PAPER

TREND OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS (PCDD/PCDFS) IN BEEHIVE MATRICES

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ABSTRACT

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDFs) are well-known persistent organic pollutants (POPs) with highly toxic potential. These compounds are released in the environment as a complex mixture of various congeners which shown significant physico-chemical differences, as well as different environmental fates. PCDD/PCDF mixtures change spatially and temporally in the environment and biota, complicating the risk assessment and regulatory control for human and animal exposure. Considering the well-known role of honeybees as bioindicators for pesticides, heavy metals and other chemicals, the present study has been developed to assess the use of honeybees and honeybee products in biomonitoring projects about PCDD/PCDFs. Three Dadant-Blatt type beehives, located since March 2017 in the headquarter of Ducati Motor Holding S.p.A. (Borgo Panigale, Bologna, Italy) have been used as monitoring stations. Honeybees, honey and beeswax have been sampled and analyzed for PCDD/PCDFs detection in June and in September of the same year. Among the analyzed

matrices, beeswax has shown the highest WHO-TEQ values, probably due to its lipidic nature capable of accumulating fat-soluble, non-volatile, persistent organic pollutants. Hexaclorodibenzo-p-dioxin (HxCDD), usually measured in vegetables and fruits, has been detected only in honey samples. Maximum levels of PCDD/PCDFs are settled by Commission Regulation (EC) No 1259/2011 of 2 December 2011, but only on animal-derived products. Considering the role of dietary-model adopted by the consumers on toxic substances dietary intake and associated exposure risks, limits on botanical derived products are needed. But more controls about bee-products are advisable also in order to reduce the exposure risk for bees and for protecting biodiversity.

Keywords: POPs, PCDD/PCDFs, honeybees, bio-indicators, environment, health

1. INTRODUCTION

Various attributes make the honeybee (*Apis mellifera*) the "ideal bioindicator" (TONG *et al*., 1975; STÖCKER, 1980; WALLWORK-BARBER, 1982; RAES *et al*., 1992; LEITA *et al*., 1996; ZHELYAZKOVA, 2012).
Due to the intense forager activity and the high sensitivity of bees toward toxic substances, the hives can give informations about environmental pollution via health-status and high mortality of bees or via the residues detection in honey, pollen, propolis, beeswax, royal-jelly, larvae and bees (CONTI and BOTRÈ, 2001; CRANE, 1984; BOGDANOV, 2006; CHAUZAT *et al*., 2011; PERUGINI *et al*., 2017).
As far as the biomonitoring of environmental pollution is concerned, honeybees have been used in a lot of investigations to evaluate different type of contaminants (KIRKHAM and COREY, 1977; BROMENSHENK *et al*., 1985; TONELLI *et al*., 1990; FRANCO *et al*., 1997; FRANCO *et al*., 1998; CELLI and MACCAGNANI, 2003; BALAYIANNIS and BALAYIANNIS, 2008; PORRINI *et al*., 2014). However, only few studies have tried to evaluate the possible application of honeybees as bioindicators for dioxin and furan detection (PORRINI *et al*., 2014; ÖZKÖK *et al*., 2018). Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDFs) are well-known persistent organic pollutants (POPs), with highly toxic potential (SEMANAINEN *et al*., 2002; Birnbaum *et al*., 2003). These compounds are released in the environment as a complex mixture of various congeners, produced primarily as by-products of chemical manufacturing activities and during the combustion of municipal and chemical waste (HUTZINGER *et al*., 1985; MENESES, 2004). Atmospheric transport and deposition processes lead to the dispersion of these compounds into soils, plant surfaces, bodies of water and sediments (VAN DEN BERG *et al*., 1994; LOHMANN and JONES, 1998). Due to the significant differences in physico-chemical properties (solubilities, volatilities, rates of degradation/metabolism, exc.) of each congener, the complex mixtures of PCDD/PCDFs change spatially and temporally in the environment and in animal tissues (SCHRENK *et al*., 1991; WEGIEL *et al*., 2018; ZHENG *et al*., 2008). Due to their lipophilic properties, PCDD/PCDFs may concentrate in fatty tissues and bioaccumulate through the food chain (TRAAG *et al*., 2006).
Considering the well-known role of honeybees as bioindicators, the present study has been developed to evaluate the distribution of PCDD/PCDFs in the hive. Due to their wide use, honey and beeswax contamination could also represent an important safety concern. Nevertheless, this investigation has the main purpose of improving data about the possible application of honeybees as bioindicators for monitoring environmental pollution and human exposure risks.

