Science of the Total Environment 867 (2023) 161462

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Biomonitoring of organic pollutants in pet dog plasma samples in North-Western Spain

Xiana González-Gómez [a], María Figueiredo-González [b], Roberto Villar-López [b], Elena Martínez-Carballo [b,*]

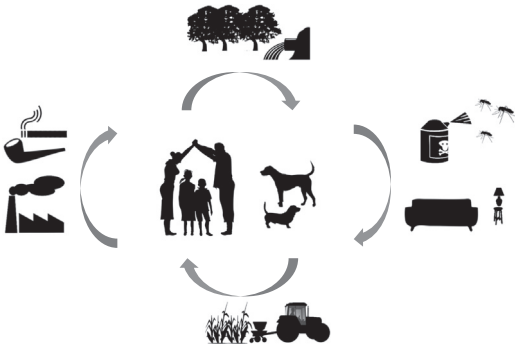
[a] Department of Analytical Chemistry, Nutrition and Food Sciences, IAQBUS - Institute of Research on Chemical and Biological Analysis, Universidade de Santiago de Compostela, Constantino Candeira S/N, Santiago de Compostela 15782, Spain

[b] Food and Health Omics, Department of Analytical and Food Chemistry, Faculty of Sciences, Campus da Auga, University of Vigo, Ourense 32004, Spain

HIGHLIGHTS

- Complementary study to assess OPs in pet dog samples is presented.
- Mean OPs detected in plasma samples were PAHs >PYRs >PCBs >OCPs >PBDEs >OPPs.
- Age, sex and size were the factors affecting OPs in selected biological samples.
- Correlation between both biological samples was found for pyrene and chrysene.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Katarzyna Kordas

Keywords:
Organic pollutants
Environmental health
Biomonitoring
Plasma
Pets

ABSTRACT

Most of organic pollutants (OPs) have the ability to interfere with biological systems causing negative effects in living beings, including humans. In the last decades, pets have been used as bioindicators of human exposure because they share the same habitat with their homeowners. We sought to determine levels of approximately 70 OPs, including polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated biphenyl ethers (PBDEs), organophosphate pesticides (OPPs), polycyclic aromatic hydrocarbons (PAHs) and pyrethroids (PYRs) in plasma samples from 39 pet dogs from Ourense (north-western Spain). The results revealed that PAHs were the dominant OPs (mean value 175 ± 319 ng/g lipid weight (lw)), followed by PYRs (132 ± 352 ng/g lw), PCBs (122 ± 96 ng/g lw), OCPs (33 ± 17 ng/g lw), PBDEs (19 ± 18 ng/g lw) and OPPs (2.1 ± 2.7 ng/g lw) in plasma samples. We have previously detected the target OPs in hair samples of pets, collected simultaneously and similar trend of some OPs has been observed. Moreover, pyrene and chrysene showed correlations between levels detected in both matrices.

1. Introduction

Fast worldwide technical development involves an important need to review links between environmental pollution and human health. OPs are potentially harmful substances for ecosystems and living beings. Many of them are known to be toxic, biodegradation-resistant, lipophilic, bioaccumulative and, even endocrine disruptors (Lau et al., 2017; Ruiz-Suárez et al., 2016; Takaguchi et al., 2019). They have been widely used as pesticides, medicines, paintings, additive flame retardants and other applications. The Stockholm Convention identified persistent OPs (POPs) to be banned of the production and use. Although the presence of POPs such as PCBs, OCPs and PBDEs have decreased in the environment during the last years, they continue to be an important public health issue due to

* Corresponding author.
E-mail addresses: xiana.gonzalez.gomez@usc.es (X. González-Gómez), mariafigueiredo@uvigo.es (M. Figueiredo-González), elena.martinez@uvigo.es (E. Martínez-Carballo).

http://dx.doi.org/10.1016/j.scitotenv.2023.161462
Received 15 September 2022; Received in revised form 3 January 2023; Accepted 4 January 2023
Available online 7 January 2023

their legacy levels (Sonne et al., 2020). Special attention should also be given to OPs that are not listed in the Stockholm Convention (OPPs, PAHs and/or PYRs) because of their negative impact on health outcomes reported in the last years such as male reproductive problems, pregnancy complications, certain cancers, obesity and brain development (Ferré et al., 2020; Henríquez-Hernández et al., 2016). They can be ubiquitous compounds that can be present in food, dust and/or soil representing a constant low-level exposure to mammals via diet, inhalation or dermal contact (Pleil et al., 2010; Ravindra et al., 2008).

