



Priority persistent contaminants in people dwelling in critical areas of Campania Region, Italy (SEBIOREC biomonitoring study) [☆]



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HIGHLIGHTS

- Blood, serum, and breast milk were obtained from many donors of Campania Region.
- Priority POPs and heavy metals As, Cd, Hg, and Pb were measured in pooled samples.
- Dioxins, PCBs, and PBDEs in serum and milk were within current values in Europe.
- Metals were within current values in Europe; As, Hg showed a relative overexposure.
- Biomarkers responded at municipality level, to possibly drive future interventions.

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ABSTRACT

To investigate if protracted living in degraded environments of the Caserta and Naples provinces (Campania Region, Italy) had an impact on exposure of local people, highly toxic persistent contaminants were measured in blood, blood serum, and human milk of a large number of healthy donors. Sampling was carried out from 2008 to 2009. Blood was collected from over 850 20–64-year old donors; by pooling, 84 blood and 84 serum samples were obtained. Milk was donated by 52 mothers: specimens were pooled into six samples. Polychlorodibenzodioxins (PCDDs), polychlorodibenzofurans (PCDFs), and polychlorobiphenyls (PCBs, dioxin-like (DL) and non-dioxin-like (Σ_6 PCBs)), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) were measured in serum (organic biomarkers) and blood (metals); these chemicals and polybromobiphenyl ethers (Σ_9 PBDEs) were analyzed in milk. PCDD + PCDF, DL-PCB, TEQ_{TOT}, and Σ_6 PCB concentration ranges (medians) in serum were 6.26–23.1 (12.4), 3.42–31.7 (11.5), 10.0–52.8 (23.9) pgTEQ₉₇/g fat, and 55.5–647 (219) ng/g fat, respectively, while in milk concentration ranges were 5.99–8.77, 4.02–6.15, 10.0–14.2 pgTEQ₉₇/g fat, and 48.7–74.2 ng/g fat. Likewise, As, Cd, Hg, and Pb findings in blood spanned 2.34–13.4 (5.83), 0.180–0.930 (0.475), 1.09–7.60 (2.60), 10.2–55.9 (28.8) µg/L, respectively; only Pb could be measured in milk (2.78–5.99 µg/L). Σ_9 PBDE levels in milk samples were 0.965–6.05 ng/g fat. Biomarkers' concentrations were

[☆] The local health agencies (ASLs) participating in the study underwent a reorganization in 2008 that determined a change of identification (before/after): ASL NA1/ASL NA1 Centro; ASL NA2/ASL NA2 Nord; ASL NA3/ASL NA2 Nord; ASL NA4/ASL NA3 Sud; ASL CE1/ASL CE; ASL CE2/ASL CE. For practical reasons, both denominations have been used in the article. Additional co-workers are listed at the end of the article under SEBIOREC Working Units, according to their original affiliations.

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found to be compatible with their current values in European countries and in Italy, and consistent with an exposure primarily determined by consumption of commercial food from the large distribution system. Based on relatively higher biomarker values within the hematic biomonitoring database, the following municipalities were flagged as possibly deserving attention for health-oriented interventions: Brusciano and Caivano (As), Giugliano (Hg), Pianura (PCDDs + PCDFs), and Qualiano–Villaricca (As, Hg). The analysis of samples' qualitative variability indicated that biomarker composition was sensitive at municipality level, a feature that can potentially drive interventions for future local risk assessment and/or management measures.

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1. Introduction

For more than two decades, many zones in the provinces of Caserta (CE) and Naples (NA), in the northern part of the Campania Region (Fig. 1), have been affected by extensive illegal dumping of mixed waste of urban as well as industrial origin, while waste has frequently been burnt in the open (Diletti et al., 2004, 2008; Esposito et al., 2014; Giovannini et al., 2014; Martuzzi and Mitis, 2007; Neugebauer et al., 2009; Rivezzi et al., 2013): a cocktail of environmental pressure factors potentially resulting in a release of organic as well as inorganic chemical contaminants. As documented by various studies (Brambilla et al., 2004; US EPA, 2006; Vassiliadou et al., 2009), the unregulated open-air burning of solid waste, including agricultural refuse, can be a significant source of polychlorodibenzodioxins and polychlorodibenzofurans (PCDDs and PCDFs, also collectively known as “dioxins”): according to Esposito et al. (2009, 2010, 2014) and Neugebauer et al. (2009), open-air waste burning may have critically contributed to the PCDD, PCDF, and dioxin-like polychlorobiphenyl (DL-PCB) contamination episodes of Campania buffalo milk, eventually ensuing in alarming non-compliances of local food produce (Borrello et al., 2008).

The territory covered by the aforesaid provinces comprises 196 municipalities and is highly inhabited. The pressure on the environment from the prolonged and diffuse local practice of illegal waste disposal/treatment and other sources of environmental degradation (including mishandled legal landfills) – altogether entailing potential risks for

human health – prompted the Italian authorities to acknowledge an emergency status (1994–2009) and declare 77 of those municipalities as “site of national interest for remediation” (Bianchi et al., 2004; Comba et al., 2006; Fazzo et al., 2008, 2011; Martuzzi et al., 2009). As described by the same authors, the results of epidemiological studies carried out in the area provide hints to potentially associate increases in cancer mortality and congenital malformations with environmental degradation and socioeconomic deprivation. However, due to the complexity and uncertainties of the exposure scenario, the assessment of a causal link between the pressure of environmental risk factors and its impact on communities' health was acknowledged to require further studies.

In the light of the above, the Campania Region allocated funds for an extensive biomonitoring community study whose aim was to: (a) analyze the hematic and mother's milk burdens of a number of priority environmental contaminants potentially associated with waste mishandling and degradation of the northern Campania environment, (b) evaluate whether the long-term coexistence with a chemically degraded environment had determined an increase of the hematic and milk chemical burdens, (c) evaluate health-related risks based on the chemical burdens assessed, in particular if significant increases were detected, and (d) if appropriate, identify the critical (dietary) exposure pathways to eventually define risk reduction policies/measures. The Istituto Superiore di Sanità (Italian National Institute for Health, Rome) was charged with the study coordination. The study itself – known as SEBIOREC and

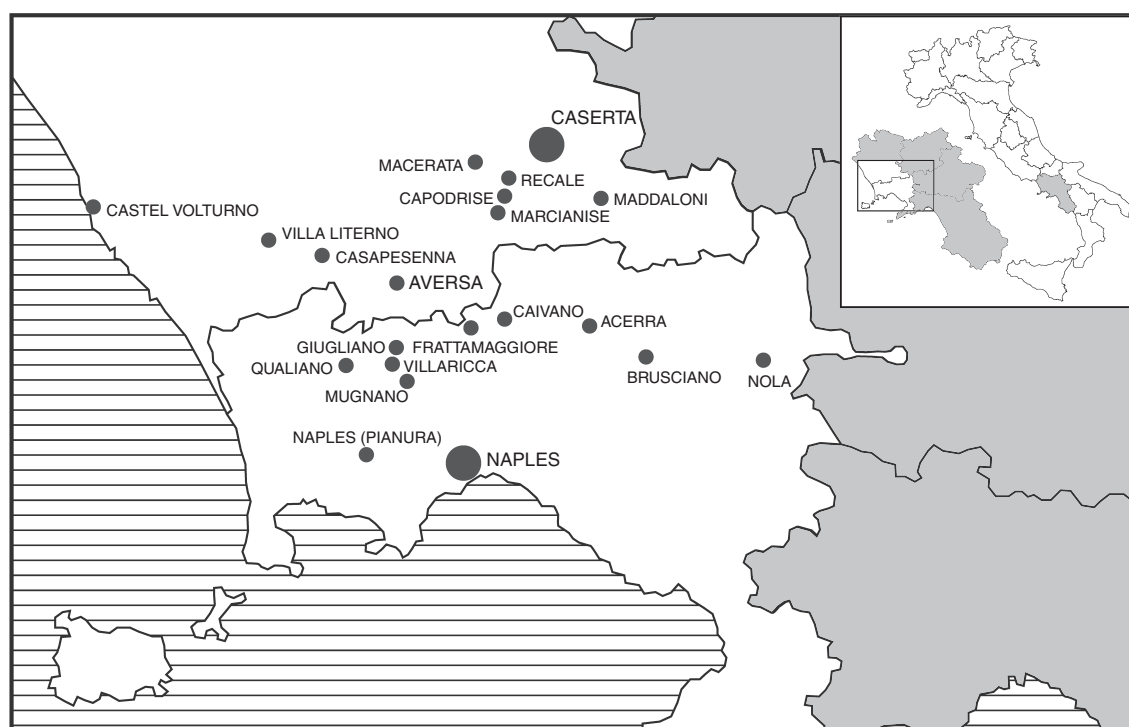


Fig. 1. Layout of the Caserta and Naples provinces, separated by a jagged line, and the municipalities where the SEBIOREC human biomonitoring study was carried out.

carried out by a group of scientific and health institutions (see affiliations) – was set out in mid-2007 and completed by the end of 2010 (De Felip and di Domenico, 2010).

2. Methods

2.1. Selection of chemical biomarkers

PCDDs, PCDFs, and DL-PCBs were recognized to have an unquestionable priority as they had well-established highly toxic properties (IARC, 1997, 2012; JECFA, 2001; SCF, 2000, 2001) and since 2001 had been detected repeatedly above regulatory levels in some Campania food products (see Introduction). Moreover, their assessed or just suspected presence above background values in environmental matrices and/or food triggered at least a psychological malaise in local people adding up to the general discomfort to co-exist with wastes freely dumped on land and mishandled. However, due to the potential complexity of the environmental scenario and degradation factors, the following additional chemical biomarkers of local concern were also included for assessment:

- non-dioxin-like polychlorobiphenyls, NDL-PCBs (EFSA, 2005; Lauby-Secretan et al., 2013);
- polybromodiphenyl ethers, PBDEs (EFSA, 2011), whose assessment was required in human milk but optional in blood serum;
- arsenic, As (EFSA, 2009a);
- cadmium, Cd (EFSA, 2009b);
- mercury, Hg (EFSA, 2012; Health Canada, 2007);
- lead, Pb (EFSA, 2010).

PCDDs, PCDFs, PCBs, and PBDEs are persistent organic pollutants (POPs, 2001), a subgroup of persistent toxic substances (PTS) of the Chemicals Programme of the United Nation Environment Programme (UNEP) (<http://www.chem.unep.ch/>), which also includes a number of heavy metals. For all of them, the chemical, physical, and toxicological properties have been well investigated, and in general a large number of scientific publications are available related to their adverse effects. Due to the potential importance of the latter, exposure to the aforesaid chemicals is traditionally a reason of health concerns, in particular as they may accumulate in food and affect its quality. A description of the toxicological properties of the chemicals is beyond the scope of this paper. Of the vast number of available citations, the recent references reported above provide start-up information.

