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ENVIRONMENTAL QUALITY AND FOOD SAFETY: BIOINDICATORS OF PERSISTENT ORGANIC POLLUTANTS EXPOSURE

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Introduction
Food of animal origin represents the main source of exposure for the general population to persistent organic pollutants (POPs). The relationship between environmental quality and POPs bioaccumulation and bioconcentration is consolidated, as well as the use of animal species as bioindicators of contaminant exposure[1]. In 2009-2010, a pilot study was conducted in Italy to evaluate sheep/goat and honeybees as potential bioindicators of environmental contamination. Dioxins and furans (PCDDs/PCDFs), dioxin-like and non dioxin-like polychlorinated biphenyls (DL-PCBs/NDL-PCBs), polybrominated diphenyl ethers (PBDEs), perfluoroalkyl acids (PFAAs), pentachlorobenzene (PeCB), hexachlorobenzene (HCB) and pentachlorophenol (PCP) were selected as POPs to be monitored in sheep/goat milk and honey. The samples were collected from areas affected by the presence of POP emission sources, and from control areas, to highlight differences in the levels of contamination.

Materials and methods

Sampling plan
In order to provide representative bioindicators, milk from sedentary sheep or goat flocks and wildflower honey were sampled. Indeed, sedentary animals better reflect the environmental quality, while wildflower honeys are produced in a longer period in comparison to monofloral ones. The samples were taken: a) nearby pollutants emission sources or contaminated areas already identified, marked as exposed (E) zones; b) control sites in remote areas, such as national or regional natural parks, marked as non-exposed (NE) zones. Samples were collected from 16 Italian regions: a) for milk, 15 samples were from E-zones, 13 from NE-zones; b) for honey, 15 sample were from E-zones, 12 from NE-zones (Table 1).

Analytical methods
For PBDEs, 9 congeners (BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183) were analyzed using ^{13}C labelled internal standards. Milk samples were extracted according to AOAC 905.02, then subjected to acidic treatment and purification on a multi-layer silica column (silica gel and acidic silica). Honey samples were dissolved in water and extracted with n-hexane, while the clean-up procedure was the same as for milk. Instrumental analysis was performed by isotope dilution gas-chromatography – high resolution mass spectrometry (GC-HRMS) at resolution > 10,000 using a DB-5 MS J&W (60 m x 0.25 mm x 0.10 μm) column. The sum of nine PBDE congeners was calculated as upper-bound (UB) concentration.
For PCDDs/PCDFs and DL-PCBs/NDL-PCBs, methods based on EPA 1613 rev. B and EPA 1668 rev. B were used, respectively. Milk samples were lyophilized and extracted with toluene by accelerated solvent extraction (ASE). Honey samples were dissolved in water and extracted with dichloromethane. In both cases, the extract was first purified on Extrelut® column acidified with sulphuric acid, and then by Power-Prep® using multi-layer silica gel, alumina and carbon columns. The PCDDs/PCDFs were separated from DL-PCBs/NDL-PCBs. Instrumental analysis was performed by GC-HRMS at resolution > 10,000 using a DB-5 MS J&W (60 m x 0.25 mm x 0.25 μm) column. PCDDs/PCDFs and DL-PCBs were expressed as UB concentration using WHO_{2005}-TEF scale. NDL-PCBs analytical sum was also calculated as UB value.

For PCP, two different analytical procedures were applied to milk and honey samples, both using ^{13}C labelled PCP as internal standard. Milk samples were extracted following the same procedure used for PCDDs/PCDFs analysis, then purified by solid-phase extraction (SPE) on a silica-gel cartridge. The final solution was analyzed by liquid-chromatography – tandem mass spectrometry (LC-MS/MS) using an XTerra C18 column (150 x 2.1 mm, 3 μm) as stationary phase, and a water/acetonitrile solution containing formic acid as mobile phase. Honey samples were extracted with water/acetonitrile, then adding magnesium sulphate, sodium chloride, and buffering salts according to the QuEChERS procedure. PCP was determined by gas chromatography – ion trap mass spectrometric detection (GC – ITD) after acetylation, using a RTX-Sil MS (30 m x 0.25 mm x 0.25 μm) column.