OPs have been detected in biological samples of pets (e.g. blood, plasma or serum) because they share similar sources of exposure with humans (see Table S1). Household animals have also been used as key environmental indicators to relate POPs with health diseases (Dirtu et al., 2013; Sonne et al., 2010; Walter et al., 2017). It has been suggested that cats show POP levels higher than dogs but also than humans because of the higher intake of dust from their grooming behaviour and the type of diet (Ali et al., 2013; Norrgran Engdahl et al., 2017; Venier and Hites, 2011). We had previously hypothesized that pet dogs may serve as good inhalation indicators of human exposure to several OPs using non-invasive biological samples (González-Gómez et al., 2018). Pets are usually exposed to these pollutants via multiple pathways where some of them have been shown to be more relevant (Braouezec et al., 2016; Mizukawa et al., 2016). Nevertheless, some studies have questioned whether dogs and other canines serve as good indicators for human health due to their different rate of OP metabolization (Ruiz-Suárez et al., 2016; Sévère et al., 2015; Storelli et al., 2009). Henríquez-Hernández et al. (2016, 2017) have highlighted that the presence of parasites, such as Dirofilaria species, could reduce levels of OPs in dogs as a result of the parasite capability to compete for the OPs burden with their host. Several researches have noted that there are species-specific differences between all these ubiquitous pollutants relating to catalytic activity and enzyme contents (Lau et al., 2017; Verreault et al., 2009).

The widespread presence of these chemicals in the indoor and outdoor environment, requires constant monitoring. Many studies have been carried out using blood as priority matrix for pollutant biomonitoring. Blood is a common and constant link between body tissues where chemicals can be stored. Nevertheless, some authors have indicated that blood only reflects a recent chemical exposure and is not recommended to provide information about easy metabolized pollutants such as PAHs or PYRs (Appenzeller et al., 2017; Esteban and Castaño, 2009). Nevertheless, there are some researches that consider preferable to measure these chemicals in blood to estimate the real level of exposure (Boada et al., 2015; Pleil et al., 2010). The objective of the present study was to evaluate the presence of selected OPs (PCBs, OCPs, PBDEs, OPPS, PAHs and PYRs) in blood plasma samples of dog pets collected in NW Spain. We have also examined the OP sources to correlate these results with those obtained previously by our research group in hair from the same pets (González-Gómez et al., 2018).

2. Experimental

2.1. Sample collection

Plasma samples were collected between 2015 and 2016 from a total of 39 pet dogs (18 females and 21 males) by venipuncture at a veterinary clinic of the city of Ourense, Galicia (NW, Spain). Breeds were distributed in as follows: dog crossbreed (19), Yorkshire Terrier (3), Schnauzers and molosoides (2), hunting dogs (5), companion dogs (3), spitzs (2) and tracking dogs (1). Age ranged from 3 months to 13 years (mean: 6.0 ± 3.9). Mean weight was 18 kg with a minimum of 3.0 kg and maximum of 62 kg. Pet food was mainly dry food (71 %), following by home-made food (17 %), the rest (8.5 % a mixture between dry and home-made food; 2.8 % dry, wet and home-made food and 2.8 % dry and wet food). With regard to life style, about 57 % of pets were coming from urban areas and 43 % from countryside. 19 % of the selected pets were from smoker owners. Epidemiological data for the individual dogs are shown in Table 1. Blood plasma was obtained by centrifugation, separated carefully into clean tubes, coded, transported to the laboratory and stored at −80 °C until analysis. An epidemiological questionnaire (sex, breed, age, weight, dietary habits, place of living) was associated to each pet. All animal owners gave informed consent.

Table 1
Epidemiological data for the individual dogs.