2.2. Participant selection and recruitment

Participants (“donors”) were recruited from the population of the aforesaid Campania provinces following interviews with a detailed questionnaire focusing on life environment and style, smoking habits, medical history, dietary habits, socio-demographic factors, work experiences, reproductive/nursing history in women, environmental risk perception, etc. Prior to sampling, each donor signed an informed consent form. Subjects were screened out when they had less than a 10-year residence in the community they formally belonged to, had a work history that might be a potential for chemical overexposure, had undergone severe and rapid weight loss over the preceding few months or years, and had been or were under a prolonged medical treatment that could alter fat metabolism. Individual specimens were made into pooled samples to increase the number of sampled subjects, improve analytical quality by having more sample matrix available, and optimize resources.

Eighty-four blood serum samples and as many heparinized blood samples were planned to be prepared to respectively investigate the presence of organic pollutants and metals; both groups of pollutants and PBDEs were to be determined also in six human milk (pooled) samples. Each pool was intended to be constituted by equivalent contributions from 10 specimens (WHO, 1996) characterized by some shared

features owing to sampling criteria. The target was to have 840 blood donors, stratified by gender and age, and 60 primiparous milk donors in their fourth to eighth nursing week, all randomly selected in geographic locations (municipalities) each typified by an estimated degree of environmental pressure (I_{PR}) and a socio-economic deprivation index value (I_D) (Martuzzi et al., 2009; Musmeci et al., 2010). I_{PR} was a synthetic hazard index primarily reflecting local activities of dumping waste. Data from census were used to calculate I_D , applying an established methodology based on variables on education, unemployment, housing ownership, surface of the dwelling, and family structure. The 1991 census data were preferred to the 2001 data to allow for long latencies (i.e. cancer mortality). Both indexes covered a scale from 1 (low) to 5 (high).

As to blood or serum, each geographic location was intended to be described by six pools of specimens drawn from 30 men and 30 women covering three age ranges (20–34, 35–49, and 50–64 years), each range being further divided into five three-year subranges in theory represented by two donors each. By combining the two indexes and other ancillary indicators (e.g. health suffering, non-compliance of local food produce, population density), risk areas A, B, and C – with presumed descending risk profiles – were identified together with the following municipalities of interest (municipality, I_{PR} – I_D , risk area, province):

- Acerra, 5–5, A, NA;
- Aversa, 5–5, A, CE;
- Brusciano, 1–5, C, NA;
- Caivano, 5–5, A, NA;
- Casapesenna, 1–5, C, CE;
- Castel Volturno, 5–4, A, CE;
- Giugliano in Campania (Giugliano hereafter), 5–4, A, NA;
- Maddaloni, 4–4, B, CE;
- Marcianise, 5–4, A, CE;
- Mugnano di Napoli (Mugnano hereafter), 1–4, C, NA;
- Naples (Pianura) (Pianura hereafter), 5–5, A, NA;
- Nola, 3–1, B, NA;
- Qualiano, 4–5, B, NA, together with Villaricca, 3–2, B, NA;
- Villa Literno, 5–5, A, CE.

To collect milk, the following Zone A municipalities were selected: Acerra, Aversa, Caivano, Giugliano, Marcianise, and Pianura; Frattamaggiore (1–5, C, NA) was considered as a potential reference of local background. Due to lack of donors as originally planned, risk area C milk specimens were also obtained from the congruent municipalities of Capodrise, Macerata, and Recale. Sampling instructions excluded that nursing mothers aside from milk would also donate blood due to the delicate postpartum condition. Further, as milk is the major route of excretion of persistent lipophilic chemicals for lactating women and the major source of exposure to said substances for breast-fed infants, it was recommended to collect milk from primiparae only to avoid body burden depauperation (Schecter et al., 1998).

2.3. Sampling of blood and human milk specimens

Detailed instructions for collecting blood and milk were provided to doctors, medical assistants, and donors in the form of written protocols as well as of oral communications. The original protocols are retrievable from De Felip and di Domenico (2010). For details, cfr. Supplementary Material (SM).

2.4. Chemical analysis

2.4.1. Persistent organic pollutants in blood serum

PCDDs, PCDFs, and DL-PCBs in serum samples (pools) were analyzed by gas chromatography coupled with high resolution mass spectrometry (HRGC–HRMS); NDL-PCBs were assayed by HRGC coupled with low resolution (LR) MS (Abballe et al., 2008, 2013). In both cases,

in-house adaptations of well-known US EPA Methods 1613B and 1668B were respectively used. HRGC–LRMS results were possibly confirmed by HRGC–HRMS. For details, cfr. SM.

The quantified congeners were the canonical 17 PCDDs and PCDFs, 12 DL-PCBs, and six “indicator” NDL-PCBs 28, 52, 101, 138, 153, and 180 (cumulatively expressed as Σ_6 PCBs). Most human data concerning PCDD, PCDF, and DL-PCB concentrations were available from the literature as 1997 dioxin toxicity equivalents (TEQ_{97}): therefore, the cumulative concentrations of PCDDs, PCDFs, DL-PCBs, and their sum (TEQ_{TOT}) were expressed accordingly by using the toxicity equivalency factors (TEF) adopted in 1997 by the World Health Organization (WHO) (Van den Berg et al., 1998) for converting to TEQ the original analytical data.

Fat-based congener limits of detection (LODs) were in the order of 1–2 pg/g for most PCDDs and PCDFs, in the 10–100 pg/g range for DL-PCBs, and in the order of 1–3 ng/g for NDL-PCBs. Repeatability standard deviation was better than $|\pm 10\%$ for individual congeners and cumulative values, while the expanded uncertainty of the method (coverage factor, $k = 2$) was less than $|\pm 25\%$ for individual congeners and less than $|\pm 20\%$ for cumulative values. IS recovery rates were considered to be acceptable when in the 40–130% range.

In serum samples, one PCDD, six PCDF, two DL-PCB, and three NDL-PCB non-detects exhibited a frequency higher than 10% (cfr. Table SM-1) (in milk samples, four PCDF and three PBDE non-detects had a frequency greater than 10%; cfr. Table SM-2). Owing to small-to-modest differences between upper and lower bound (UB-LB) values, all the cumulative figures reported in this article are medium bound (MB) estimates obtained by posing a non-detect value equal to $LOD \times 0.5$.

2.4.2. Persistent organic pollutants in human milk

As described for serum samples, PCDDs, PCDFs, and DL-PCBs in milk samples (pools) were analyzed by HRGC–HRMS, while NDL-PCBs and PBDEs were assayed by HRGC–LRMS (Abballe et al., 2008, 2013). Nine PBDE congeners were quantified: PBDEs 28, 47, 99, 100, 153, 154, 183, 197, and 209, cumulatively expressed as Σ_9 PBDEs. Again, HRGC–LRMS results were possibly confirmed by HRGC–HRMS. LODs of PCDD, PCDF, DL-PCB, and NDL-PCB congeners were approximately one tenth of those reported for serum samples; PBDE LODs were around 0.03 ng/g fat. Cfr. SM.

2.4.3. Heavy metals in blood

As, Cd, Hg, and Pb were analyzed in blood samples (pools) by means of dynamic-reaction-cell inductively-coupled plasma mass spectrometry DRC-ICP-MS technique (D’Ilio et al., 2010). Quantification was carried out by DRC-ICP-MS operating in the standard mode. Cfr. SM.

Method LODs for As, Cd, Hg, and Pb in blood were respectively 0.7, 0.03, 0.2, and 0.2 $\mu\text{g/L}$. For each element, the within-laboratory reproducibility in the analytical working range was better than or in the order of $|\pm 10\%$, with an expanded uncertainty of less than $|\pm 30\%$ (coverage factor, $k = 2$). Recovery rates were considered acceptable when in the 90–110% range.

2.4.4. Heavy metals in human milk

As, Cd, Hg, and Pb were analyzed in milk samples (pools) with a DRC-ICP-MS-based procedure (D’Ilio et al., 2008; Rivero Martino et al., 2000). Method LODs were 3, 0.08, 0.3, and 0.5 $\mu\text{g/L}$, respectively. Cfr. SM.

2.5. Statistical analysis

The non-parametric Kruskal–Wallis and Mann–Whitney U tests (Statistica, Version 8.0) were used to investigate the statistical significance of the quantitative differences between groups in contaminants’ concentrations in serum, blood, and human milk pooled samples. Concentrations were used directly as obtained from sample analysis, i.e. uncorrected for possible confounders (raw data).

The principal component factor analysis (PCFA) (Statgraphics XVI), concerned with correlations between variables only, was adopted to explore the qualitative variability of POP data (Fielding, 2007; Izenman, 2008): the found correlations allowed to characterize the classifying factors (“classifiers”). With regard to serum pools, the PCFA was carried out on the separate age groups (or ranges) while investigating the effects of the following classifiers: risk area, health district (ASL), municipality, and gender. Congeners with a non-detect frequency across the database greater than 10% were excluded from the analysis to maximize the PCFA structure and its diagnostic potential (this exclusion threshold was also adopted for milk results). The “varimax” method was used to maximize the explained variability (variance (%)) of the loadings in each factor group. The diagnostic power of factor loadings and scores was provided by their positive values greater than a default of 0.3 (Fielding, 2007). Factor loadings and scores provided information on the most important congeners in each factor.

Sample characterization with blood metal data was carried out by applying the general linear models (GLMs) (Statgraphics XVI) and using the same classifiers defined for serum pools. Normality of the original and log-transformed values of each data set was always tested at the start. The hypothesis of normality was not rejected for $P > 0.05$ (Shapiro and Wilk’s test), a condition resulting after log-transformation. Regressions were multiple variables GLMs. In the metal database, non-detects were absent.

As to milk pools, the relevant classifiers – eating habits, extra exposure, municipality, and smoking habits (Table 1) – were entered as numerical descriptors in the PCFA. Due to the limited number of milk pools analyzed, subgroups of variables were studied.

3. Results and discussion

3.1. Recruitment and sampling

3.1.1. Blood and serum specimens and pools

Blood sampling was carried out between January 2008 and October 2009. A total of 876 serum specimens were collected respectively from 429 and 447 male (M) and female (F) donors; in parallel, 423 and 436 heparinized blood specimens were obtained for a total of 859 specimens. In both cases, 84 pooled samples were obtained (cfr. Tables SM-3 and SM-4).

Most ($\geq 60\%$) pools were compliant with pooling design: of the non-compliant samples, three of serum and eight of blood were made with a number of contributions less than 10, whereas many of the remaining pools had an excess number of contributions. Irregular pool compositions were also determined by other deviations from original sampling design such as an altered availability of age-stratified donors and/or the amounts of serum and blood specimens, often less than expected: amounts were respectively in the ranges of approximately 5–20 and 1–5 mL/donor, which allowed to make pooled samples with volumes of 50–100 and 6–10 mL, appropriate for confirmatory analyses and independent quality checks. In any case, the final contents of non-compliant pools resulted from balancing the contributions available so as to minimize the extent of non-compliance with respect to the scheduled patterns of age and quantitative composition.

A screening of questionnaires revealed that several questions and entries had been filled in incompletely or unreliably, often as unchecked personal expressions. For the difficulty to extract the correct information, the evaluation of questionnaire data and their correlations with the exposure biomarkers described in this article will be reported elsewhere (Bianchi et al., in preparation). On the whole, pools had compositions reflecting closely the sampling/pooling design as to the age distribution of donors and their analytical contributions. The level of smoking habits in the different pools was comparable, whereas differences were detected as to parity – women that had different lactation/breastfeeding episodes during the 10-year residence period given as a screening-out condition – and consumption of foods if produced locally.