2. MATERIALS AND METHODS

2.1. Beehives location

Monitoring station in the headquarter of Ducati Motor Holding S.p.A. (Borgo Panigale, Bologna, Italy), was placed in an important Italian industrial area, at less than 7 Km from Bologna Central Station. Bologna city represents one of the most populated cities in Italy (2783 p/Km^2) and its province extends for about 3702 Km^2 representing in Italy, the most productive industrial area for metalworking and engine sector. The monitoring station, consisting of three beehives (BH1, BH2, BH3), Dadant-Blatt type with 12 frames have been

located in the headquarter of Ducati Motor Holding S.p.A. (Borgo Panigale, Bologna, Italy) at the end of March 2017. The hives, homogeneous in colony strength (colonies bring up on 10 frames) were placed into a wooden gazebo open frontally and laterally, with roof on top, at the distance of 40 cm between them. As suggested by PORRINI *et al*, (2014), all the beehives have been provided with cages for dead bees sampling (under-basket type) and were periodically monitored.

2.2. Sampling

Honeybees, honey and beeswax were sampled from each beehive in June and in September 2017.
Bees have been collected alive in airtight container directly from the combs, taking care to not involve the queen. Honey and wax have been collected as two honeycomb centrifuged for honey extraction.
Stored at −20°C for 24 hours, each sample (50g) was homogenized with liquid nitrogen by a crushing mill (IKA, Wilmington, NC), then analyzed for PCDD/PCDFs detection. The analyzed congeners have been reported in Table 1.

2.3. Chemical analysis

Determination of PCDD/Fs were performed following analytical methods based on international norms for dioxin analysis, such as EPA 1613 (EPA, 1994), and following the requirements of European Directives related to this subject.
Extraction of the fat fraction, including the compounds of interest, was performed in Soxhlet apparatus with solvents (hexane/dichlormethane or hexane/diethyl ether). Beeswax samples were directly dissolved in 20 ml of hexane. Sample extracts were purified in a 4 cm diameter multilayer column, containing (top to bottom) Na2SO4 , 44% H2SO4/silica, 22 %H2SO4/silica, NaOH/silica and AgNO3/silica. PCDD/Fs were eluted with hexane. The purified extracts were fractionated in SPE pre-packed carbon tubes (Supelclean Envi-Carb), from SUPELCO (Bellefonte, PA, USA). The obtained PCDD/F fractions were evaporated to 15 μl under nitrogen stream and corresponding PCDD/F 13C syringe standards (1,2,3,4-TeCDD and 1,2,3,7,8,9-HxCDD) were added.
Samples were analysed in a 6890N gas chromatograph (Agilent, Santa Clara, CA, USA), coupled to an Autospec Ultima high resolution mass spectrometer (Micromass, Manchester, UK), operating in electronic impact ionization mode and at 10,000 resolving power. For the PCDD/F analysis, samples were injected (2 μl) on splitless mode (1 min) into the injector at 280 ºC. The chromatograph was fitted with a RTX-5MS column (60 m x 0.25 mm i.d., 0.25 μm) from Restek (Bellefonte, PA, USA). Carrier gas was helium at 250 kPa constant pressure mode. The temperature program was 150 ºC (held for 1 min), increased at 30 min-1 to 200 ºC, increased at 3 ºC min-1 to 235 ºC (held for 10 min) and increased at 6 min-1 to 300 ºC (held 17 min). Monitored masses were those proposed by EPA 1613 method (EPA, 1994). Samples were quantified according to the isotopic dilution method, with the use of 13C12-labelled PCDD/F as internal standards. Among 200 PCDDs and 70 PCDFs, 17 congeners considered dangerous from a toxicological point of view (Council Regulation (EU) 1259/2011), have been evaluated for the present investigation (Table 1).

Table 1. LOQ, LOD and WHO-TEFs (Van den Berg *et al*., 2006) of researched PCDD/PCDF congeners.

