Journal of Food Protection, Vol. 74, No. 2, 2011, Pages 261–269
doi:10.4315/0362-028X.JFP-10-176

Bioaccumulation of Dioxin-like Substances and Selected Brominated Flame Retardant Congeners in the Fat and Livers of Black Pigs Farmed within the Nebrodi Regional Park of Sicily

GIANFRANCO BRAMBILLA,[1]* STEFANIA PAOLA DE FILIPPIS,[1] ANNA LAURA IAMICELI,[1] NICOLA IACOVELLA,[1] VITTORIO ABATE,[1] VINCENZO ARONICA,[2] VINCENZO DI MARCO,[2] AND ALESSANDRO DI DOMENICO[1]

[1]*Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy; and* [2]*Istituto Zooprofilattico Sperimentale della Sicilia, Via S. Andrea 96, 98051 Barcellona, Messina, Italy*

MS 10-176: Received 23 April 2010/Accepted 22 October 2010

ABSTRACT

An observational study was designed to assess the bioaccumulation of polychlorodibenzodioxins (PCDD) and polychlorodibenzofurans (PCDF), dioxin-like polychlorobiphenyls (DL-PCB), and 13 selected polybromodiphenylethers (PBDE) in autochthonous pigs reared in the Nebrodi Park of Sicily (Italy). Perirenal fat and liver samples were drawn from animals representative of three different outdoor farming systems and from wild pigs and then analyzed for the chemicals mentioned previously. The highest concentrations of PCDD+PCDF and DL-PCB were detected in the fat (0.45 and 0.35 pg World Health Organization toxicity equivalents [WHO-TE] per g of fat base [FB], respectively) and livers (12.7 and 3.28 pg WHO-TE per g FB) of the wild group, whereas the free-ranging group showed the lowest levels (0.05 and 0.03 pg WHO-TE per g FB in fat and 0.78 and 0.27 pg WHO-TE per g FB in livers). The sum of PBDE congeners was highest in wild pigs (0.52 ng/g FB in fat and 5.64 ng/g FB in livers) and lowest in the farmed group (0.14 ng/g FB in fat and 0.28 ng/g FB in livers). The contamination levels in fat and livers of outdoor pigs had mean concentration values lower than those levels reported for intensively indoor-farmed animals. In wild pigs, bioaccumulation was associated with their free grazing in areas characterized by bush fires. The results of this study aid to emphasize the quality of the environment as a factor to guarantee food safety in typical processed pig meat products, specifically from outdoor and extensive Nebrodi farming systems.

Rural farmed animals may undergo combined environmental exposures to persistent organic pollutants such as polychlorodibenzodioxins (PCDD) and polychlorodibenzofurans (PCDF), dioxin-like polychlorobiphenyls (DL-PCB), and polybromodiphenylethers (PBDE), which originate mainly from soil and plants. Within this construct, environment quality plays a relevant role in determining their overall bioaccumulation and subsequent carryover to animal produce *(8, 27)*. Previous studies reported higher levels of the above-mentioned contaminants in free-ranging hen eggs *(19, 37)* and milk of grazing dairy animals *(25, 29)* than those levels found in the same foods from intensively farmed animals fed on commercial feeds *(10)*.

At times, the recorded levels in products from outdoor-rearing systems exceeded the pertinent cumulative PCDD, PCDF, and DL-PCB regulatory maximum levels (ML) in food of animal origin, according to current EU legislation (Regulation No. 1881/06/EC). For pig meat and livers, levels have been posed at 1.5 pg World Health Organization toxicity equivalents (WHO-TE) per g of fat base (FB) and at 12 pg WHO-TE per g whole weight, respectively, as cumulative values (PCDD+PCDF and DL-PCB). This could represent a cause for possible concern when confronted with the general ''healthy'' claims associated with the friendly animal welfare and environmentally benign practices of organic farming *(31, 38)*. As far as pigs are concerned, very few studies have dealt with exposure and bioaccumulation of persistent organic pollutants in outdoor and not intensive farming; the available literature has mainly focused on toxicokinetics in meat from high-inbred animals because of exposure to contaminated feed *(13, 15, 18, 28)*.

Information available is sparse and indicates that outdoor-farmed pigs might be more exposed than the corresponding indoor animals might. However, the recorded PCDD, PCDF, and DL-PCB concentrations in fat and offal of outdoor animals could not be directly associated with the characterization of the environment at farm *(22, 32)*. Therefore, this work's intent was to study the bioaccumulation of the contaminants mentioned previously in the autochthonous black pig feeding substantially on natural pastures and reared under different extensive farming systems within the preserved area of the Regional Nebrodi Park of Sicily (Italy). The acquired information could provide answers to food safety issues for locally processed meat production and, at the same time, endorse the above-mentioned sustainable farming practices, i.e., that outdoor

* Author for correspondence. Tel: +39-06-49902764; Fax: +39-06-49902836; E-mail: gianfranco.brambilla@iss.it.

and wild pigs be recognized as sensitive sentinels to monitor the overall quality of the environment *(36)*.

MATERIALS AND METHODS

Site description, animals, and sampling. The study was carried out in the autumn of 2007 within the Regional Nebrodi Park of Sicily. Within the park (which spans over 85,000 ha [850 km^2] in the Messina District, between the Thyrrenian Sea and Mt. Etna) (Fig. 1), different traditional rural farming systems were identified. (i) Outdoor farms, i.e., wild black pigs caught when young were reared in 1-ha (0.01-km^2) paddocks until the slaughtering age of 18 months (ca. 120-kg body weight) and fed on natural pastures characterized by the presence of chestnuts and acorns, and some local cereal and field bean supplements were provided in periods of low pasture. (ii) En plein air (in the open air), i.e., wild pigs caught when young were confined in 5-ha (0.05-km^2) allotments and fed as described above. (iii) Free ranging, i.e., wild piglets were caught and put in 20- to 40-ha (0.2- to 0.4-km^2) areas where they were free to graze in woods composed primarily of the genera *Fagus* and *Quercus* (beech and oak), with a feeding practice limited to supplements. (iv) Wild pigs without space confinement (>1,000 ha [10 km^2]) and without external feed supply were caught just before slaughter.