Table 1
General information on human milk donors and qualitative descriptors (normalized) of the derived pooled samples. Sampling period: October 2008 through December 2009. For difficulties in finding all required donors for Marcianise and Macerata samples, specimens were also gathered from other municipalities of congruent risk areas.

Municipality or locality	Naples (Pianura)	Giugliano	Caivano	Acerca	Marcianise ^a	Macerata ^b	Rome
Risk area	Zone A	Zone A	Zone A	Zone A	Zone A	Zone C	–
ASL (local health agency)	NA1	NA2	NA3	NA4	CE1, CE2	CE1, NA3	– ^c
Number (N) of donors in pool	10	10	11	10	5	7	10
Age, median and range (years)	32; 26–39	32; 21–37	26; 22–36	26; 19–34	31; 24–35	30; 22–35	33; 25–38
Pool, parity rating ^d	0.90	1.00	1.00	1.00	1.00	1.00	1.00
Pool, extra exposure ^e	0.50	0.00	0.27	0.40	0.20	0.14	0.00
Pool, smoking habits ^f	0.80	0.55	0.32	0.40	0.40	0.14	0.50
Pool, eating habits ^g	0.02	0.00	0.28	0.07	0.00	0.00	0.00

^a Five specimens collected in the areas of Aversa (1) and Marcianise (4).

^b Seven specimens collected in the areas of Capodrise (1), Frattamaggiore (1), Macerata (4), and Recale (1).

^c Hospital “Umberto I”, Italian State University “Sapienza”, Rome.

^d Based on the number of primiparae in pool (N_p/N): primiparae only, 1; absence of primiparae, 0.

^e Donors' protracted previous or ongoing exposure to potential source(s) of chemical risk factors: all donors in pool exposed, 1; no donor in pool exposed, 0.

^f All donors in pool active (>5 cigarettes/day per donor) smokers only, 1; all donors in pool passive smokers only, 0.5; all donors in pool active and passive smokers, 1.5; no donor in pool active or passive smoker, 0.

^g Based on a consumption list comprising 26 food items either from the general distribution or from local/own production: consumption of local/own food only, 1; no consumption of local/own food, 0.

3.1.2. Human milk specimens and pools

Milk sampling was carried out between October 2008 and December 2009. As detailed in Table 1, 53 milk specimens were gathered from as many mothers living in municipalities within the provinces of Caserta and Naples. The individual amounts of milk collected (approximately 20–100 mL/mother) were often less than originally instructed but anyway adequate to provide six pooled samples with volumes of at least 50 mL each. In general, there was enough sample for confirmatory analyses, if required.

As the Rome area had provided milk donors in two preceding WHO-coordinated milk studies (Abballe et al., 2008; Ingelido et al., 2007; Larsen et al., 1994; Schecter et al., 1992), the same area was used in this study primarily to have a generic time-related reference of the current POP levels in the aforesaid matrix.

In general, donors were healthy women with no relevant gynecological and obstetrical history, had not been assuming drugs that could interfere with the study, and had not had a severe weight loss/variation in the last 10 years aside from the recent pregnancy. With the exception of Pianura pool (whose parity was 0.90 due to a secundipara's contribution), all the other pools had only contributions from primiparae. The age distribution of all the mothers enrolled in the six Campania groups was 19–39 years, a range comprising that of Rome mothers (25–38 years); however, the median of the latter (33 years) fell slightly above the upper end of the Campanian median range (26–32 years). Not always were mothers' height and weight recorded: on the whole, the available body mass indexes (BMIs) ranged from 21.2 to 31.6 (median, 25.4) kg/m², but no average BMI values could be estimated at pool level.

Data concerning lifestyle and dietary habits were gathered from answers mostly expressing subjective evaluations that could not be confirmed reliably, thereby having only a qualitative valence. For this reason, the pool descriptors reported in Table 1 were obtained by assigning a “yes–no”-type index to each donor in the pool: the normalized pool score was the index sum through the pool divided by the number of pool donors.

With the exception of Giugliano and Rome mothers (no extra exposures reported in both cases), many mothers declared environmental or else occupational exposures – unquantifiable as to frequency and/or magnitude – in general to volatile chemicals but also to some pesticides: each donor's index of extra exposure occurrence or absence was rated respectively either 1 or 0.

In all pools there were contributions from mothers that were either active (>5 cigarettes/day per donor) or passive smokers, or both (passive smoking may have been misjudged as the question was posed too broadly): the index values used to rate an active, passive, or active and

passive smoker, and a non-smoking mother were 1, 0.5, 1.5, and 0, respectively.

The questionnaire section dealing with dietary habits was coarsely profiled on 26 food macro-aggregations, this aspect introducing additional uncertainty in evaluating the available answers as the relevance of specific food contributions could not in general be accounted for. On the whole, food consumption at individual level exhibited many common traits between mothers, occasional deviances being smoothed down by the pooling operation. For this reason, only the question associated with each of the 26 food items, investigating whether food was from the general distribution or from local/own production – index values 0 and 1, respectively – was considered to be relevant: before normalization at pool level, the index of each donor was normalized dividing the total score (ranging between 0 and 26) by 26.

3.2. Descriptive statistics of biomonitoring results

The results obtained – in bulk and stratified by risk area, provincial district, age range, and gender – are statistically described and compared respectively in Tables 2 and 3. The descriptors X_{MIN}, Q₂₅, Q₅₀, X̄, Q₇₅, X_{MAX}, and N (where Q and X̄ stand respectively for percentile and arithmetic mean) were used by default.

The application of Kruskal–Wallis and Mann–Whitney U tests provided a self-explanatory evidence that significant ($P < 0.05$), eventually highly significant, differences could be detected for: risk areas and PCDDs + PCDFs; provincial districts and As; age ranges and organic pollutants or heavy metals (except As); genders and PCDDs + PCDFs, Hg, and Pb. Synoptical box-plot representations of the statistical analysis outcome are visible in Figs. 2 and 3, respectively for organic pollutants and heavy metals. Cfr. Table SM-5 for pool-specific results and exemplification of specimen/sample labeling.

3.2.1. Persistent organic pollutants in blood serum samples

PCDD + PCDF, DL-PCB, TEQ_{TOT}, and Σ₆PCBs cumulative concentrations (Table 2, “All data”) fall respectively in the ranges 6.26–23.1, 3.42–31.7, 10.0–52.8 pgTEQ₉₇/g fat, and 55.5–647 ng/g fat; the related median and mean estimates are 12.4 and 12.6, 11.5 and 12.2, 23.9 and 24.9 pgTEQ₉₇/g fat, and 219 and 232 ng/g fat. The relative contribution of DL-PCBs to TEQ_{TOT} is characterized by a generally increasing trend with increasing TEQ_{TOT} (and age range), being on average around 48% and spanning approximately from 27 to 67%. The sum of NDL-PCB congeners 138, 153, and 180 (Σ₃PCBs) is a substantially quantitative estimate (≥95%) of Σ₆PCBs over the entire range of measured concentrations.

Table 2Statistical descriptors of organic and inorganic pollutant concentrations respectively measured in serum and blood pools. Values rounded off to three figures.^a

Chemical ^b	X _{MIN}	Q ₂₅	Q ₅₀	⟨X⟩	Q ₇₅ ^c	X _{MAX}	Chemical	X _{MIN}	Q ₂₅	Q ₅₀	⟨X⟩	Q ₇₅ ^c	X _{MAX}	N
<i>All data</i>														
PCDDs + PCDFs	6.26	9.62	12.4	12.6	15.1	23.1	Arsenic	2.34	4.65	5.83	6.37	7.57	13.4	84
DL-PCBs	3.42	7.51	11.5	12.2	16.1	31.7	Cadmium	0.180	0.352	0.475	0.492	0.577	0.930	84
TEQ _{TOT}	10.0	17.3	23.9	24.9	31.2	52.8	Mercury	1.09	1.90	2.60	2.86	3.37	7.60	84
Σ ₆ PCBs	55.5	131	219	232	310	647	Lead	10.2	19.7	28.8	29.6	38.4	55.9	84
<i>Risk area A</i>														
PCDDs + PCDFs	7.09	9.83	12.5	12.8	15.1	20.4	Arsenic	2.34	4.35	5.61	6.00	6.87	13.4	48
DL-PCBs	3.42	7.47	11.0	11.9	16.0	22.6	Cadmium	0.240	0.423	0.510	0.526	0.630	0.930	48
TEQ _{TOT}	11.0	17.2	24.4	24.7	31.1	38.2	Mercury	1.09	1.80	2.49	2.74	3.36	6.36	48
Σ ₆ PCBs	55.5	131	210	228	302	550	Lead	10.2	20.6	29.6	31.0	41.1	55.9	48
<i>Risk area B</i>														
PCDDs + PCDFs	8.91	11.0	13.5	14.2	16.8	23.1	Arsenic	2.64	4.66	5.70	6.02	7.61	10.3	18
DL-PCBs	3.81	7.08	12.7	13.7	18.7	31.7	Cadmium	0.180	0.328	0.375	0.425	0.508	0.670	18
TEQ _{TOT}	14.1	18.1	26.4	27.9	33.6	52.8	Mercury	1.61	2.12	2.76	3.01	3.52	6.98	18
Σ ₆ PCBs	95.7	126	240	264	354	647	Lead	13.6	19.2	23.6	26.0	33.7	44.4	18
<i>Risk area C</i>														
PCDDs + PCDFs	6.26	8.19	9.80	10.7	12.7	18.4	Arsenic	4.19	5.39	7.04	7.51	8.97	13.2	18
DL-PCBs	3.64	8.35	11.5	11.6	14.3	22.6	Cadmium	0.270	0.358	0.420	0.471	0.508	0.890	18
TEQ _{TOT}	10.0	16.7	21.8	22.3	25.9	41.0	Mercury	1.73	2.05	2.58	3.02	3.42	7.60	18
Σ ₆ PCBs	86.6	144	208	211	258	355	Lead	14.4	19.2	31.8	29.4	36.5	46.7	18
<i>Caserta provincial district</i>														
PCDDs + PCDFs	7.31	9.10	10.7	12.0	14.6	23.1	Arsenic	2.34	3.95	4.55	4.72	5.24	9.72	36
DL-PCBs	3.71	8.12	11.0	12.4	16.2	25.4	Cadmium	0.250	0.343	0.460	0.488	0.565	0.930	36
TEQ _{TOT}	11.9	16.9	21.8	24.3	32.2	48.6	Mercury	1.09	1.87	2.61	2.60	2.86	6.36	36
Σ ₆ PCBs	82.1	142	216	242	302	550	Lead	10.2	18.9	25.7	27.3	34.8	50.8	36
<i>Naples provincial district</i>														
PCDDs + PCDFs	6.26	10.3	12.9	13.1	15.3	21.2	Arsenic	2.64	6.12	6.98	7.62	8.93	13.4	48
DL-PCBs	3.42	7.20	12.2	12.1	16.1	31.7	Cadmium	0.180	0.373	0.480	0.495	0.580	0.930	48
TEQ _{TOT}	10.0	17.8	25.1	25.3	30.8	52.8	Mercury	1.42	1.99	2.58	3.05	3.63	7.60	48
Σ ₆ PCBs	55.5	120	221	225	318	647	Lead	13.6	21.1	32.6	31.3	40.5	55.9	48
<i>Age range 20–34 years</i>														
PCDDs + PCDFs	6.26	8.20	9.35	9.56	11.0	14.7	Arsenic	2.34	3.81	5.37	5.53	6.74	10.3	28
DL-PCBs	3.42	5.44	6.68	6.51	7.75	9.93	Cadmium	0.180	0.290	0.360	0.383	0.460	0.580	28
TEQ _{TOT}	10.0	14.3	16.5	16.1	18.3	22.3	Mercury	1.09	1.65	2.15	2.43	2.81	6.98	28
Σ ₆ PCBs	55.5	93.2	112	115	131	166	Lead	10.2	15.7	19.3	21.4	25.7	35.2	28
<i>Age range 35–49 years</i>														
PCDDs + PCDFs	7.68	10.1	12.8	13.0	15.1	20.4	Arsenic	2.66	4.46	5.85	6.46	7.60	13.4	28
DL-PCBs	7.75	10.4	12.0	12.0	14.3	16.1	Cadmium	0.180	0.320	0.410	0.430	0.535	0.780	28
TEQ _{TOT}	16.5	21.8	25.1	25.0	28.9	32.7	Mercury	1.45	2.04	2.54	2.90	3.30	7.60	28
Σ ₆ PCBs	159	200	224	228	255	298	Lead	16.4	22.9	29.8	31.9	44.0	50.9	28
<i>Age range 50–64 years</i>														
PCDDs + PCDFs	8.09	12.8	15.1	15.4	18.2	23.1	Arsenic	3.94	5.29	6.31	7.17	8.93	12.9	28
DL-PCBs	6.78	15.9	17.9	18.2	21.1	31.7	Cadmium	0.300	0.440	0.495	0.566	0.670	0.930	28
TEQ _{TOT}	17.3	29.9	33.6	33.6	36.9	52.8	Mercury	1.71	2.58	3.14	3.26	3.86	6.36	28
Σ ₆ PCBs	134	310	344	354	406	647	Lead	18.8	29.7	37.0	35.9	41.5	50.9	28
<i>Male gender</i>														
PCDDs + PCDFs	6.26	8.91	11.5	11.7	14.0	20.4	Arsenic	2.64	4.70	5.76	6.50	7.61	13.4	42
DL-PCBs	3.42	7.32	10.8	11.8	16.1	21.8	Cadmium	0.180	0.380	0.470	0.490	0.550	0.930	42
TEQ _{TOT}	10.0	16.7	22.8	23.5	30.6	38.6	Mercury	1.09	2.20	2.70	3.16	3.80	7.60	42
Σ ₆ PCBs	55.5	138	208	232	314	550	Lead	17.2	26.0	34.6	35.0	41.8	55.9	42
<i>Female gender</i>														
PCDDs + PCDFs	7.09	10.5	12.9	13.6	16.8	23.1	Arsenic	2.34	4.55	5.85	6.24	7.55	13.2	42
DL-PCBs	3.64	7.63	12.0	12.7	16.0	31.7	Cadmium	0.250	0.340	0.480	0.495	0.615	0.930	42
TEQ _{TOT}	10.8	18.5	24.8	26.2	32.7	52.8	Mercury	1.18	1.75	2.40	2.55	3.10	6.36	42
Σ ₆ PCBs	86.6	126	224	232	298	647	Lead	10.2	16.4	21.5	24.0	31.1	44.0	42