	LOQ (pg/g)	LOD (pg/g)	WHO 2005 TEFs
2, 3, 7, 8 - Tetrachlorodibenzo-p-dioxin (TCDD)	0.04	0.02	1
1, 2, 3, 7, 8 - Pentachlorodibenzo-p-dioxin (PeCDD)	0.05	0.025	1
1, 2, 3, 4, 7, 8 - Hexachlorodibenzo-p-dioxin (HxCDD)	0.10	0.05	0.1
1, 2, 3, 6, 7, 8 - Hexachlorodibenzo-p-dioxin (HxCDD)	0.10	0.05	0.1
1, 2, 3, 7, 8, 9 - Hexachlorodibenzo-p-dioxin (HxCDD)	0.10	0.05	0.1
1, 2, 3, 4, 6, 7, 8 - Heptachlorodibenzo-p-dioxin (HpCDD)	0.25	0.13	0.01
1, 2, 3, 4, 6, 7, 8, 9 - Octachlorodibenzo-p-dioxin (OCDD)	0.50	0.25	0.0003
2, 3, 7, 8 - Tetrachlorodibenzofuran (TCDF)	0.04	0.02	0.1
1, 2, 3, 7, 8 - Pentachlorodibenzofuran (PeCDF)	0.05	0.025	0.03
2, 3, 4, 7, 8 - Pentachlorodibenzofuran (PeCDF)	0.05	0.025	0.3
1, 2, 3, 4, 7, 8 - Hexachlorodibenzofuran (HxCDF)	0.10	0.05	0.1
1, 2, 3, 6, 7, 8 - Hexachlorodibenzofuran (HxCDF)	0.10	0.05	0.1
2, 3, 4, 6, 7, 8 - Hexachlorodibenzofuran (HxCDF)	0.10	0.05	0.1
1, 2, 3, 7, 8, 9 - Hexachlorodibenzofuran (HxCDF)	0.10	0.05	0.1
1, 2, 3, 4, 6, 7, 8 - Heptachlorodibenzofuran (HpCDF)	0.25	0.13	0.01
1, 2, 3, 4, 7, 8, 9 - Heptachlorodibenzofuran (HpCDF)	0.25	0.13	0.01
1, 2, 3, 4, 6, 7, 8, 9 - Octachlorodibenzofuran (OCDF)	0.50	0.25	0.0003

2.4. WHO-TEF and WHO-TEQ

The concepts of toxic equivalency factor (TEF) and total toxic equivalent (TEQ) have been developed and introduced by the World Health Organization (WHO) to facilitate risk assessment and regulatory control of exposure to PCDD/PCDFs mixtures. The WHO-TEF estimates the toxic potential of each congener comparing its affinity for a cytosolic receptor protein (aryl hydrocarbon receptor – AhR) with the highest affinity associated to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Table 1).
The WHO-TEQ is operationally defined by the sum of each compound concentration multiplied by its TEF value and represents an evaluation of the total 2,3,7,8-TCDD–like activity of the PCDD/PCDFs mixture, as well as of their total potential toxicity (VAN DEN BERG *et al*., 1998, VAN DEN BERG *et al*., 2006; VAN DEN BERG *et al*., 2013).
Two different methods can be used for WHO-TEQ evaluation. Usually, it is calculated as *lower-bound* for environmental matrices, considering the undetectable concentrations equal to zero. Instead, for high-lipid-content food products it is calculated with the upper-bound method, considering the undetectable concentrations equal to the detection limit of each congener (LOD) (Commission Regulation (EU) No 589/2014). For the present study the WHO-TEQs were calculated on honeybees, honey and beeswax PCDD/PCDFs concentrations with both lower-bound and upper-bound methods (Table 2).

3. RESULTS AND DISCUSSION

Ingestion of contaminated food is the principal way of human exposure to PCDD/PCDFs, accounting for 90% if compared to other ways such as inhalation and dermal contact (SWEETMAN *et al*., 2000). This concern about the human health impact and continuous

encouragement from scientific committees to monitor food samples across Europe, have led to numerous international and local studies on the concentration of dioxins in particular food items or on the estimation of the daily intake from food (European Commission, Brusseles, June 2002; FOCANT *et al*. 2002; KARL *et al*., 2002). Seafood represents the most contaminated foodstuff and the congeners most frequently detected in all type of analyzed foodstuff were OCDD and HpCDD, as well as PeCDD. Regarding dietary intake evaluation on human health it was carry out combining data on consumption habits with the different concentrations of PCDD/PCDFs expressed in WHO-TEQ found in food samples (BORDAJANDI *et al*., 2004).