At slaughter, perirenal fat and liver samples from pigs (n = 10) representative of each typology were drawn and pooled on an equal gravimetric basis for the assessment of chemicals of interest. For the observational study carried out, the perirenal fat was selected for analysis of persistent organic pollutants to facilitate sampling and to have a matrix less sensitive to possible short-term variations of pollutant intake-uptake, thus more representative of overall long-term exposure *(28)*.

An inventory of possible occasional sources of exposure was carried out in each farm considered in this study *(2)*.

Standards and reagents. *n*-Hexane (Carlo Erba, Rodano, Italy), acetone, concentrated sulfuric acid (International Organization for Standardization analytical grade), cyclohexane, dichloromethane, sodium sulfate (Riedel de Haen, Seelze, Germany), and diatomaceous earth (EXtrelut, Merck, Darmstadt, Germany) were of the best commercial quality. Gases (with purities of 5.5) were purchased from Rivoira (Turin, Italy). Certified unlabeled and fully ^{13}C-labeled PCDD, PCDF, and DL-PCB standards (purities of 99%) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). Certified unlabeled and fully ^{13}C-labeled PBDE standards (purity of 98%) were provided by Sigma-Aldrich (St. Louis, MO).

PCDD, PCDF, DL-PCB, and PBDE determination. The analytical procedure was adapted from U.S. Environmental Protection Agency Method No. 1613 *(33)* and validated in-house. In brief, after fortification with ^{13}C-labeled congeners, each 10-g test portion from a 10-sample homogenate rested for 24 h at 4°C. After addition of 70 g of anhydrous sodium sulfate, the mixture was extracted twice with 200 ml of *n*-hexane after a 90-min sonication. Each organic extract was collected and prepurified by eluting it through a column of EXtrelut impregnated with concentrated sulfuric acid. Then, a Power-Prep apparatus (Fluid Management Systems, Watertown, MA) was utilized for an automated cleanup with three sequential chromatographic steps on columns packed with silica gel, alumina, and graphitic carbon, respectively, suitable for adequate separation of the analytes of interest.

PCDD, PCDF, and the non–*ortho*-substituted DL-PCB were quantified by high-resolution gas chromatography on a column 60 m long, with a 0.25-mm inside diameter (BPX-DXN, Scientific Glass Engineering Analytic Science, Ringwood, Victoria, Australia), coupled to a high-resolution VG AutoSpec mass spectrometer (Waters/Micromass, Manchester, UK) (HRGC-HRMS) used in the ''selected ion-monitoring mode'' (SIM); the mono-*ortho*-substituted DL-PCB were determined by HRGC on an HT5 column 60 m long, with a 0.25-mm inside diameter (Scientific Glass Engineering Analytic Science) coupled to a low-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA) (HRGC-LRMS[SIM]). In both cases, the analytical values were converted to WHO-TE units by using the 1997 WHO toxic equivalency factors (TEF) *(34)*. The selected PBDE congeners (28, 47, 49, 71, 85, 99, 100, 153, 154, 183, 197, 206, and 209) were determined by HRGC-LRMS(SIM) on a BP-1 column 12 m long, with a 0.25-mm inside diameter (Scientific Glass Engineering Analytic Science) *(20)*. Recovery rates for the ^{13}C-labeled internal standards were within the range of 70 to 110%. All cumulative results were estimated as upper-bound values affected by an extended uncertainty within $|\pm 25\%|$.

Animal welfare. Pigs were transported to the slaughterhouse, stunned, and slaughtered under the supervision of the appointed veterinarian officer, in compliance with Regulation No. 882/2004/EC of the European Parliament and the Council of the European Union.

Statistics. Observational data from Nebrodi pigs pooled samples were compared with the PCDD+PCDF and DL-PCB frequency tables and summary statistics describing the WHO-TE means and the 25th, 50th, 75th, and 90th percentiles (P25, P50, P75, and P90), respectively, in pig fat and meat, based on EU monitoring plans 1998 to 2008, as reported by the European Food Safety Agency (EFSA) *(10)*.

RESULTS

Slaughter inspection. All recruited animals were healthy and did not show any anamnesis, pathological signs, or lesions such as chloracne, hypospadia, cleft palate, or sex ratio alterations *(24)* that could be related to high exposure to persistent organic pollutants at both ante- and postmortem inspections at slaughter; their meat and offal were compliant with food consumption regulations.

Inventoried risk factors. The following PCDD, PCDF, and DL-PCB risk factors were inventoried in the area: (i) use of pentachlorophenol-treated wood for fencing outdoor farms; (ii) free-ranging farm proximity to a 1972 electric transformer, still containing dielectric fluid with PCB; and (iii) occurrence of spontaneous bush fires within the park during 2007 (Fig. 1).

Persistent organic pollutants bioaccumulation in pig perirenal fat and kidneys. The concentrations of PCDD, PCDF, and DL-PCB congeners in perirenal fat and liver samples of pigs from the different farming systems are reported in Table 1. The data available are graphically reduced in Figures 2 and 3, which exhibit the PCDD, PCDF, and DL-PCB profiles of detected congeners, respectively, plotted in analytical and WHO-TE units.

Most of selected PBDEs were not detected in the fat and liver samples analyzed (Table 2). PBDE 47, usually not reported as a prevalent congener in products of animal origin *(18)*, was determined in both matrices in all pig

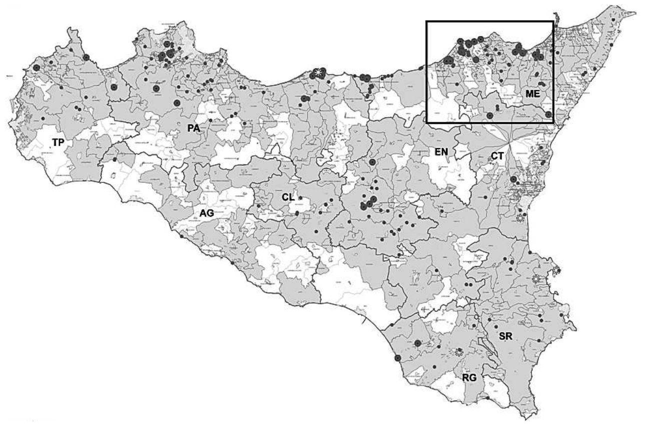


FIGURE 1. *The Nebrodi Park area (box). The map also shows the locations where severe (●) and moderate (•) bush and grassland fires occurred during the summer of 2007, per the records of the Sicilian Regional Department of Civil Protection.*

groups, and did not show appreciable differences in bioaccumulation between fat and livers.