^a Values are medium bound estimates in pgWHO-TEQ₉₇/g fat for PCDDs + PCDFs, DL-PCBs, and TEQ_{TOT}, and in ng/g fat for Σ₆PCBs. Metal concentrations are in μg/L blood.^b TEQ_{TOT} = PCDDs + PCDFs + DL-PCBs; Σ₆PCBs = NDL-PCBs 28 + 52 + 101 + 138 + 153 + 180.^c Estimate only indicative when N = 18.

The following evaluations (similarly carried out for metals) were obtained by averaging the ratios of the four descriptors Q₂₅, Q₅₀, ⟨X⟩, and Q₇₅ (Table 2) when applied to two data subsets.

When serum pool contents are compared based on risk area (A, B, or C) attribution, statistically significant differences are detected only for PCDDs + PCDFs, specifically between risk areas A/C ($P = 0.029$) and B/C ($P = 0.0079$). The PCDD + PCDF pattern in risk areas can be

represented as [dioxins]_B ≥ [dioxins]_A > [dioxins]_C, their concentrations in risk area C being on average some 26 and 18% lower than those measured in pools from risk areas B and A, respectively.

No significant differences are observed when pool contents are compared based on provincial district attribution, although a marginally significant ($P = 0.091$) difference is noticed for PCDDs + PCDFs ([dioxins]_{NA} ≥ [dioxins]_{CE}).

Table 3Summary of multiple and two-group statistical comparisons performed on the organic and inorganic pollutant concentration subsets.^a

Chemical	Risk area (A, B, C)				Provincial district (CE, NA) ^b		Age range (20–34, 35–49, 50–64 years)				Gender (F, M) ^c
	N _A = 48; N _B = 18; N _C = 18				N _{CE} = 36; N _{NA} = 48		N _{20–34} = 28; N _{35–49} = 28; N _{50–64} = 28				N _F = 42; N _M = 42
	P _{A/B/C}	P _{A/B}	P _{A/C}	P _{B/C}	P _{CE/NA}	P _{20–64}	P _{20–34/35–49}	P _{20–34/50–64}	P _{35–49/50–64}	P _{F/M}	
<i>Persistent organic pollutants (in blood serum)</i>											
PCDDs + PCDFs ^d	0.020	0.26	0.029	0.0079	0.091	<0.0001	<0.0001	<0.0001	0.0096	0.033	
DL-PCBs ^d	0.76	0.53	0.88	0.49	0.88	<0.0001	<0.0001	<0.0001	<0.0001	0.60	
TEQ _{TOT} ^{d,e}	0.30	0.36	0.27	0.17	0.53	<0.0001	<0.0001	<0.0001	<0.0001	0.22	
Σ ₆ PCBs ^f	0.54	0.36	0.77	0.28	0.51	<0.0001	<0.0001	<0.0001	<0.0001	0.99	
<i>Heavy metals (in blood)</i>											
Arsenic ^g	0.11	0.56	0.030	0.21	0.0001	0.079	0.42	0.025	0.17	0.90	
Cadmium ^g	0.071	0.045	0.13	0.33	0.70	0.0001	0.36	0.0001	0.002	0.93	
Mercury ^g	0.49	0.30	0.40	0.89	0.16	0.004	0.15	0.001	0.057	0.038	
Lead ^g	0.33	0.13	0.70	0.42	0.11	0.0001	0.0001	0.0001	0.13	0.0001	

^a Multiple and two-group comparisons carried out with the Kruskal–Wallis and Mann–Whitney U tests, respectively.^b CE, Caserta; NA, Naples.^c F, female; M, male.^d Original data in pgWHO-TEQ₉₇/g fat.^e TEQ_{TOT} = PCDDs + PCDFs + DL-PCBs.^f Σ₆PCBs = NDL-PCBs 28 + 52 + 101 + 138 + 153 + 180. Original data in ng/g fat.^g Original data in µg/L.

The concentrations of organic pollutants in serum pools appear to be strongly ($P < 0.0001$ in all cases but one, $P < 0.0096$) age-dependent and, as expected (Harden et al., 2004; Needham et al., 2006), follow the general pattern $[\text{POPs}]_{50-64} > [\text{POPs}]_{35-49} > [\text{POPs}]_{20-34}$. On average, bioaccumulation with increasing age is given by the following empirical relationships:

- $[\text{dioxins}]_{50-64} = (1.21 \pm 0.04) [\text{dioxins}]_{35-49}$;
- $[\text{dioxins}]_{35-49} = (1.33 \pm 0.07) [\text{dioxins}]_{20-34}$;
- $[\text{dioxins}]_{50-64} = (1.61 \pm 0.04) [\text{dioxins}]_{20-34}$;
- $[\text{DL-PCBs}]_{50-64} = (1.50 \pm 0.02) [\text{DL-PCBs}]_{35-49}$;
- $[\text{DL-PCBs}]_{35-49} = (1.85 \pm 0.05) [\text{DL-PCBs}]_{20-34}$;
- $[\text{DL-PCBs}]_{50-64} = (2.78 \pm 0.11) [\text{DL-PCBs}]_{20-34}$;
- $[\text{TEQ}_{\text{TOT}}]_{50-64} = (1.33 \pm 0.04) [\text{TEQ}_{\text{TOT}}]_{35-49}$;
- $[\text{TEQ}_{\text{TOT}}]_{35-49} = (1.54 \pm 0.03) [\text{TEQ}_{\text{TOT}}]_{20-34}$;
- $[\text{TEQ}_{\text{TOT}}]_{50-64} = (2.06 \pm 0.04) [\text{TEQ}_{\text{TOT}}]_{20-34}$;
- $[\Sigma_6\text{PCBs}]_{50-64} = (1.56 \pm 0.02) [\Sigma_6\text{PCBs}]_{35-49}$;
- $[\Sigma_6\text{PCBs}]_{35-49} = (2.02 \pm 0.09) [\Sigma_6\text{PCBs}]_{20-34}$;
- $[\Sigma_6\text{PCBs}]_{50-64} = (3.14 \pm 0.12) [\Sigma_6\text{PCBs}]_{20-34}$.

An attempt was carried out to model the trends of POP concentrations (y) vs. age (x) in spite of the limited number of x coordinates available: for the fittings, the latter were identified with the intermediate points of the three age ranges, each x coordinate being associated with 28 y values. The following conventional models were tested:

- $y = m x + q$ (a)
- $\ln[y] = m x + q$ (b)
- $\ln[y] = m \ln[x] + q$ (c)

Based on r^2 , r , and $F_{1,82}$ estimates, fittings appeared to improve remarkably from (a) to (c), always being formally highly significant ($P_r \ll 0.001$; $P_F \ll 0.001$). When reverted to linear coordinates, model (c) regression equations below yielded the average power functions described in Fig. 4:

- $\ln[\text{dioxins, pgTEQ}_{97}/\text{g fat}] = (0.629 \pm 0.082) \ln[\text{age, years}] + (0.17 \pm 0.30)$
- $\ln[\text{DL-PCBs, pgTEQ}_{97}/\text{g fat}] = (1.39 \pm 0.10) \ln[\text{age, years}] - (2.74 \pm 0.36)$
- $\ln[\text{TEQ}_{\text{TOT}}, \text{pgTEQ}_{97}/\text{g fat}] = (0.979 \pm 0.075) \ln[\text{age, years}] - (0.47 \pm 0.28)$
- $\ln[\Sigma_6\text{PCBs, ng/g fat}] = (1.49 \pm 0.09) \ln[\text{age, years}] - (0.17 \pm 0.32)$

The slopes (increase rates) are greatest, and similar, for DL- and NDL-PCBs ($m > 1$), whereas the PCDD + PCDF trend exhibits a slight downwards bend ($m < 1$): the two rates are partially compensated in TEQ_{TOT} data, whose trend in linear coordinates is almost linear ($m \approx 1$). The PCDD + PCDF's downwards bend rendered (only) by model (c) may reflect the effects of a diminished human exposure (Alcock et al., 2000) following the strong risk reduction measures that have been applied in the western world since the 1980s to abate the environmental release of PCDDs, PCDFs, and PCBs (COM(2001) 593 final; Decision 2179/98/EC; Directive 2000/76/EC); in the European Union (EU), these measures have been accompanied since the early 2000s by the development of an effective body of rules to limit their occurrence in food (Recommendation 2011/516/EU; Regulations (EU) 1259/2011 and 277/2012), the major exposure pathway for humans. The differences in PCB and PCDD + PCDF trends may indicate that reduction of exposure to the latter has been more effective than to PCBs, at least in the Campania Region: congruent results can be obtained by running model (c) on the two gender-specific data sets. Fig. 4 also suggests the age when the relative contributions to TEQ_{TOT} of PCDDs + PCDFs and DL-PCBs become equivalent (46 years), the contribution of the latter being respectively lower and higher than that of PCDDs + PCDFs before and after such time point.