Honey and other bee-products are included in nutritional habits of a lot of country and currently are widely used also as dietary supplements for health purposes. However these product were never been taking into account for human dietary exposure calculation and then never analysed for PCDD/PCDFs evaluation. This study has been performed for improving this lack o data profile on food PCDD/PCDFs concentration and to evaluate a possible application of honeybee as bio-indicators for PCDD/PCDFs monitoring in the environment. Results are showed in Tables 2 and 3.

Octachlorodibenzo-p-dioxin (OCDD), as well as being reported in other studies regarding PCDD/PCDFs, is the congener most frequently detected during the present investigation (BORDAJANDI *et al*., 2004; DOMINGO *et al*., 1999). It has been quantified in all the analysed matrices (Figure 1 and Figure 2). Aside for one honeybee sample collected in September (BH1), that reported 0.07 pg/g of 2, 3, 7, 8 – tetraclorodibenzofuran (TCDF), OCDD was the only congener detected in honeybee samples. This trend is confirmed also for honey samples. TCDF has been quantified only in one sample of honey collected in September from BH1 (0.07 pg/g), while interesting concentrations of OCDD have been detected in all honey samples collected in June and in September (Figs. 1 and 2).

Detectable concentrations of 1, 2, 3, 4, 7, 8 - hexaclorodibenzo-p-dioxin and 1, 2, 3, 6, 7, 8 - hexaclorodibenzo-p-dioxin (HxCDD), usually measured in vegetables and fruits (DOMINGO *et al*., 1999), have been detected, in June and in September, only in honey samples (Figs. 1 and 2). Honey is a natural product that honeybees make from blossom's nectar or from secretions coming from living parts of plants (ÖZKÖK *et al*., 2017), but ÖZKÖK *et al*. (2018) monitoring PCDD/PCDFs in honeybee pollen (honey component) had encountered different results. 2, 3, 7, 8 – TCDF, 1, 2, 3, 7, 8 – PeCDD, 2, 3, 4, 7, 8 – PeCDF, showed the higher concentrations with both analytical methods employed for the study. However, mentioned studies confirm with present data, that maximum levels should be established also for cereals, vegetables and bee-products, in which not negligible concentrations have been reported (ÖZKÖK *et al*., 2018). Moreover, vegetables represent the most frequent consumed food for a healthy diet and dietary-model adopted by the consumers should be considered important for assessing daily pollutants intake for humans (DOMINGO *et al*., 1999; SCHECTER *et al*., 2006).

The most toxic PCDD/PCDF congeners are 2,3,7,8-substituted tetra-, penta-, and hexachloro compounds that, in addition to OCDD, have the greatest tendency to bioaccumulate (COHEN *et al*., 2002; BOCIO and DOMINGO, 2005). Nevertheless, the highest concentrations registered during the present investigation and associated to heptaclorodibenzo-p-dioxin (HpCDD) and octachlorodibenzo-p-dioxin (OCDD), have been detected in beeswax samples collected in June (Fig. 1).

Table 2. Concentrations detected in June 2017.

Analyzed congeners	Bees					Honey					WAX				
	BH1* (pg/g)	BH2* (pg/g)	BH3* (pg/g)	Average* (pg/g)	SD	BH1* (pg/g)	BH2* (pg/g)	BH3* (pg/g)	Average* (pg/g)	SD	BH1* (pg/g)	BH2* (pg/g)	BH3* (pg/g)	Average* (pg/g)	SD
2,3,7,8 - Tetraclorodibenzo-p-diossina (TCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,7,8 - Pentaclorodibenzo-p-diossina (PeCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,4,7,8 - Esaclorodibenzo-p-diossina (ExCDD)	<LOD	<LOD	<LOD	ND	ND	0,11	0,11	0,15	0,12	0,02	<LOD	<LOD	<LOD	ND	ND
1,2,3,6,7,8 - Esaclorodibenzo-p-diossina (ExCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	0,12	0,12	0,08	0,07	<LOD	<LOD	<LOD	ND	ND
1,2,3,7,8,9 - Esaclorodibenzo-p-diossina (ExCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,4,6,7,8 - Eptaclorodibenzo-p-diossina (EpCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	2,01	1,39	1,73	1,71	0,31
Octaclorodibenzo-p-diossina (OCDD)	0,52	0,56	<LOD	0,36	0,31	0,54	0,52	0,51	0,52	0,02	12,05	9,08	9,97	10,37	1,52
2,3,7,8 - Tetraclorodibenzofurano (TCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,09	0,07	0,08	0,08	0,01
1,2,3,7,8 - Pentaclorodibenzofurano (PeCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,07	0,07	0,08	0,07	0,01
2,3,4,7,8 - Pentaclorodibenzofurano (PeCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,4,7,8 - Esaclorodibenzofurano (ExCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND

1,2,3,6,7,8 - Esaclorodibenzo furano (ExCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
2,3,4,6,7,8 - Esaclorodibenzo furano (ExCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,7,8,9 - Esaclorodibenzo furano (ExCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,4,6,7,8 - Eptaclorodibenzo furano (EpCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,41	1,20	0,86	0,82	0,40
1,2,3,4,7,8,9 - Eptaclorodibenzo furano (EpCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,25	<LOD	<LOD	0,08	ND
Octaclorodibenzofu rano (OCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,84	0,66	0,88	0,79	0,12

Table 3. Concentrations detected in September 2017.

	Bees					Honey					WAX				
Analyzed congeners	**BH1* (pg/g)**	**BH2* (pg/g)**	**BH3* (pg/g)**	**Average* (pg/g)**	**SD**	**BH1* (pg/g)**	**BH2* (pg/g)**	**BH3* (pg/g)**	**Average* (pg/g)**	**SD**	**BH1* (pg/g)**	**BH2* (pg/g)**	**BH3* (pg/g)**	**Average* (pg/g)**	**SD**
2,3,7,8 - Tetraclorodibenzo-p-diossina (TCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,7,8 - Pentaclorodibenzo-p-diossina (PeCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,4,7,8 – Esaclorodibenzo-p-diossina (ExCDD)	<LOD	<LOD	<LOD	ND	ND	0,11	0,16	0,15	0,14	0,03	<LOD	<LOD	<LOD	ND	ND
1,2,3,6,7,8 – Esaclorodibenzo-p-diossina (ExCDD)	<LOD	<LOD	<LOD	ND	ND	0,11	0,18	0,12	0,14	0,04	<LOD	<LOD	<LOD	ND	ND
1,2,3,7,8,9 - Esaclorodibenzo-p-diossina (ExCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,4,6,7,8 - Eptaclorodibenzo-p-diossina (EpCDD)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,58	1,36	1,36	1,10	0,45
Octaclorodibenzo-p-diossina (OCDD)	0,77	0,90	0,79	0,82	0,07	0,52	0,59	0,52	0,54	0,04	5,68	8,42	10,04	8,05	2,20
2,3,7,8 - Tetraclorodibenzofurano (TCDF)	<LOD	<LOD	0,07	0,07	0,00	<LOD	<LOD	0,07	0,07	0,00	0,14	0,15	0,16	0,15	0,01

1,2,3,7,8 - Pentaclorodibenzofurano (PeCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,05	0,08	0,05	0,06	0,02
2,3,4,7,8 - Pentaclorodibenzofurano (PeCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,09	0,08	0,05	0,07	0,02
1,2,3,4,7,8 - Esaclorodibenzofurano (ExCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	0,12	0,10	0,10	0,11	0,01
1,2,3,6,7,8 - Esaclorodibenzofurano (ExCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
2,3,4,6,7,8 - Esaclorodibenzofurano (ExCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,7,8,9 - Esaclorodibenzofurano (ExCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,4,6,7,8 - Eptaclorodibenzofurano (EpCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
1,2,3,4,7,8,9 - Eptaclorodibenzofurano (EpCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND
Octaclorodibenzofurano (OCDF)	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	<LOD	ND	ND	<LOD	<LOD	1,31	1,31	0,00