DISCUSSION

Impact of the environmental quality on PCDD, PCDF, and DL-PCB bioaccumulation in the considered farming systems. All PCDD, PCDF, and DL-PCB TE concentrations found in perirenal fat samples always appeared below the pertinent ML for pig meat or fat stated in Regulation No. 1881/06/EC (1.0 pg WHO-TE per g FB for PCDD+PCDF and 1.5 pg WHO-TE per g FB as cumulative values, respectively), and even lower than the alert levels reported in Recommendation 2006/88/EC (0.6 pg WHO-TE per g FB for PCDD+PCDF and 0.5 pg WHO-TE per g FB for DL-PCB, respectively). The PCDD+PCDF and DL-PCB contamination levels recorded in farmed, en plein air, and free-ranging pigs are lower than those levels on average reported for outdoor pig meat in the United Kingdom (0.23 and 0.39 pg WHO-TE per g FB, respectively) *(32)* and by the EFSA for intensively farmed pig meat (0.47 and 0.46 pg WHO-TE per g FB, respectively) and fat (0.92 and 0.95 pg WHO-TE per g FB, respectively), and on a cumulative basis, close or even lower than the P25 of the pertinent EFSA distributions (P25 pig fat = 0.20 pg WHO-TE per g FB; P20 pig meat = 0.24 pg WHO-TE per g FB) *(10)*. To evaluate the data summarized in Tables 1 and 2, the ''dilution'' of contamination in the fat mass should be taken into account: in low-inbred black pigs, fat represents an average of 45% of carcass weight *(23)* versus a 30% average reported for high-inbred industrial lines *(16)*.

Liver data highlight better the differences of exposure between the different groups: wild pigs revealed a contamination roughly three times higher than the contamination recorded in the livers of outdoor, en plein air, and free-ranging pigs. Because of their unlimited grazing area, wild animals could come in close contact with occasional contamination sources and with recently burned areas (Fig. 1), where PCDD+PCDF concentrations in topsoil can be as high as 5 pg international TEF/g dry weight *(7)*, a target level below which animal grazing is generally considered to be safe *(12)* (for clarity's sake, it is pointed out that 1997 WHO-TEF and ''international'' TEF generally provide similar TE values when the matrix is soil). In wild pigs, a more efficient induction of aryl-hydrocarbon receptors of liver cell membranes—receptors capable of isolating PCDD, PCDF, and DL-PCB from the fat content of the liver *(1)*—and the probable occurrence of starvation periods that require a mobilization of fat from adipose tissue to the liver could be the main factors determining the relevant differences recorded in PCDD, PCDF, and DL-PCB concentrations (FB) in the two selected matrices. The calculated liver-to-fat ratio concentrations (FB) for PCDD, PCDF, and DL-PCB congeners yielded the following figures: 22, 52, 13, and 20, respectively, for farmed, en plein air, free-ranging, and wild pigs. The highest value of 52 for the en plein air group could be because of recent exposures recorded in liver and not yet transferred to the less perfused perirenal fat *(35)*.

Observational studies from residue monitoring plans in the United Kingdom *(32)* provide a liver-to-fat ratio of 33, based on mean values in outdoor pigs (18.8 and 1.8 pg WHO-TE per g FB, respectively, for cumulative PCDD+PCDF and DL-PCB concentrations in livers) and a liver-to-fat ratio of 12 in indoor pigs (7.3 and 1.6 pg WHO-TE per g FB for the same chemicals in livers). These results are consistent with previous reports on other farmed species such as chicken *(30)* and cattle (meat and dairy) *(11, 25)*, indicating the liver is the most bioaccumulating organ, and that observational levels could encroach on the pertinent alert levels (4 pg WHO-TE per g FB and 4 pg WHO-TE per g FB for PCDD+PCDF and DL-PCB congeners, respectively) and ML (6 pg WHO-TE per g FB for PCDD+PCDF and a 12 pg WHO-TE per g FB, as cumulative) in such a matrix, even when recorded contamination in fat can be considered at background levels.

The observed patterns in Nebrodi pigs could be ascribed to a variety of occasional and different exposures (Figs. 2 and 3); the diversity between the profiles detected in fat and livers could be explained by the fact that liver is more sensitive to recent exposures than is the less perfused perirenal fat. Despite the low contamination levels found and the relevant number of nondetects—n = 12, 15, and 13 in farmed, en plein air, and free-ranging pigs, respectively (Table 1)—some farmed pig fat samples on analytical bases have profiles useful for detecting possible exposure differences in the three farming systems (Fig. 2). In free-ranging pigs, the predominant hepta-CCD and octa-CCD contributions among PCDD+PCDF congeners suggest a possible exposure to pentachlorophenol-treated timbers *(17)*, as inventoried among possible local farm risk factors, even if such exposure does not have any effect on the rise of contamination on a WHO-TE basis (Fig. 3). The higher contamination levels recorded in the wild pig group (Table 1) are characterized by the appreciable analytical contribution of the mono-*ortho*-substituted DL-PCB. Their profile could reflect the influence of exposures in areas with bush and grassland fires, where the PCDF fraction could represent up to 50% of PCDD and PCDF TE emissions *(14, 21)*. In such a context, we also consider heat-stressed PCB,