As to gender influence, PCDDs + PCDFs are the only organic pollutants exhibiting a significant ($P = 0.033$) gender-driven bioaccumulation ($[\text{dioxins}]_F > [\text{dioxins}]_M$), their concentrations in male donor pools ("M-samples") being on average some 14% lower than in female donor pools ("F-samples"). It can be noticed that the aforesaid difference is significant only when the entire data set is considered: when the latter is broken down by age range ($N = 28$ each range), the probability value changes from non-significant ($P = 0.36$), to marginally significant ($P = 0.089$), to almost significant ($P = 0.054$) as age increases. In other words, data seem to indicate that a slightly selective mechanism is at work that favors bioaccumulation in women (Harden et al., 2004; Needham et al., 2006), but requires years-long exposures and/or a fair number of data to be statistically detectable.

Lastly, our results for PCDDs + PCDFs, DL-PCBs, TEQ_{TOT}, and Σ₆PCBs in blood serum are in good agreement with their current values obtained in studies carried out since 2000 in different European populations (Cao et al., 2008; Costopoulou et al., 2006; De Felip et al., 2004a, 2008; Fromme et al., 2009; Kvale et al., 2009; Porpora et al., 2006, 2009; Reis et al., 2007; Thomas et al., 2006; Wittsiepe et al., 2008).

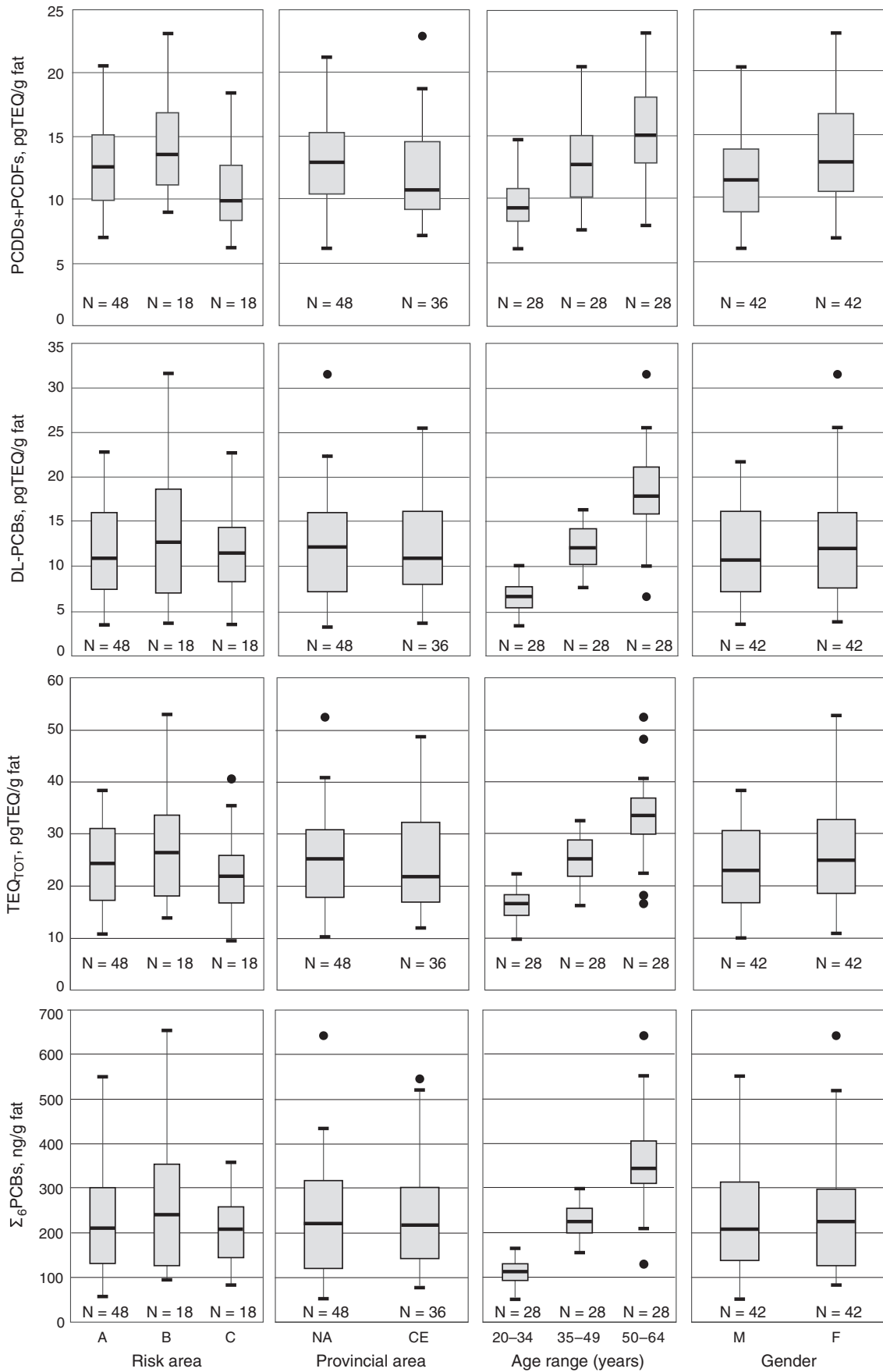


Fig. 2. Box plots of POP concentrations in blood serum pools. Boxes provide the Q₂₅, Q₅₀, and Q₇₅ estimates (the latter are only indicative when N = 18). Dots represent outliers.

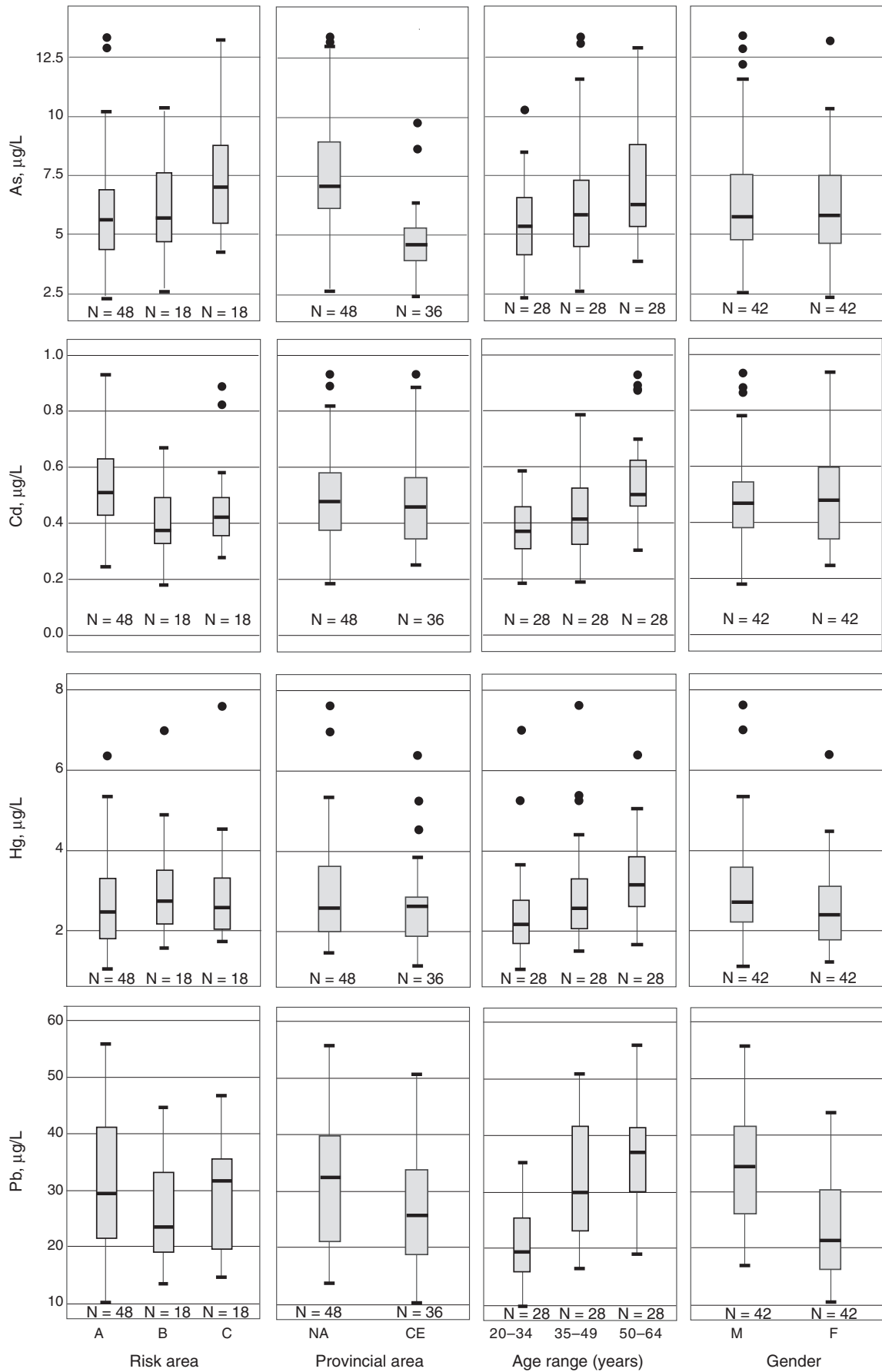


Fig. 3. Box plots of heavy metal concentrations in blood pools. Boxes provide the Q₂₅, Q₅₀, and Q₇₅ estimates (the latter are only indicative when N = 18). Dots represent outliers.