PFOS/PFOA were extracted with acetonitrile, previously adding their respective ^{13}C labelled internal standards and dissolving honey in water. The extract was purified onto an Oasis WAX 150 mg cartridge. The instrumental analysis was performed by LC-MS/MS using a Symmetry C18 (150 mm x 2.1 mm, 3 μm) column and water/methanol (both containing 0.01% formic acid) as mobile phase.

PeCB/HCB in milk samples were analyzed by the same procedure used for PBDEs, previously adding a ^{13}C labelled HCB internal standard. Honey samples were dissolved in water and extracted with n-hexane, then purified on an Extrelut® column impregnated with sulphuric acid. The analytical determination was accomplished by GC-HRMS using a HT8-PCB (60 m x 0.25 mm x 0.25 μm) column.

Results and discussion

Milk samples
The results are summarised in Table 2. The Mann-Whitney U non-parametric test for independent groups was used to compare the results for E/NE-zones. The statistical evaluation was not performed for PCP and PFOA due to the high frequency of results below the limit of quantification.
Statistically significant differences (p = 0.05) between zones were found:
• for PCDDs/PCDFs (max 1.88, Lazio E-zone - min 0.04, Tuscany NE-zone, pg WHO$_{2005}$-TEQ/g fat);
• for DL-PCBs (max 3.33, Lombardy E-zone – min 0.06 Lazio NE-zone, pg WHO$_{2005}$-TEQ/g fat).
Four samples from E-zones were above the PCDDs/PCDFs and DL-PCBs action levels designed to prompt competent authorities and operators to identify a source of contamination and to take measures for its reduction or elimination. In particular, the action level was exceeded for PCDDs/PCDFs in Colleferro (Lazio), and for DL-PCBs in Val Trompia (Lombardy), Milazzo (Sicily) and Crotone (Calabria). These sites are characterized by the presence of industrial plants, some of them have been also classified as contaminated sites of national interest (Table 1).
PBDE sum was in the range 146-920 pg/g fat with predominant congeners in the order 47 > 99 > 100 > 154 reflecting past use of technical mixtures constituted of penta- and hexa-bromodiphenylethers as flame retardants[2]. Regarding PFAAs, the maximum values for PFOS (0.26 ng/g, Sardinia E-zone) and PFOA (0.02 ng/g, Umbria NE-zone) were below the mean values reported in the literature[3].
For HCB, the obtained values were in the range 1.69 – 9.27 ng/g fat (EU maximum level 0.01 mg/kg fat), while for PeCB concentrations between 0.05 and 0.57 ng/g fat were recorded were recorded. All samples were negative for PCP (< 5 ng/g).

Honey samples
The Mann-Whitney U test could not be performed for honey samples because most of results were below the LOQ values. This matrix seemed not suitable for POP levels monitoring, nevertheless useful data were obtained for heavy metals and trace elements as a part of the study not discussed here. In conclusion, honey contamination appears mainly attributable to beekeeping practices (e.g. acaricides and antibiotics use) rather than environmental contaminants, excluding heavy metals and trace elements.

Acknowledgements

References
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Table 1. Sampling sites for sheep/goat milk and honey

Region	Contaminated area (milk – honey)	Control area (milk – honey)
Sardinia	Fiume Santo (SS)[1] – Sulcis Iglesiente Guspinese (CI)[2]	Sassari – Lula (NU)
Sicily	Milazzo (ME)[2]	Nebrodi Regional Park (ME)
Tuscany*	Le Strillaie (GR)[2]	Grosseto
Marche	Basso bacino del fiume Chienti (MC)[2]	Gola della Rossa Regional Park AN)
Lazio	Colleferro (RM)[3] – Segni (RM)[6]	Monti Simbruini Regional Park (RM)
Abruzzo	Bussi Popoli(PE)[2]	Gran Sasso Monti della Laga National Park (TE)
Campania	Valle del Sarno (SA)[2]	Cilento National Park (SA)
Trentino	Valsugana (TN)[4]	---
Molise	Campobasso Guglionesi II (CB)[2]	Abruzzo National Park (IS)
Umbria	Terni Papigno (TR)[2]	Monti Sibillini National Park (PG)
Basilicata	Val Basento (MT)[2]	Pollino National Park (MT)
Lombardy	Val Trompia (BS)[4]	---
Apulia	Taranto[2]	Gargano National Park (FG)
Emilia- Romagna	Formigine (MO)[5]	Appennino Tosco Emiliano National Park (BO)
Calabria	Crotone[2]	Sila National Park (CS)
Piedmont**	Carisio (VC)[7]	---