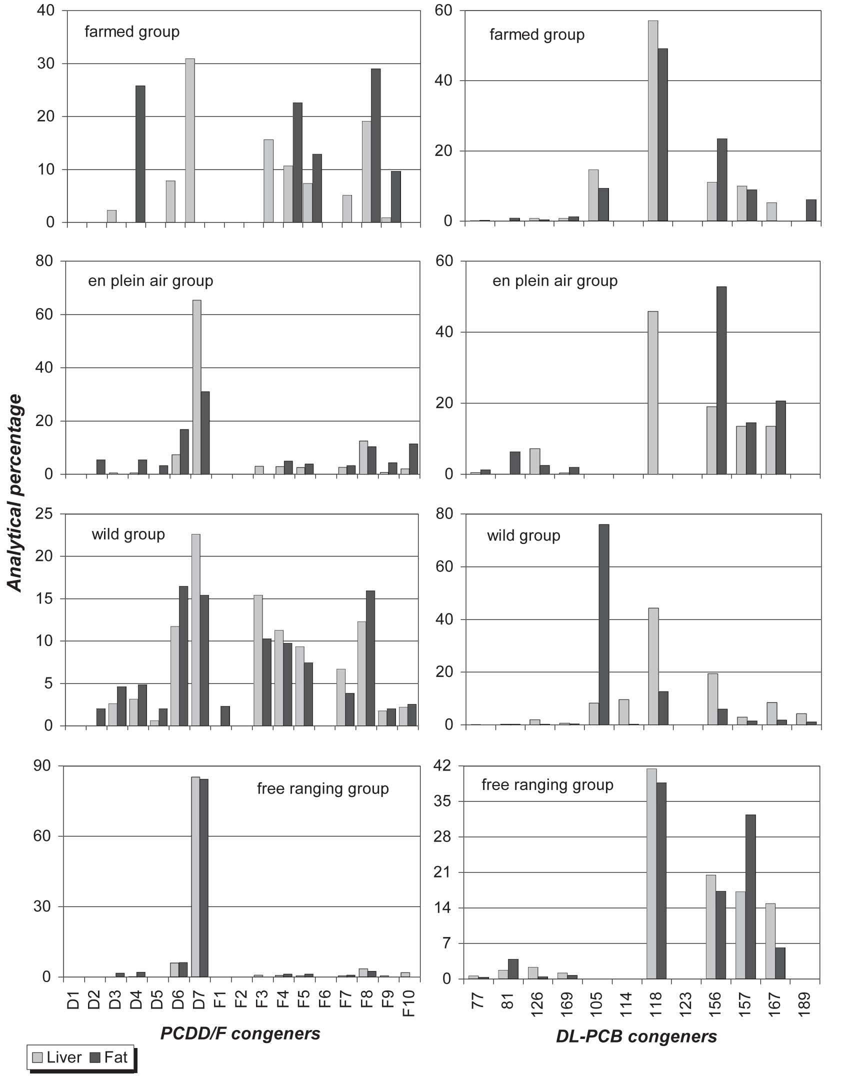
TABLE 1. *PCDD, PCDF, and DL-PCB found in fat and liver samples from four different pig farms in the Nebrodi Park area*[a]

	Farm no., type:							
	78992, farmed		80020, en plein air		80866, wild		79007, free ranging	
Congeners	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)
2,3,7,8-T_4CDD	<0.02	<0.18	<0.02	<0.37	<0.02	<0.24	<0.01	<0.13
1,2,3,7,8-P_5CDD	0.10	<0.23	<0.01	<0.51	0.08	<0.30	<0.01	<0.14
1,2,3,4,7,8-H_6CDD	<0.01	0.70	<0.01	0.92	0.18	2.83	0.04	<0.14
1,2,3,6,7,8-H_6CDD	0.10	0.69	0.08	<0.42	0.19	3.37	0.05	0.13
1,2,3,7,8,9-H_6CDD	0.06	<0.24	<0.01	<0.49	0.08	0.68	<0.01	<0.13
1,2,3,4,6,7,8-H_7CDD	0.31	9.91	<0.01	3.13	0.64	12.5	0.15	3.88
O_8CDD	0.57	88.4	<0.01	12.3	1.00	24.1	2.04	55.2
2,3,7,8-T_4CDF	<0.01	<0.14	<0.01	<0.30	0.09	<0.18	<0.01	<0.10
1,2,3,7,8-P_5CDF	<0.01	<0.16	<0.01	<0.34	<0.01	<0.20	<0.01	<0.11
2,3,4,7,8-P_5CDF	<0.01	4.01	<0.01	6.23	0.40	16.4	<0.01	0.51
1,2,3,4,7,8-H_6CDF	0.09	3.97	0.07	4.26	0.38	12.0	0.03	0.46
1,2,3,6,7,8-H_6CDF	0.07	3.44	0.04	2.94	0.29	9.95	0.03	0.36
1,2,3,7,8,9-H_6CDF	<0.01	<0.19	<0.01	<0.41	<0.01	<0.25	<0.01	<0.13
2,3,4,6,7,8-H_6CDF	0.06	3.61	<0.01	2.04	0.15	7.16	0.02	0.34
1,2,3,4,6,7,8-H_7CDF	0.19	16.9	0.09	7.65	0.62	13.1	0.06	2.32
1,2,3,4,7,8,9-H_7CDF	0.08	1.03	0.03	0.37	0.08	1.89	<0.01	0.31
O_8CDF	0.21	2.72	<0.02	<0.46	0.10	2.39	<0.01	1.26
PCB 77	0.17	0.51	0.18	0.46	0.17	1.24	0.12	0.60
PCB 81	0.85	<0.17	0.67	<0.37	1.86	3.43	1.33	1.63
PCB 126	0.34	7.81	0.31	5.40	2.43	28.7	0.17	2.15
PCB 169	0.27	0.41	0.94	5.01	3.55	8.57	0.24	1.09
PCB 105	<2.90	<14.00	7.06	92.0	39.75	121	<2.20	<15.2
PCB 114	<2.96	<13.7	<2.04	<24.9	2.81	142	<2.14	<19.6
PCB 118	<2.78	49.5	37.1	358	158	655	13.13	37.7
PCB 123	<3.11	<14.4	<2.16	<26.2	<2.18	<22.3	<2.24	<21.4
PCB 156	7.09	20.6	17.7	69.7	75.1	287	5.89	18.7
PCB 157	1.95	14.6	6.75	62.8	19.0	43.4	11.00	15.6
PCB 167	2.77	14.6	<2.00	33.4	23.1	126	2.10	13.5
PCB 189	<4.64	<23.0	4.66	<40.5	12.8	62.5	<3.43	<33.3