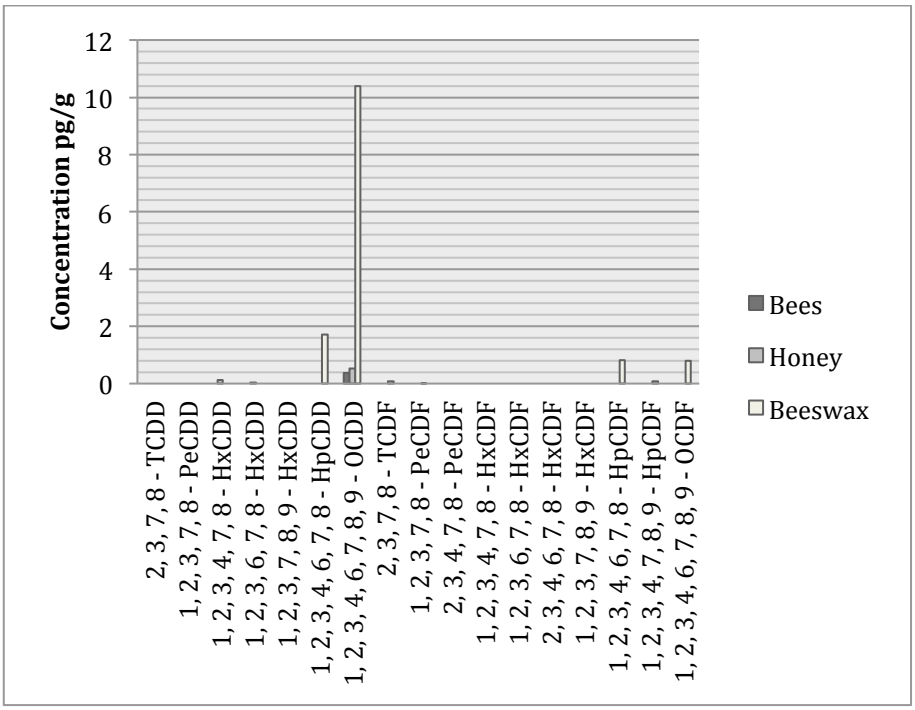

Figure 1. Average concentrations of PCDD/PCDFs detected in hive matrices in June 2017.

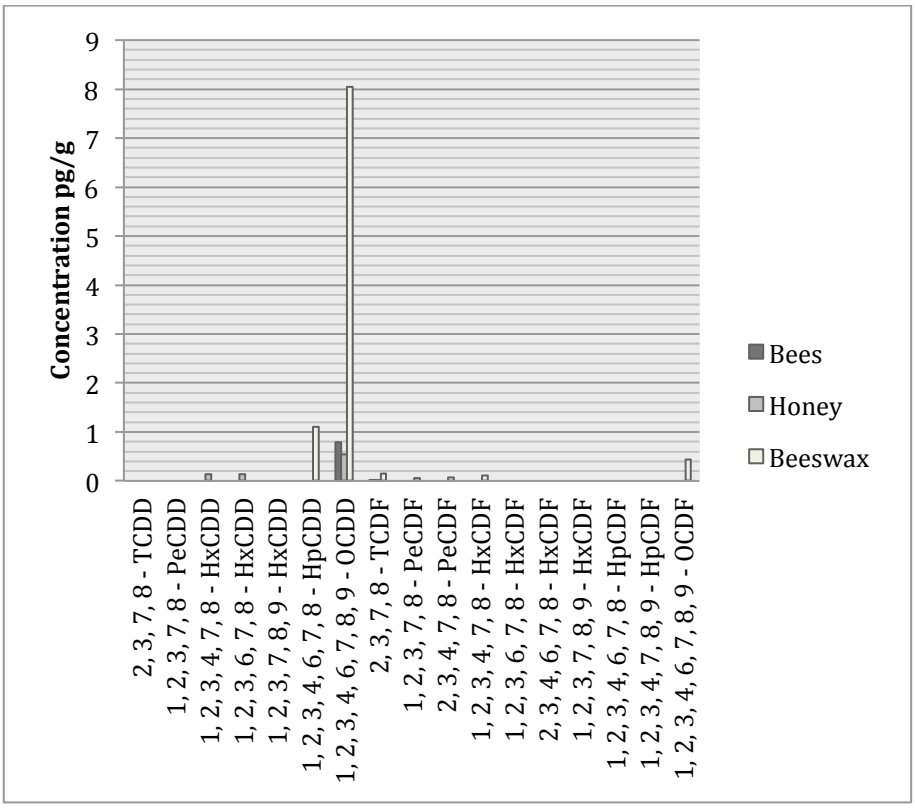

Figure 2. Average concentrations of PCDD/PCDFs detected in hive matrices in September 2017.

Among the analysed matrices, beeswax has shown the highest number of detectable congeners. HpCDD, OCDD, TCDF, pentachlorodibenzofuran (PeCDF), heptachlorodibenzofuran (HpCDF) and octachlorodibenzofuran (OCDF) have all been detected in beeswax (Figs. 1 and 2). A larger number of congeners have been quantified in beeswax samples collected in September as compared to those collected in June (Fig. 2) and indeed, the highest WHO-TEQ values calculated for the present investigation have been associated to them (Table 4).