*Only milk samples taken; **Only honey samples taken
[1]Thermal power plant; [2]Contaminated site of national interest; [3]Metal industries, organochlorine pesticides production, cement kilns, waste incinerators; [4]Steel plants; [5]Ceramic factories; [6]Cement kiln; [7]Aluminum smelter.

Table 2. Analytical results for sheep/goat milk samples

Region	Group	PBDEs[a] pg/g fat	PCDDs/PCDFs pg WHO$_{2005}$-TEQ/g fat	DL-PCBs pg WHO$_{2005}$-TEQ/g fat	NDL-PCBs ng/g fat	PeCB ng/g fat	HCB ng/g fat	PFOS ng/g	PFOA ng/g
Sardinia	E-zone	257	0.17	0.49	2.4	0.379	3.929	0.2557	<0.0090
	NE-zone	419	0.09	0.59	2.4	0.134	2.620	0.0040	<0.0092
Sicily	E-zone	511	0.72	2.91	6.4	0.166	2.801	0.0355	ND[b]
	NE-zone	363	0.29	0.28	2.4	<0.086	2.492	0.0037	ND[b]
Tuscany	E-zone	442	0.61	0.63	2.4	0.354	3.903	0.0033	<0.0080
	NE-zone	292	0.04	0.28	2.4	<0.082	3.255	0.0026	<0.0077
Marche	E-zone	276	0.35	0.76	4.1	0.267	4.983	0.1365	<0.0086
	NE-zone	321	0.69	0.67	3.5	0.084	3.836	0.0018	<0.0082
Lazio	E-zone	379	1.88	1.30	2.7	0.177	5.104	0.0041	<0.0082
	NE-zone	376	0.20	0.06	2.4	0.286	4.150	0.0045	<0.0079
Abruzzo	E-zone	146	0.11	0.66	2.6	0.193	3.388	0.0054	<0.0085
	NE-zone	335	0.10	0.68	4.4	0.195	4.896	0.0148	<0.0087
Campania	E-zone	432	0.71	1.31	2.4	0.143	3.548	0.0078	<0.0085
	NE-zone	351	0.31	0.95	5.3	0.242	2.291	0.0049	<0.0084
Trentino	E-zone	518	0.51	1.71	4.6	0.538	9.270	0.0160	<0.0087
Molise	E-zone	567	0.11	0.80	2.8	<0.235	2.501	0.0053	<0.0085
	NE-zone	298	0.11	0.72	2.7	<0.113	1.686	0.0054	<0.0084
Umbria	E-zone	244	0.73	1.63	5.0	0.145	3.057	0.0034	<0.0084
	NE-zone	320	0.11	0.67	3.0	0.444	4.600	0.0039	0.0188
Basilicata	E-zone	423	1.17	0.59	3.7	0.066	2.952	0.0033	<0.0090
	NE-zone	274	0.19	0.34	3.5	<0.069	3.224	<0.0056	ND[b]
Lombardy	E-zone	920	0.30	3.33	13.3	0.239	3.098	0.0121	<0.0081
Apulia	E-zone	277	0.53	1.74	9.1	0.570	5.008	0.0137	<0.0083
	NE-zone	398	0.28	0.54	2.8	0.080	1.809	0.0124	<0.0083
Emilia-Romagna	E-zone	645	0.75	0.94	3.5	<0.201	1.926	<0.0057	ND[b]
	NE-zone	488	0.09	0.67	3.1	0.059	2.374	0.0388	<0.0085
Calabria	E-zone	457	0.10	2.64	10.3	0.046	3.765	0.0056	<0.0101
	NE-zone	327	0.12	1.91	5.4	<0.170	3.687	0.0028	<0.0096

[a] Sum of BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183
[b] Not determined due to the signal from procedural blanks