TABLE 1. *Continued*

	Farm no., type:							
	78992, farmed		80020, en plein air		80866, wild		79007, free ranging	
Congeners	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)
PCDDs, analytical	1.18	100	0.16	18.2	2.19	44.0	2.31	59.8
PCDFs, analytical	0.70	36.1	0.32	25.0	2.12	63.6	0.20	5.89
PCDDs+PCDFs, analytical	1.88	136	0.48	43.2	4.31	107	2.51	65.7
Non-*ortho* PCBs, analytical	1.63	8.90	1.86	11.2	8.01	41.9	1.86	5.52
Mono-*ortho* PCB, analytical	28.2	164	79.5	707	333	1359	42.1	175
DL-PCBs, analytical	29.8	173	81.4	718	341	1400	44.0	180
PCDDs, toxicological	0.14	0.68	0.04	1.10	0.15	1.36	0.03	0.35
PCDFs, toxicological	0.03	3.33	0.02	4.21	0.30	11.3	0.02	0.43
PCDD+PCDF, toxicological	0.17	4.01	0.06	5.31	0.45	12.7	0.05	0.78
Non-*ortho* PCBs, toxicological	0.04	0.79	0.02	0.59	0.28	2.96	0.02	0.23
Mono-*ortho* PCBs, toxicological	0.01	0.03	0.02	0.13	0.07	0.32	0.01	0.04
DL-PCBs, toxicological	0.05	0.82	0.04	0.62	0.35	3.28	0.03	0.27
Cumulative PCDDs/Fs+DL-PCBs, toxicological	0.23	4.83	0.10	6.03	0.80	16.0	0.08	1.05

[a] Cumulative values are expressed both on analytical and toxicological (1998 WHO-TE) bases, with the upper-bound approach. Values are rounded to a maximum of three numbers.

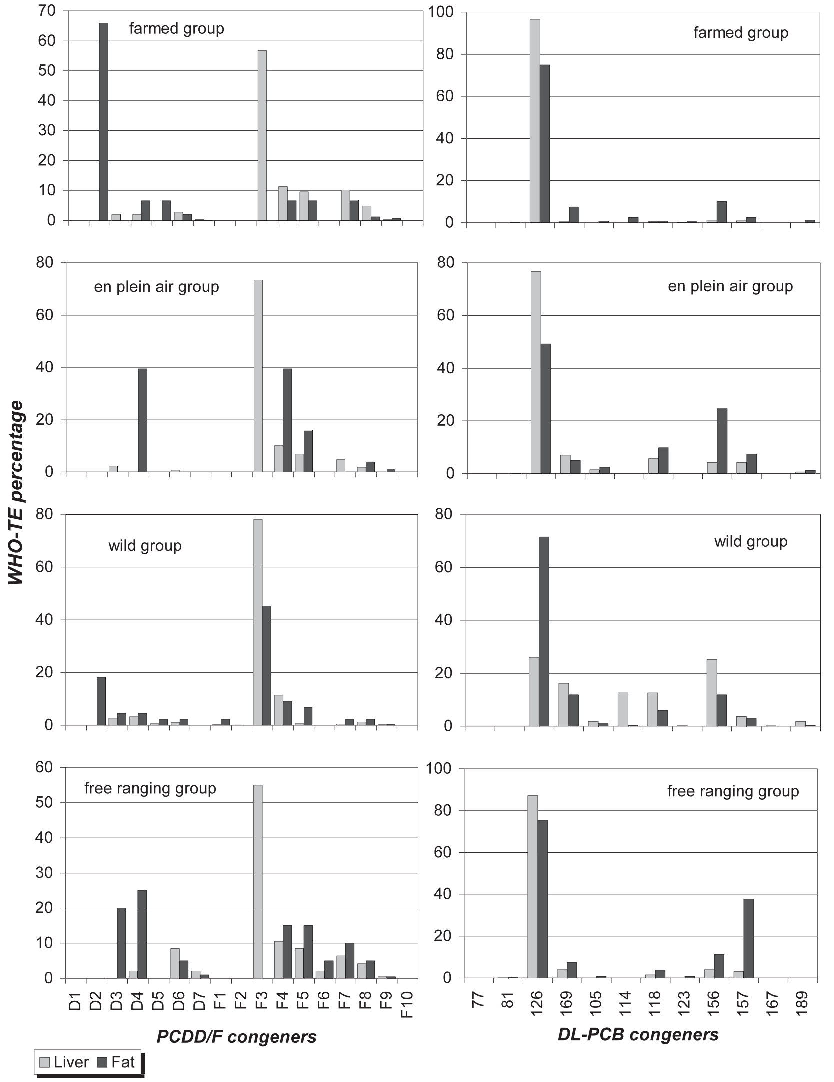


D1, 2,3,7,8-T_4CDD; **D2**, 1,2,3,7,8-P_5CDD; **D3**, 1,2,3,4,7,8-H_6CDD; **D4**, 1,2,3,6,7,8-H_6CDD; **D5**, 1,2,3,7,8,9-H_6CDD; **D6**, 1,2,3,4,6,7,8-H_7CDD; **D7**, O_8CDD; **F1**, 2,3,7,8-T_4CDF; **F2**, 1,2,3,7,8-P_5CDF; **F3**, 2,3,4,7,8-P_5CDF; **F4**, 1,2,3,4,7,8-H_6CDF; **F5**, 1,2,3,6,7,8-H_6CDF; **F6**, 1,2,3,7,8,9-H_6CDF; **F7**, 2,3,4,6,7,8-H_6CDF; **F8**, 1,2,3,4,6,7,8-H_7CDF; **F9**, 1,2,3,4,7,8,9-H_7CDF; **F10**, O_8CDF.

FIGURE 2. *PCDD+PCDF–detected (left) and DL-PCB–detected (right) congener profiles, expressed as percentages of PCDD+PCDF and DL-PCB analytical sums, respectively, in the livers and perirenal fat of pigs from selected farms.*

i.e., originating from old transformers seated on electric line poles within fire areas; these should not overlooked as a possible emission contributor *(5)*. By comparison, the analytical profile recorded during the Belgian Dioxin Affair of 1999, and more recently as a follow-up of the surveillance activity on tainted fat in Irish pork meat, are both dominated by 2,3,4,7,8-P_5CDF and 1,2,3,4,7,8-H_6CDF *(9, 15, 26),* along with a roughly 70% contribution of the mono-*ortho*-substituted DL-PCB, on the total TE *(4)* (Fig. 4). However, in the case of Nebrodi wild black pigs, such contribution of mono-*ortho* congeners on the cumulative TE in fat does not surpass 10%, thus suggesting the contamination levels in fat, lower than the regulatory ML (Table 1), can be ascribed eventually to time-restricted, occasional exposures to PCB leakages.