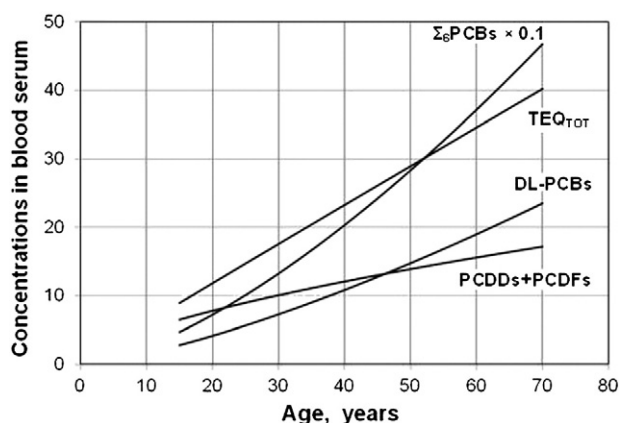


Fig. 4. Average trends of POP concentrations (y) in blood serum pools with age (x, years) based on the power functions obtained from fitting model $\ln[y] = m \ln[x] + q$, allowing supralinear, linear, and sublinear relationships, and by reverting to linear coordinates the regression equations (all highly significant). The NDL-PCB y values are scaled down by a factor 10.

$$\text{PCDDs} + \text{PCDFs} (\text{pgWHO-TEQ}_{97}/\text{g fat}) : y = 1.18 x^{0.629}$$

$$\text{DL-PCBs} (\text{pgWHO-TEQ}_{97}/\text{g fat}) : y = 0.0645 x^{1.39}$$

$$\text{TEQ}_{\text{TOT}} (\text{pgWHO-TEQ}_{97}/\text{g fat}) : y = 0.627 x^{0.979}$$

$$\text{NDL-PCBs} (\text{ng/g fat}) : y = 0.841 x^{1.49}$$

3.2.2. Persistent organic pollutants in human milk samples

As summarized in Table 4, PCDD + PCDF, DL-PCB, TEQ_{TOT} , and $\Sigma_6\text{PCBs}$ cumulative concentrations in the six Campania samples and in Rome pool fall respectively in the ranges 5.99–8.77, 4.02–6.97, 10.0–14.2 $\text{pgTEQ}_{97}/\text{g fat}$, and 48.7–108 ng/g fat . The Rome data appear to be placed at the upper end of each distribution, or next to it and within analytical uncertainty for PCDDs + PCDFs: in other words, relative to Rome mothers the milk sampled from Campania donors tends to be somewhat less contaminated, the contaminant levels also being substantially unrelated to the risk area origin of the samples. All the above concentrations are noticeably lower than those measured in the pools of milk collected in the 1998–2001 period in the Rome and Venice areas (respectively: 9.40–14.8, 11.0–19.3, 20.4–34.2 $\text{pgTEQ}_{97}/\text{g fat}$, and 195–318 ng/g fat) (Abballe et al., 2008; Ingelido et al., 2007), a possible confirmation that exposure to the aforesaid contaminants maintained a negative trend. As for blood serum samples, $\Sigma_3\text{PCBs}$ is a quantitative (>97%) estimate of $\Sigma_6\text{PCBs}$.

The SEBIOREC outcome can be compared with two recent works in which individual milk specimens were analyzed. According to Diletti et al. (2008), who described the preliminary results from a subgroup of 44 specimens collected in “exposed” and “not exposed” areas of CE and NA provinces, the PCDD + PCDF and DL-PCB concentrations

covered the ranges 5.5–14.9 (mean, 8.8) and 4.0–21.2 (mean, 8.2) $\text{pgTEQ}_{97}/\text{g fat}$, respectively: all the pertinent results of our study fall within the aforesaid ranges and, in particular, below the means shown. Our PCDD + PCDF findings are also in good agreement with those reported by Giovannini et al. (2014) and Rivezzi et al. (2013) concerning 94 individual milk specimens from primiparae of the same areas (range, 3.8–19.0 $\text{pgTEQ}_{97}/\text{g fat}$). Ulaszewska et al. (2011) measured the concentrations of PCDDs + PCDFs, DL-PCBs, TEQ_{TOT} , and $\Sigma_6\text{PCBs}$ plus DL-PCB 118 in milk from 21 donors living in Giugliano, obtaining the values respectively of 1.26–9.44 (mean, 3.78 ± 2.09), 1.81–10.46 (mean, 4.87 ± 2.21), 4.22–18.95 (mean, 8.65 ± 3.55) $\text{pgTEQ}_{97}/\text{g fat}$, and 23.59–160.49 (mean, 72.42 ± 29.20) ng/g fat (these latter figures include DL-PCB 118 and are overestimated by an average 10% relative to $\Sigma_6\text{PCBs}$): all the results we reported for Giugliano fall well within the aforesaid pertinent ranges, and in general comply with the mean estimates shown within the associated uncertainties (standard deviations).

At last, it can be observed that our results for PCDDs + PCDFs, DL-PCBs, TEQ_{TOT} , and $\Sigma_6\text{PCBs}$ in milk are in excellent agreement with their current values obtained in recent studies carried out in different European populations (Bordajandi et al., 2008; Cao et al., 2008; Colles et al., 2008; Costopoulou et al., 2006; Croes et al., 2012; Jaraczewska et al., 2006; Lignell et al., 2009; Malisch and van Leeuwen, 2003; Malisch et al., 2008; Pratt et al., 2012; Raab et al., 2008; Schuhmacher et al., 2009; Vandentorren et al., 2011; Vieth et al., 2011; Wittsiepe et al., 2007a, 2007b).

In the same pools, the $\Sigma_9\text{PBDEs}$ cumulative concentrations are comprised between 0.965 and 6.05 ng/g fat (Table 4): this range is substantially comparable with that of the 1998–2001 study (1.55–4.06 ng/g fat) (Abballe et al., 2008; Ingelido et al., 2007) by taking into account the uncertainties underlying both analytical outcomes and that the congener array used in this study includes PBDEs 197 and 209. By far the lowest $\Sigma_9\text{PBDE}$ level was measured in an (incomplete) risk area A pool with milk collected by ASLs CE1 + CE2, whereas the highest level was found in the only pool nominally from risk area C (ASLs NA3 + CE1). PBDEs 47, 99, 100, and 153 give always a major contribution to cumulative concentrations, greater than 60% in zone A pools of ASLs NA1, NA2, and CE1 + CE2, and in Rome sample. However, in pools of ASLs NA3, NA4, and NA3 + CE1 such contribution decreases (respectively: 55, 42, and 37%) as the cumulative concentrations of PBDEs 183, 197, and 209 concurrently increase to 43, 56, and 62%.

As extensively reviewed by Frederiksen et al. (2009), the PBDE levels detected in environmental, food, and human matrices exhibit a very large variability, in part to be ascribed to the intrinsic uneven diffusion of the chemicals and in part to a lack of a standardized selection of congeners for the analysis, a factor that can hinder a direct comparison of data from different sources. However, our findings for milk are on average in agreement with the PBDE values reported by recent studies

Table 4

Concentrations of organic and inorganic pollutants in human milk pools obtained from donors of different areas in the Campania Region and of the metropolitan area of Rome over the period October 2008 through December 2009. Values rounded off to three figures.^a

ASL ^b – risk area	Municipality or locality	N specimens (N pools)	Fat (%)	PCDDs + PCDFs	DL-PCBs	TEQ_{TOT} ^c	$\Sigma_6\text{PCBs}$ ^d	$\Sigma_9\text{PBDEs}$ ^e	Arsenic	Cadmium	Mercury	Lead
NA1 – Zone A	Naples (Pianura)	10 (1)	4.2	6.28	6.15	12.4	74.2	1.48	<3	<0.1	<0.3	4.45
NA2 – Zone A	Giugliano	10 (1)	4.3	6.29	5.52	11.8	70.5	1.75	<3	<0.1	<0.3	5.99
NA3 – Zone A	Caivano	11 (1)	3.5	6.17	4.99	11.2	51.1	1.91	<3	<0.1	<0.3	4.79
NA4 – Zone A	Acerra	10 (1)	3.7	8.77	5.38	14.2	62.6	3.42	<3	<0.1	<0.3	2.78
CE1, CE2 – Zone A	Marcianise ^f	5 (1)	4.0	5.99	4.02	10.0	48.7	0.965	<3	<0.1	<0.3	3.75
CE1, NA3 – Zone C	Macerata ^g	7 (1)	3.7	7.00	5.79	12.8	67.7	6.05	<3	<0.1	<0.3	5.28
Hospital Umberto I	Rome	10 (1)	3.5	7.26	6.97	14.2	108	2.16	<3	<0.1	<0.3	2.59

^a Values are medium bound estimates in $\text{pgWHO-TEQ}_{97}/\text{g fat}$ for PCDDs + PCDFs, DL-PCBs, and TEQ_{TOT} , and in ng/g fat for $\Sigma_6\text{PCBs}$ and $\Sigma_9\text{PBDEs}$. Metal concentrations are in $\mu\text{g/L}$.

^b Azienda Sanitaria Locale (local health agency).

^c $\text{TEQ}_{\text{TOT}} = \text{PCDDs} + \text{PCDFs} + \text{DL-PCBs}$.

^d $\Sigma_6\text{PCBs} = \text{NDL-PCBs } 28 + 52 + 101 + 138 + 153 + 180$.

^e $\Sigma_9\text{PBDEs} = \text{PBDEs } 28 + 47 + 99 + 100 + 153 + 154 + 183 + 197 + 209$.

^f Specimens collected in the areas of Aversa (N = 1) and Marcianise (N = 4).

^g Specimens collected in the areas of Capodrise (N = 1), Frattamaggiore (N = 1), Macerata (N = 4), and Recale (N = 1).

concerning different European populations (Bordajandi et al., 2008; Colles et al., 2008; Fång et al., 2010; Jaraczewska et al., 2006; Kotz et al., 2005; Lacorte and Ikononou, 2009; Lignell et al., 2009; Raab et al., 2008; Schuhmacher et al., 2009; Thomsen et al., 2010).

3.2.3. Correlation between TEQ_{05} and TEQ_{97} values in blood serum and human milk samples

Compared with 1997 WHO-TEFs, the 2005 WHO-TEF system (Van den Berg et al., 2006) yields lower TEQ estimates, in particular for DL-PCBs.

