration was well below the maximum limit permitted. None of the samples was contaminated with mercury.

In conclusion, levels of organic pollutants (PCDD/Fs and PCBs) and mineral components in cold-pressed citrus essential oils were the object of this study. The contamination found in essential oil samples was characterized by higher concentrations of PCDDs compared to PCDFs, little presence of PCDF, and relatively high concentrations of congeners OCDD, HpCDF, and OCDF. The data reported reveal a widespread pollution characteristic of a typical contamination in rural areas. With regard to heavy metal contamination (As, Hg, and Pb), all samples met criteria enforced by current European legislation and should be considered safe for human health.

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