Impact of the environmental quality on PBDE bioaccumulation in the considered farming systems. The PBDE upper-bound concentrations found in the perirenal fat of Nebrodi pigs (Table 2) are in agreement with the data available from literature for meat fat. A U.S. Food and Drug Administration study based on a food consumption survey *(18)* reported an average PBDE contamination of 2.6 ng/g FB in pig meat for eight selected congeners, with a range spanning from 0.190 to 16.3 ng/g FB. In The Netherlands, the average medium bound level found in pig meat was 1.0 ng/g FB *(6),* while in background pig fat samples from Australia, within a context of extensive forest fires, the average cumulative concentration of PBDEs 47, 85, 99, 100, 138, 153, and 154 was 5.6 ± 2.3 ng/g FB *(3)*. The sparse data concerning PBDE occurrence and toxicokinetics in food producing animals do not allow further interpretation of the data obtained. In general, the presence of PBDEs (which are of strict anthropogenic origin) in pigs reared within a preserved area could be determined by the use of recycled foam insulation lining animal pens and/or



D1, 2,3,7,8-T$_4$CDD; **D2**, 1,2,3,7,8-P$_5$CDD; **D3**, 1,2,3,4,7,8-H$_6$CDD; **D4**, 1,2,3,6,7,8-H$_6$CDD; **D5**, 1,2,3,7,8,9-H$_6$CDD; **D6**, 1,2,3,4,6,7,8-H$_7$CDD; **D7**, O$_8$CDD; **F1**, 2,3,7,8-T$_4$CDF; **F2**, 1,2,3,7,8-P$_5$CDF; **F3**, 2,3,4,7,8-P$_5$CDF; **F4**, 1,2,3,4,7,8-H$_6$CDF; **F5**, 1,2,3,6,7,8-H$_6$CDF; **F6**, 1,2,3,7,8,9-H$_6$CDF; **F7**, 2,3,4,6,7,8-H$_6$CDF; **F8**, 1,2,3,4,6,7,8-H$_7$CDF; **F9**, 1,2,3,4,7,8,9-H$_7$CDF; **F10**, O$_8$CDF.

FIGURE 3. *PCDD+PCDF–detected (left) and DL-PCB–detected (right) congener profiles, expressed as percentages of PCDD+PCDF and DL-PCB WHO-TE, respectively, in the livers and perirenal fat of pigs from selected farms.*

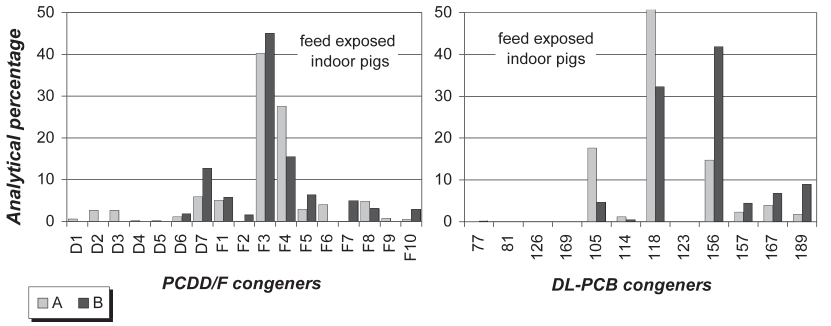
plastic tubing for water, and to the intake of ashes from discarded plastic containers burned by fires *(3)*.

Statistics. The practicability of the analysis carried out in field conditions within the Nebrodi Park relied on the pool approach to fat and livers of 10 animals representative of well-defined and homogeneously farmed groups; the pool approach is in line with the common practice used to test bulk milk on dairy farms, insofar as it is acknowledged that the homogeneous groups of animals belonging to the same farm have the same sources of exposure, with almost the same timing. Such a pool approach, however, limited the number of the observations; this fact, along with the number of congeners with nonquantifiable levels, basically hampered the statistical evaluation of data, as well as a principal component analysis whose potencies rely on the number of data and variables available. In the near future, when we amass a significant amount of data on the Nebrodi pigs, we will do a sound statistical evaluation of our findings, in part concerning possible time trends of the considered contaminants with respect to the assessed geo-referenced background levels.

In conclusion, a preserved quality of grazing areas supports reduced exposure to PCDD, PCDF, DL-PCB, and PBDEs in rural- and outdoor-farmed pigs, with FB levels of the previously mentioned contaminants lower than those FB levels reported in intensively indoor-farmed animals fed commercial feeds. Wild pigs, due to their unrestricted migration within the Nebrodi Park, represent the group most sensitive, and their livers a matrix more suitable for the assessment of the overall environmental quality of this natural area, where bush and grassland fires could represent the main source of PCDD, PCDF, and DL-PCB emissions. Further work is in progress to trace wild pig paths within the Nebrodi Park, with the aid of a geographical information system, in an attempt to correlate bioaccumulation data with

TABLE 2. *Upper-bound levels of selected PBDE congeners found in perirenal fat and livers of pigs belonging to different farming systems*

	Farm no., type:							
	78992, farmed		80020, en plein air		80866, wild		79007, free ranging	
Congener	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)	Fat (pg/g FB)	Liver (pg/g FB)
PBDE 28	<0.01	<0.10	<0.01	<0.23	<0.01	<0.03	<0.01	<0.05
PBDE 47	0.33	0.33	0.06	0.14	0.20	0.16	0.06	0.13
PBDE 49	<0.01	<0.05	<0.01	<0.11	<0.01	<0.02	<0.01	<0.03
PBDE 71	<0.01	<0.07	<0.01	<0.15	<0.01	<0.03	<0.01	<0.04
PBDE 85	<0.01	<0.11	<0.01	<0.30	<0.01	<0.06	<0.01	<0.05
PBDE 99	0.43	<0.10	0.05	<0.26	0.10	<0.05	0.11	<0.04
PBDE 100	0.09	<0.11	<0.01	<0.30	0.04	<0.05	0.02	<0.05
PBDE 153	0.05	<0.13	0.01	<0.46	0.02	0.08	0.03	<0.12
PBDE 154	0.05	<0.18	0.01	<0.54	0.03	0.06	0.02	<0.12
PBDE 183	0.03	<0.12	0.01	<0.30	0.02	0.06	0.02	<0.10
PBDE 197	<0.02	<0.15	<0.01	<0.36	<0.03	<0.06	<0.02	<0.09
PBDE 206	<0.002	<0.02	<0.001	<0.04	<0.003	0.11	<0.002	<0.01
PBDE 209	0.57	<0.16	0.14	<0.30	<0.03	4.86	<0.01	<0.12
Total PBDEs	1.63	1.64	0.34	3.49	0.52	5.64	0.32	0.97