Table 4. Average WHO-TEQ *lower-* and *upper-bound* values, calculated on the average PCDD/PCDF concentrations measured in honeybee, honey.

	June 2017			September 2017		
	Honeybees	**Honey**	**Beeswax**	**Honeybees**	**Honey**	**Beeswax**
WHO-TEQ *lower bound*	0.0001 pg/WHO-TEQ/g	0.0088 pg/WHO-TEQ/g	0.0382 pg/WHO-TEQ/g	0.0025 pg/WHO-TEQ/g	0.0164 pg/WHO-TEQ/g	0.0630 pg/WHO-TEQ/g
WHO-TEQ *upper bound*	0.1884 pg/WHO-TEQ/g	0.1913 pg/WHO-TEQ/g	0.2159 pg/WHO-TEQ/g	0.1894 pg/WHO-TEQ/g	0.1971 pg/WHO-TEQ/g	0.2181 pg/WHO-TEQ/g

Higher values of WHO-TEQ lower- and upper-bound have been registered in September then in June for all the analyzed matrices (Table 2). The highest amounts of WHO-TEQ lower- and upper-bound have been associated to the beeswax samples in June and in September (Table 2). Beeswax composition, consisting in a mixture of fatty acids, fatty alcohols, paraffinic hydrocarbons and free fatty acids, is capable of accumulating of fat soluble, non-volatile and persistent pollutants (TULLOCH, 1980; JOHNSON *et al*., 2010; SERRA-BONVEHÍ and ORANTES-BERMEJO, 2010; RAVOET *et al*., 2015; PERUGINI *et al*., 2017). However, the mechanisms of beeswax contamination have not been well investigated yet. Beeswax is made by young worker bees who have never been out of the hive and its contamination could be the result of chemicals transmigration between different matrices, as well as the result of degradation/metabolism processes allowed by the bees consuming contaminated pollen and nectar. Currently, regarding PCDD/PCDFs, a possible bioaccumulation phenomenon in the hive cannot be excluded. Similarly to animal's fat-tissues, beeswax is the main reservoir for PCDD/PCDFs mixtures that change in the "hive tissues" during the exposure time according to specific degradation/metabolism processes.
Although further studies would be advisable, honeybees, honey and beeswax data suggest the possible use of them as indicators for PCDD/PCDFs distribution in the environment (bees), in vegetable foodstuffs (honey) and in animal fat-tissues (beeswax).

4. CONCLUSIONS

"Honeybees monitoring stations" have been confirmed as an effective and inexpensive method for controlling the levels of PCDD/PCDFs, as well as other pollutants, in the environment.
Nevertheless, honey and beeswax contamination also represents an important concern for beekeeping practices and for honeybee products consumer health.

Honey is an important food product in many countries and beeswax finds important applications in food, cosmetic and pharmaceutical industries, representing possible sources of exposure for humans (PERUGINI *et al*., 2017). Considering the beekeeping common practice to recycle not controlled beeswax for wax foundation sheets production, it can become a source of subsequent recirculation of pollutants in the hive, with serious risks for honeybee health and for biodiversity protection (MULLIN *et al*., 2010; WU *et al*., 2011; WU *et al*., 2012).
Based on WHO-TEF and WHO-TEQ concepts, the Commission Regulation (EC) No 1259/2011 of 2 December 2011 set PCDD/PCDFs maximum levels modifying those established by the Commission Regulation (EC) No 1881/2006 of 19 December 2006. These limits are settled mainly for animal-derived products and expressed as pg WHO-PCDD/F-TEQ/g, but currently, no maximum levels are applied to cereals, fruit and vegetables, or to honey and other honeybee products.
According to many studies this lack should be revised in order to guarantee risk assessment and regulatory control of exposure to PCDD/PCDFs mixtures, taking into account real nutritional habit in different countries. Cereals, vegetables and bee-products have long been considered with a "low-impact" for humans daily intake, but recent studies (ÖZKÖK *et al*., 2018) have demonstrated that interesting concentrations can also be found in honeybee pollen (honey component) as well in cereals and vegetables (BORDAJANDI *et al*., 2004; DOMINGO *et al*., 1999; FOCANT *et al*., 2002; KARL *et al*., 2002; SCHECTER *et al*., 2006).

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