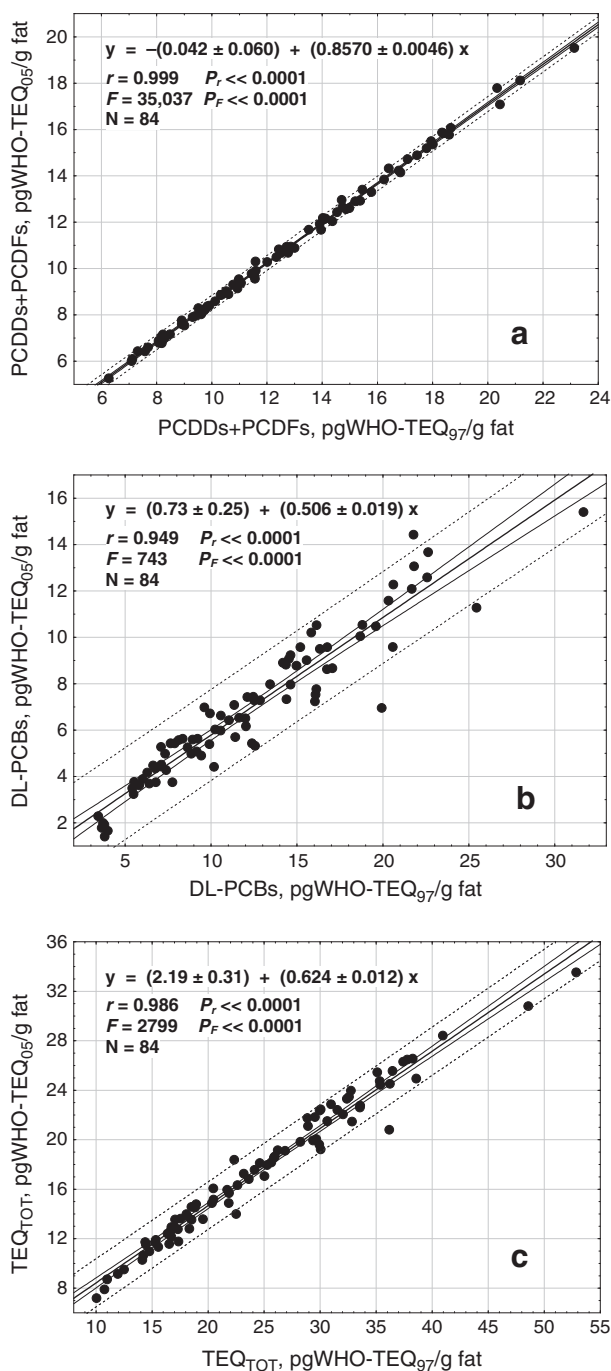


Fig. 5. WHO-TEQ₀₅ vs. WHO-TEQ₉₇. All linear regressions are highly significant, with a decreasing level of significance following the order PCDDs + PCDFs (a) > TEQ_{TOT} (c) > DL-PCBs (b). The pictures show the linear regression lines and their 95% confidence limits (continuous lines); the outer broken lines define the 95% prediction boundaries.

The impact of using the later TEF system is well represented in Fig. 5 exhibiting the TEQ₀₅-vs.-TEQ₉₇ trends in blood serum pools: all linear regressions are highly significant, with a decreasing level of significance following the order PCDDs + PCDFs > TEQ_{TOT} > DL-PCBs. Within the ranges of reported values (cfr. Table SM-5), the relative underestimation that affects PCDD + PCDF TEQ₀₅ values is on average in the order of 14–15% (Wittsiepe et al., 2007b), whereas underestimation increases with increasing concentration for TEQ_{TOT} (from 16 to 33%) and DL-PCBs (from 28 to 47%), tending to flatten out for higher x values. For $x = \langle X \rangle$ pgTEQ₉₇/g fat, the corresponding TEQ₀₅ values are underestimated by approximately an average 15, 29, and 43%, respectively. In agreement with regression statistical qualifiers, the adherence of experimental points to regression line is visibly very good for PCDDs + PCDFs; the deviations from regression lines that stand out in DL-PCB and to a lesser extent in TEQ_{TOT} graphics, likely reflect the relevant changes in 2005 TEFs relative to the 1997 values, particularly pronounced for most DL-PCBs. From Fig. 5, it can be inferred that uncertainty on regression predictiveness increases noticeably with decreasing significance level of regression.

As to the seven milk samples, the mean underestimations (%) in PCDD + PCDF, TEQ_{TOT}, and DL-PCB TEQ₀₅ values are respectively 18.4 ± 1.6 , 26.8 ± 2.2 , and 37.1 ± 3.2 .

3.2.4. Heavy metals in blood samples

As, Cd, Hg, and Pb concentrations fall respectively in the ranges 2.33–13.4, 0.180–0.935, 1.10–7.60, and 10.2–55.9 $\mu\text{g/L}$ blood (Table 2, “All data”); the related median and mean estimates are 5.83 and 6.87, 0.475 and 0.492, 2.60 and 2.86, and 28.8 and 29.6 $\mu\text{g/L}$ blood.

When blood pool contents are compared based on attribution to risk area (A, B, or C), statistically significant differences are detected only for As and Cd, respectively between risk areas A/C ($P = 0.030$) and risk areas A/B ($P = 0.045$). The patterns of the aforesaid metals in risk areas can be represented as $[\text{As}]_C \geq [\text{As}]_B \geq [\text{As}]_A$ and as $[\text{Cd}]_A \geq [\text{Cd}]_C \geq [\text{Cd}]_B$. On average, As concentrations in risk area A are some 21% lower than those measured in risk area C, while Cd levels in blood pools from risk area B are approximately 22% lower than those detected in risk area A.

No significant differences are detected for Cd, Hg, and Pb when blood pool contents are compared based on CE and NA provincial district attribution. However, a highly significant ($P = 0.0001$) difference is observed for As: on average, concentrations in CE pools are approximately 37% lower than in NA pools.

Metals' behavior with increasing age is not as consistent as that shown by POPs although, generally speaking, metal concentrations show a positive trend as well. As exhibits increasing blood levels that can be represented as $[\text{As}]_{50-64} \geq [\text{As}]_{35-49} \geq [\text{As}]_{20-34}$, leading to a significant ($P = 0.025$) difference only between the extreme age ranges, that is $[\text{As}]_{50-64} = (1.30 \pm 0.09) [\text{As}]_{20-34}$. Cd exhibits a similar pattern ($[\text{Cd}]_{50-64} > [\text{Cd}]_{35-49} \geq [\text{Cd}]_{20-34}$). In particular, $[\text{Cd}]_{50-64} = (1.46 \pm 0.06) [\text{Cd}]_{20-34}$ ($P = 0.0001$) and $[\text{Cd}]_{50-64} = (1.29 \pm 0.07) [\text{Cd}]_{35-49}$ ($P = 0.002$); Hg accumulation pattern can be described as $[\text{Hg}]_{50-64} \geq [\text{Hg}]_{35-49} \geq [\text{Hg}]_{20-34}$, with a significant ($P = 0.001$) difference between the extreme age ranges according to $[\text{Hg}]_{50-64} = (1.44 \pm 0.10) [\text{Hg}]_{20-34}$, and a marginally significant ($P = 0.057$) difference between the eldest and the intermediate age ranges. Pb accumulation pattern can be expressed as $[\text{Pb}]_{50-64} \geq [\text{Pb}]_{35-49} > [\text{Pb}]_{20-34}$, with highly significant ($P = 0.0001$) differences between the intermediate and the youngest age ranges ($[\text{Pb}]_{35-49} = (1.55 \pm 0.11) [\text{Pb}]_{20-34}$) and between the extreme age ranges ($[\text{Pb}]_{50-64} = (1.78 \pm 0.15) [\text{Pb}]_{20-34}$).

As to gender influence, only Hg and Pb exhibit a significant (respectively: $P = 0.038$ and 0.0001) differential accumulation of the type $[\text{metal}]_M > [\text{metal}]_F$, their concentrations in F-samples being on average some 17 and 33% lower than in M-samples.

All the As, Cd, Hg, and Pb concentrations measured in this study fall below the corresponding X_{MAX} values (15.5, 3.87, 15.0, and 215 $\mu\text{g/L}$,

respectively) reported by Alimonti et al. (2011) for the Italian general population as represented by over 1400 urban adults of both sexes sampled in 2008–2010 in five regions (Campania Region not included). From a comparison of the results in Table 2 (cfr. also Table SM-5) with the percentiles reported by the aforesaid authors, 62% of As values appear to be higher than Q_{95} (5.32 $\mu\text{g/L}$), all Cd values are lower than Q_{90} (1.11 $\mu\text{g/L}$), all Hg concentrations but one are higher than Q_{50} (1.15 $\mu\text{g/L}$), with six values falling above Q_{95} (5.16 $\mu\text{g/L}$), while 98% of Pb values are in the range Q_{10} – Q_{95} (9.04–51.7 $\mu\text{g/L}$): thus, in particular in the province of Naples, there seems to be a relative overexposure to As and, to a lesser extent, Hg that may tentatively be attributed to the volcanic nature of the region and/or local anthropogenic activities, as suggested by the work of Albanese et al. (2013). The above observations for As, Cd, Hg, and Pb are in substantial agreement with the findings of recent investigations in Europe (Batárviová et al., 2006; Becker et al., 2002; Beneš et al., 2000; Černá et al., 2001; Heitland and Köster, 2006; Wilhelm et al., 2004).

3.2.5. Heavy metals in human milk

Table 4 summarizes the As, Cd, Hg, and Pb levels measured in the seven milk pools studied. The first three elements were never quantifiable at the respective LODs of 3, 0.1, and 0.3 $\mu\text{g/L}$. Pb was measured in all samples (2.59–5.99 $\mu\text{g/L}$).

On the whole, these results are in accord with the sparse pertinent values recently reported for European countries (Abballe et al., 2008; Almeida et al., 2008; Dorea, 2004; Gundacker et al., 2002; Koyashiki et al., 2010; Krachler et al., 2000; Leotsinidis et al., 2005; Turconi et al., 2004; Ursinyova and Masanova, 2005). In particular, As, Cd, and Hg potential occurrence would come at the lower ends of the respective concentration ranges deducible from the aforesaid articles. As to Pb, its presence seems to be characterized by a remarkable variability of concentrations, possibly spanning three orders of magnitude: our results are consistent with some of the average estimates reported. However, the obvious inconsistency between these results and those reported by Abballe et al. (2008) (0.849–1.07 $\mu\text{g/L}$) cannot be explained, especially when considering that 10 years elapsed between samplings and that an important source of exposure to airborne Pb started to be phased out already in the mid-1990s by the introduction of unleaded fuel.

3.3. Analysis of analytical profiles in serum and blood

3.3.1. Polychlorodibenzodioxins and polychlorodibenzofurans

As deducible from the number of PCDD, PCDF, and DL-PCB factors and their loadings (cfr. Tables SM-6 and SM-7), congener compositions are different in the three age ranges and the chemical families analyzed. The gender is the most important classifier of the 20–34-year age range: the highest PCDD and PCDF scores for both Factors 1 and 2 are given by M-samples, whereas F-samples are not adequately characterized. Similarly, there are municipalities that are relatively well typified, but only Casapesenna, Castel Volturno, and Nola show a stronger characterization by both genders. The classification of the 35–49-year age range is similar to that of the lower one: on the whole, M-samples are typified by both factors whereas F-samples are not satisfactorily characterized. Among the municipalities, the most relevant are Aversa and Villa Literno (Factor 1), Giugliano (Factors 1 and 2), and Maddaloni (Factor 2), all typified by both genders. Therefore, different contamination sources seem to be brought out by samples that reflect eventual municipality-specific emissions. The 50–64-year age range is described by three factors, thus confirming that age is a critical determinant in influencing the body burden of the aforesaid chemicals (Harden et al., 2004; Needham et al., 2006). The gender is again the principal driver of data classification: M-samples are represented by all three factors whereas, considering a two-gender classification, Giugliano and Pianura are typified only by Factor 3, Castel Volturno by Factors 1 and 3, and Nola by Factors 1, 2, and 3.

Contamination sources from specific municipalities seem to influence all the age groups investigated. In particular, Castel Volturno, Giugliano, and Nola are those most frequently characterized: these municipalities, located in different provinces, are more distant from each other than other towns dealt with (Fig. 1), and the differential exposure suggested by the PCFA may reflect this net physical separation. Despite a general profile uniformity observed in the lower age ranges, a differential municipality-dependent exposure could exist but only exposures that last long enough may allow to detect it. As to the gender, the highest Factor 1 scores do not deviate from the aforesaid evaluations but they seem to be influenced by the low rate of F-sample classification.