D1, 2,3,7,8-T_4CDD; **D2**, 1,2,3,7,8-P_5CDD; **D3**, 1,2,3,4,7,8-H_6CDD; **D4**, 1,2,3,6,7,8-H_6CDD; **D5**, 1,2,3,7,8,9-H_6CDD; **D6**, 1,2,3,4,6,7,8-H_7CDD; **D7**, O_8CDD; **F1**, 2,3,7,8-T_4CDF; **F2**, 1,2,3,7,8-P_5CDF; **F3**, 2,3,4,7,8-P_5CDF; **F4**, 1,2,3,4,7,8-H_6CDF; **F5**, 1,2,3,6,7,8-H_6CDF; **F6**, 1,2,3,7,8,9-H_6CDF; **F7**, 2,3,4,6,7,8-H_6CDF; **F8**, 1,2,3,4,6,7,8-H_7CDF; **F9**, 1,2,3,4,7,8,9-H_7CDF; **F10**, O_8CDF.

FIGURE 4. *PCDD+PCDF–detected (left) and DL-PCB–detected (right) congener profiles, expressed as percentages of PCDD+PCDF and DL-PCB analytical sums found in the fat from intensive farmed pigs fed on PCB-contaminated feeds in Ireland (A) (26) and Belgium (B) (15), respectively.*

a more detailed site characterization and subsequent identification of the occasional source(s) of exposure.

ACKNOWLEDGMENTS

This work was funded by a grant from the Assessorato all'Agricoltura e Foreste della Regione Siciliana, ''Environment, Animal Welfare, and Animal Health Monitoring, within the Nebrodi Park,'' project no. 2006-2008. The authors thank Antonella Pilozzi for her administrative assistance and Fabiola Ferri for the graphic work.

REFERENCES

1. Ábalos, M., E. Abad, A. Estévez, M. Solé, A. Buet, L. Quirós, B. Piad, and J. Rivera. 2008. Effects on growth and biochemical responses in juvenile gilthead seabream *Sparus aurata* after long-term dietary exposure to low levels of dioxins. *Chemosphere* 73: S303–S310.
2. Brambilla, G., G. Cherubini, S. De Filippis, M. Magliuolo, and A. di Domenico. 2004. Review of aspects pertaining to food contamination by polychlorinated dibenzodioxins, dibenzofurans, and biphenyls at the farm level. *Anal. Chim. Acta* 514:1–8.
3. Burniston, D. A., R. K. Symons, M. Croft, M. Trout, and W. Korth. 2003. Determination of polybrominated dyphenyl ethers (PBDEs) in Australian pig fat. *Organohal. Compd.* 61:167–170.
4. Covaci, A., J. J. Ryan, and P. Schepens. 2002. Patterns of PCBs and PCDD/PCDFs in chicken and pork fat following a Belgian food contamination incident. *Chemosphere* 47:207–217.
5. de Felip, E., A. di Domenico, M. Falleni, F. Ferri, N. Iacovella, G. Menale, P. Tafani, G. Tommasino, and L. Turrio Baldassarri. 1994. Polychlorodibenzodioxin and polychlorodibenzofuran levels in dielectric fluids containing polychlorobiphenyls. *Toxicol. Environ. Chem.* 46:239–260.
6. de Mul, A., R. de Winter-Sorkina, P. E. Boon, G. van Donkersgoed, M. I. Bakker, and J. D. van Klaveren. 2005. Dietary intake of brominated diphenyl ether congeners by the Dutch population. Dutch National Institute for Public Health and the Environment report 310305004 of July 2005. Available at: http://rivm.openrepository.com/rivm/bitstream/10029/7298/1/310305004.pdf. Accessed 30 September 2010.
7. Eun-Jung, K., O. Jeong-Eun, and C. Yoon-Seok. 2003. Effects of forest fire on the level and distribution of PCDD+PCDFs and PAHs in soil. *Sci. Total Environ.* 311:177–189.

8. European Commission Scientific Committee on Animal Nutrition. 2000. Opinion on the dioxin contamination of feedingstuffs and their contribution to the contamination of food of animal origin, adopted on 6 November 2000. Scientific Committee on Animal Nutrition, European Commission. Available at: http://ec.europa.eu/food/committees/scientific/out55_en.pdf. Accessed 30 September 2010.
9. European Food Safety Authority. 2008. Statement of EFSA on the risks for public health due to the presence of dioxins in pork from Ireland (question no. EFSA-Q-2008-777). *EFSA J.* 911:1–15.
10. European Food Safety Authority. 2010. Results of the monitoring of dioxin levels in food and feed. *EFSA J.* 8:1385–1420.
11. Feil, V., J. K. Huwe, J. K. Zaylskie, R. G. Davison, K. L. Anderson, V. L. Marchello, and M. Tiernan. 2000. Chlorinated dibenzo-*p*-dioxin and dibenzofuran concentrations in beef animals from a feeding study. *J. Agric. Food Chem.* 48:6163–6173.
12. Fiedler, H. 2003. Dioxins and furans (PCDD/PCDF), chap. 6, p. 125–201. *In* H. Fiedler (ed.), The handbook of environmental chemistry, vol. 3, part O: persistent organic pollutants. Springer, Berlin.
13. Fries, G. F. 1996. A model to predict concentrations of lipophilic chemicals in growing pigs. *Chemosphere* 32:443–451.
14. Gullett, B., A. Touati, and L. Oudejans. 2008. PCDD+PCDF and aromatic emissions from simulated forest and grassland fires. *Atmos. Environ.* 42:7997–8006.
15. Hoogenboom, L. A. P., C. A. Kan, T. F. H. Bovee, G. van der Weg, C. Onstenk, and W. A. Traag. 2004. Residues of dioxins and PCBs in fat of growing pigs and broilers fed contaminated feed. *Chemosphere* 57:35–42.
16. Huwe, J. K. 2006. Uptake of dioxin-like compounds in growing swine: correlation between experimental and predicted data. *Organohal. Compd.* 68:197–200.
17. Huwe, J. K., K. Davison, V. J. Feil, G. L. Larsen, M. K. Lorentzsen, R. Zaylskie, and T. O. Tiernan. 2004. Levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in cattle raised at agricultural research facilities across the USA and the influence of pentachlorophenol-treated wood. *Food Addit. Contam.* 21:182–194.
18. Huwe J. K., and G. Larsen. 2005. Polychlorinated dioxins, furans, and biphenyls, and polybrominated diphenyl ethers in a US meat market basket and estimates of dietary intake. *Environ. Sci. Technol.* 39:5606–5611.
19. Kijlstra, A., W. A. Traag, and L. A. P. Hoogenboom. 2007. Effect of flock size on dioxin levels in eggs from chickens kept outside. *Poult. Sci.* 86:2042–2048.
20. Ingelido, A. M., T. Ballard, E. Dellatte, A. di Domenico, F. Ferri, A. R. Fulgenzi, T. Herrmann, N. Iacovella, R. Miniero, O. Papke, M. G. Porpora, and E. De Felip. 2007. Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in milk from Italian women living in Rome and Venice. *Chemosphere* 67:S301–S306.
21. Meyer, C., T. Beer, J. Müller, R. Gillet, I. Weeks, J. Powell, K. Tolhurst, L. McCaw, G. Cook, D. Marney, and R. Symons. 2004. Dioxin emission from bushfires in Australia. National Dioxins Program technical report no. 1. Australian Government, Department of Environment and Heritage, Canberra.
22. Mortimer, D. N., M. Gem, M. Rose, A. Fernandes, and S. White. 2008. Dioxins in liver: a regulatory conundrum. *Organohal. Compd.* 70:883–886.
23. Pugliese, C., G. Calagna, V. Chiofalo, V. M. Moretti, S. Margiotta, O. Franci, and G. Gandini. 2004. Comparison of the performances of Nero Siciliano pigs reared indoors and outdoors: joints composition, meat, and fat traits. *Meat Sci.* 68:523–528.
24. Rhind, S. M. 2002. Endocrine disrupting compounds and farm animals: their properties, actions, and routes of exposure. *Domest. Anim. Endocrinol.* 23:179–187.
25. Schulz, A. J., T. Wiesmüller, H. Appuhn, D. Stehr, K. Severin, D. Landmann, and J. Kamphues. 2005. Dioxin concentration in milk and tissues of cows and sheep related to feed and soil contamination. *J. Anim. Physiol. Anim. Nutr.* 89:72–78.
26. Scortichini, G., and G. Diletti. 2009. Personal communication.
27. Smith, P. N., G. P. Cobb, C. Godard-Codding, D. Hoff, S. T. McMurry, T. R. Rainwater, and K. D. Reynolds. 2007. Contaminant exposure in terrestrial vertebrates. *Environ. Pollut.* 150:41–64.
28. Spitaler, M., C. Iben, and H. Tausch. 2005. Dioxin residues in the edible tissue of finishing pigs after dioxin feeding. *J. Anim. Physiol. Anim. Nutr.* 89:65–71.
29. Stachel, B., E. L. Christoph, R. Götz, T. Herrmann, F. Krüger, T. Kühn, J. Lay, J. Löffler, O. Päpke, H. Reincke, C. Scröter-Kermani, R. Schwartz, E. Steeg, D. Stehr, S. Uhlig, and G. Umlauf. 2006. Contamination of the alluvial plain, feedingstuffs, and foodstuffs with polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD+PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs), and mercury from the River Elbe in the light of the flood event in August 2002. *Sci. Total Environ.* 364:96–112.
30. Stephens, R. D., M. X. Petreas, and D. G. Hayward. 1995. Biotransfer and bioaccumulation of dioxins and furans from soil: chickens as a model for foraging animals. *Sci. Total Environ.* 175:253–273.
31. Toma, L., and E. Mathis. 2007. Environmental risk perception, environmental concern, and propensity to participate in organic farming programmes. *J. Environ. Manage.* 83:145–157.
32. U.K. Food Standard Agency. 2006. Limits for dioxins in liver. Communication to the European Commission, United Kingdom Food Standard Agency, ENV 376, 17 July 2006. U.K. Food Standard Agency, London.
33. U.S. Environmental Protection Agency. 1994. Method 1613, revision B: tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS. U.S. Environmental Protection Agency, Office of Water Engineering and Analysis Division, Washington, DC.
34. van den Berg, M., L. Birnbaum, A. T. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J. P. Giesy, A. Hanberg, R. Hasegawa, S. W. Kennedy, T. Kubiak, J. C. Larsen, R. F. X. van Leeuwen, A. K. Liem, C. Nolt, R. E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern, and T. Zacharewski. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, and PCDFs for humans and wildlife. *Environ. Health Perspect.* 106:775–792.
35. van den Berg, M., J. J. De Jongh, H. Poiger, and J. R. Olson. 1994. The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Crit. Rev. Toxicol.* 24:1–74.
36. van der Schalie, W. H., H. S. Gardner, Jr., J. A. Bantle, C. T. De Rosa, R. A. Finch, J. S. Reif, R. H. Reuter, L. C. Backer, J. Burger, L. C. Folmar, and W. S. Stokes. 1999. Animals as sentinels of human health hazards of environmental chemicals. *Environ. Health Perspect.* 107:309–315.
37. van Overmeire, I., L. Pussemier, V. Hanot, L. De Temmerman, M. Hoenig, and L. Goeyens. 2006. Chemical contamination of free-range eggs from Belgium. *Food Addit. Contam.* 23:1109–1122.
38. Williams, C. M. 2002. Nutritional quality of organic food: shades of grey or shades of green? *Proc. Nutr. Soc.* 61:19–24.