3.3.2. Dioxin-like polychlorobiphenyls

Based on the number of pertinent factors, the capability of DL-PCB chemical characterization looks higher than that of PCDDs and PCDFs. The 20–34-year age range is typified by four DL-PCB factors (cfr. Tables SM-6 and SM-8). The M-samples are well represented by Factors 1 and 3, whereas F-samples, although exhibiting some scattered positive scores, do not show any sex-specific pattern: however, when present concurrently in both genders, positive scores lead to a better municipality classification than yielded by PCDDs and PCDFs. The following municipalities have common characteristics although belonging to different provinces: Casapesenna, Castel Volturno, and Marciánise in CE province, and Acerra, Caivano, Giugliano, and Mugnano in NA province. The observed differences and their relatively clear occurrence in this young age range suggest that exposure to DL-PCBs is current. The 35–49-year age range is as well described by four factors. The distribution of M-samples is scattered across the factors, while F-samples are described mainly by Factor 2. Among the municipalities, the pattern previously observed is confirmed in that Factor 3 characterizes exclusively Aversa, Castel Volturno, and Villa Literno, while Factors 2 and 4 characterize Caivano, Mugnano, and Nola. The 50–64-year age range is represented by three factors: this suggests that the body burden increase with increasing age could be counterbalanced by a decrease of the variability of exposure factors following a possible reduction of the activity level typical of mature age. M-samples are described mainly by Factors 1 and 2, while F-samples essentially by Factor 3. The municipalities Aversa, Castel Volturno, and Caivano are characterized by Factors 1 and 2, while Acerra only by Factor 2.

The trends observed for PCDDs and PCDFs are confirmed and possibly highlighted by DL-PCBs: a higher number of samples testify the presence of different contamination sources associated with the municipalities of interest. These sources can vary at intra- and inter-municipality level and their impact on a pool will depend on the exposure context experienced by the donors composing the pool. The municipalities marked by these congeners in the 20–34-year age range are Acerra, Casapesenna, Castel Volturno, and Caivano, while in the 50–64-year age range are Acerra, Aversa, Castel Volturno, and Caivano; in the 35–49-year age range, for DL-PCB 126 only, are present Aversa, Castel Volturno, Villa Literno, and Nola. In addition, in the latter age range, the pools involved in the characterization are equally subdivided between F- and M-samples.

3.3.3. Non-dioxin-like polychlorobiphenyls

NDL-PCB data do not show any clear-cut pattern (e.g. by the municipality and/or gender). This outcome may depend on the low number of variables available for the assessment (namely, NDL-PCBs 138, 153, and 180). As these three congeners are the predominant NDL-PCBs in the general population and in biological material (De Felip et al., 2004b), they may not be the most appropriate markers to trace differences, if any, in exposure sources.

3.3.4. Heavy metals

As to the heavy metals measured in blood pools, no classifier (risk area, health district (ASL), municipality, and gender) is discriminative

Table 5

Results by age range from the application of general linear models (GLMs) to heavy metal data. Only As and Pb highlighted significant differences respectively for municipality and gender (figures in bold).

Age range (years)	20–34	35–49	50–64
<i>Arsenic</i>			
Model, <i>P</i>	< 0.001	0.008	0.166
r^2 (df adjusted) \times 100	77.7	61.8	27.4
Gender, <i>P</i>	0.904	0.137	0.956
Municipality, <i>P</i>	< 0.001	0.007	0.138
<i>Cadmium</i>			
Model, <i>P</i>	0.232	0.656	0.856
r^2 (df adjusted) \times 100	20.9	0.0	0.0
Gender, <i>P</i>	0.336	0.828	0.168
Municipality, <i>P</i>	0.220	0.604	0.927
<i>Mercury</i>			
Model, <i>P</i>	0.134	0.218	0.343
r^2 (df adjusted) \times 100	31.0	22.2	11.7
Gender, <i>P</i>	0.727	0.011	0.181
Municipality, <i>P</i>	0.112	0.511	0.374
<i>Lead</i>			
Model, <i>P</i>	0.026	< 0.001	0.065
r^2 (df adjusted) \times 100	51.6	77.5	41.4
Gender, <i>P</i>	< 0.001	<< 0.001	0.008
Municipality, <i>P</i>	0.180	0.140	0.154

in the 50–64-year age range. The application of GLMs highlights that the risk area and health district are not significant by themselves but the health district becomes significant when nested with the municipality: in the other two age ranges, municipality and gender classifiers are significant for As ($P < 0.01$) and Pb ($P < 0.001$), respectively (Table 5). The NA province municipalities generally exhibit somewhat higher As concentrations – as also shown by the descriptive statistics – with maximum levels occurring in Bruscianno, Caivano, Giugliano, Mugnano, and Qualiano–Villaricca pools. As to Pb data, M-samples result to be more characterized than F-samples, a situation confirming what was highlighted by the descriptive statistics and by the PCFA across the three age groups on dioxin-like compounds.

3.4. Analysis of analytical profiles in human milk

The PCFA of PCDD congeners explains a cumulative variability of 80.6%. With the exception of Giugliano and Rome, which are not characterized, Campania samples are however typified by different congener aggregations, possibly shared by different municipalities. The classifier extra exposure (Table 1) marks specifically the Acerra, Caivano, and Pianura samples but cannot be correlated to any one of the congeners considered. Likewise, the PCFA of PCDF congeners explains a cumulative variability of 84.7% of total database variability. With the exception of the mixed pooled sample obtained with milk from Capodrise, Frattamaggiore, Macerata, and Recale (hereafter identified with Macerata) and Rome pool, both not characterized, the remaining Campania samples are typified. The extra exposure characterizes the pools from Acerra, Caivano, and Giugliano; similarly to what observed for PCDD congeners, the classifier does not appear to be linked to any one of PCDF congeners. Among both all the municipalities considered and the PCDD and PCDF congener families, the Rome pool exhibits no shared characterizing profile elements.

As to DL-PCB congeners, the PCFA explains a cumulative variability of 81.3%. With the exception of the mixed pool from Macerata, all NA province municipality pools and Rome pool are typified by some differences suggesting an influence from local exposure sources. Of the classifiers, the extra exposure and smoking habits specifically represent Acerra and Pianura and, to a much lesser extent, Rome; however, no congener(s) may be linked to the aforesaid classifiers.

The PCFA of PBDE congeners explains a cumulative variability of 66.2% of total database variability. In particular, Caivano, Pianura, and

Rome pools are typified by shared congener aggregations which, however, are different from those of the other typified pool (Acerra). The classifier extra exposure characterizes specifically the municipalities of Acerra and Pianura.

A municipality-specific exposure can be observed for all the chemical families, this indicating that the exposure can depend on local factors, but the number of milk samples is too low to draw any further conclusions.

The relationships between PCDD, PCDF, and DL-PCB congener distributions in blood (serum) and milk samples were assessed by PCFA. As to the hematic data sets, only the 20–34-year age range was considered due to its general overlapping with the age ranges of milk donors. No correlations were found between serum and milk data sets: the databases resulting from combining the sets together were described by two factors for PCDDs and PCDFs (cumulative variability of 83.3%) and one factor for DL-PCBs (cumulative variability of 84.4%). Data sets were clearly diverse as all milk samples were characterized by negative scores. All congeners included in the statistical analysis (with the exception of 2,3,4,7,8-P₅CDF) were on average less concentrated in milk than in hematic samples. On the whole, these observations are in agreement with the findings of other authors (Mannetje et al., 2012; Todaka et al., 2010; Wittsiepe et al., 2007a), i.e. the pathway from exposure source(s) to excretion step(s) can significantly influence congener concentrations and composition.

4. Conclusions

The aim of the SEBIOREC study was to investigate if protracted living in potentially or ascertained degraded environments – primarily consequent to mixed waste mishandling and/or illegal dumping, with concurrent release of dangerous persistent chemicals – had an impact on exposure of local people. For the study, highly toxic persistent contaminants were measured in blood, serum, and human milk pooled samples obtained by collecting a large number of individual specimens from local donors. The municipalities of interest were located in risk areas A, B, and C (with presumed descending risk levels), the latter virtually acting as control area: however, at least based on raw data the differences between areas were in general not clear-cut and quite chemical-dependent, this partly compromising a default control role of risk area C pools.

In general, biomarkers' concentrations were found to be compatible with their current values as deducible from European scientific literature and other domestic investigations: the overall outcome of this study is indeed consistent with an exposure primarily determined by a dietary intake derived from the large commercial food distribution system. However, as each municipality was represented by six blood or serum pools, the occurrence of four or more pools of the same municipality in the upper quartile of a pollutant's data distribution was viewed as a potential signal of a relative local overexposure to that specific risk factor, possibly deserving attention for future health-oriented actions (De Felip and di Domenico, 2010). The municipalities distinguished by such feature are Qualiano–Villaricca (As and Hg, five pools), Bruscianno and Caivano (As, four pools), Giugliano (Hg, four pools), and Pianura (PCDDs + PCDFs, four pools).

Long-term exposures helped for a better characterization of the database: for instance, the 45-year-long observation period utilized for the study would suggest that measures to reduce human exposure to PCDDs, PCDFs, and PCBs were more effective for PCDDs and PCDFs than for PCBs. In other words, as to the current human exposure to contamination source(s) in the areas of interest, PCBs seem to deserve specific attention.

In general, age could be an important accumulation factor but its role was substantially chemical-specific.

The CE and NA provinces were probed by including many municipalities: according to a qualitative analysis (PCFA), biomarkers' composition in blood or serum samples was found to be sensitive to municipality, not

to province. PCDDs, PCDFs, and DL-PCBs were sample-discriminative, whereas NDL-PCBs were the least effective possibly for the limited number of congeners assayed. The municipalities most frequently marked by the organic contaminants essentially belong to CE province rather than to NA province, a fact suggesting that the sources of the aforesaid contaminants are diffused, at least in the CE area. On the contrary, metals show a more defined prioritization as Bruscianno, Caivano, Giugliano, Mugnano, and Qualiano–Villaricca of NA province are municipalities clearly characterized by the presence of As, Hg, although not effective in differentiating municipalities, can result of concern due to a possible diffuse environmental presence again in NA province. As said, NA province is a typical volcanic territory, where not uncommon geological formations containing As may be a source of the metal to water supplies used for drinking water; on the other hand, Hg may have an anthropogenic origin. PCFA also supplied hints of possibly different exposure sources at municipality level, which may be of interest to explore in depth. At the same time, unexpected results were also produced: for instance, municipalities belonging to different provinces and relatively far apart – e.g. Castel Volturno, Giugliano, and Nola; Castel Volturno and Caivano – had somewhat more characterization traits in common (PCDD + PCDF and DL-PCB congeners, respectively) than other municipalities of a same province and near to one another. Therefore, the analysis of biomarker distribution can potentially drive the direction for future local risk assessment and/or management actions.

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Conflict of interest statement

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Appendix A. Supplementary data

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