Sample	Age*	Sex	Weight (kg)	Size	Housing	Diet	Smoker owner
1	Adult	Male	25	Normal	Indoor	Dry	No
2	Adult	Male	6.3	Normal	Indoor	Dry	Yes
3	Senior	Female	10	Normal	Indoor	Dry	No
4	Senior	Male	10	Normal	Indoor	Dry	No
5	Pre-adult	Female	26.4	Normal	Outdoor	Dry	Yes
6	Adult	Male		Normal	Indoor	Dry	No
7	Senior	Male	4.1	Toy	Indoor	Dry and homemade	No
8	Senior	Male	26.5	Normal	Indoor	Dry	No
9	Adult	Male	23	Normal	Outdoor	Homemade	Yes
10	Senior	Female	3.0	Toy	Indoor	Dry	No
11	Senior	Female	23	Toy	Outdoor	Homemade	No
12	Pre-adult	Male	46	Normal	Outdoor	Dry	No
13		Female	4.2	Toy	Indoor	Dry	Yes
14	Senior	Female	22	Normal	Indoor	Dry	No
15	Adult	Male	17	Normal	Indoor	Dry	No
16	Adult	Male	15	Normal	Outdoor	Dry and homemade	No
17	Senior	Female	24.5	Normal	Outdoor	Dry	Yes
18	Senior	Male	4.0	Toy	Indoor	Dry	No
19	Senior	Female	18	Normal	Outdoor	Dry	No
20	Senior	Male	62	Normal	Outdoor	Homemade	No
21	Pre-adult	Male	27	Normal	Outdoor	Homemade	Yes
22	Senior	Female	30	Normal	Outdoor	Dry	No
23	Adult	Female	15	Normal	Outdoor	Homemade	No
24	Adult	Female	5.1	Toy	Indoor	Dry	Yes
25	Adult	Male	8.8	Toy	Outdoor	Homemade	No
26	Adult	Female	13	Normal	Indoor	Dry	No
27	Adult	Female	25	Normal	Outdoor	Dry	No
28	Pre-adult	Male	16	Toy	Indoor	Dry	No
29	Senior	Female	3.0	Normal	Indoor	Dry	No
30	Adult	Male	3.6	Normal	Indoor	Dry and wet	No
31	Adult	Female	11	Normal	Outdoor	Dry	No
32	Adult	Female	25	Normal	Outdoor	Dry	No
33	Adult	Male	27	Normal	Outdoor	Dry, homemade and wet	No
34		Female					
35	Senior	Male	6	Toy	Indoor	Dry	No
36	Pre-adult	Female	3.5	Toy	Indoor	Dry	
37		Male					
38	Adult	Male	25	Normal	Indoor		No
39	Adult	Male	35	Normal	Indoor		No

*Pre-adult: <1 year old; adult: between 1 and 9 years old; senior: >9 years.

2.2. Sample preparation, OP determination and quality control

Information about all chemicals, including internal and surrogate standards, as well as, detection parameters are available in the supplementary material (Appendix S1, Tables S2 and S3). Briefly, plasma analysis was performed as previously described by Sinha et al. (2006) with slight modifications. Briefly, aliquots of plasma (500 μL) were spiked with surrogate standards and hydrolysed with 175 μL of acetic acid, vortexed for 1 min and sonicated for 10 min. The extraction of analytes was carried out with 0.75 mL methanol and 4.5 mL hexane using low speed shaker for 1 h. Centrifugation at 1640 ×*g* during 20 min was performed to obtain the Hex layer. Finally, the extract was evaporated to dryness, reconstituted in 0.10 mL of acetone containing 50 ng of the internal standards and the analyte protectants (APs). Quantification of targeted compounds was performed by GC-QqQ-MS (Fernández-Cruz et al., 2017; González-Gómez et al., 2018; Fernández-Cruz et al., 2020) (Table S4). Lipid weight (lw) was determined using the methodology described by Bernert et al. (2007).

The set of samples analysed each day was processed together with a reagent blank, to assess contamination during the extraction process, and a spiked plasma sample at an intermediate concentration (10 ng/g) to

calculate the extraction efficiency in the series. Chemicals or labelled compounds of OPPs (Chlorpyrifos-D_{10}), of OCPs (α,γ-HCH-D_6; DDE-D_8; HCB-$^{13}C_6$), of PAHs (Chrysene-D_{12}), of PCBs (PCB 14, 65 and 166), of PBDEs (PBDE 77) and of PYRs (*cis*-Permethrin-$^{13}C_6$) were chosen as surrogates.

Internal linear calibration was used to quantify the targeted OPs in the selected plasma and hair samples using the following internal standards: DDT-D_8 for DDT; diazinon-D_{10} for OPPs; PCB 30 and 195 for PCBs; *trans*-cypermethrin-D_6 for PYRs and PBDE 166 for PBDEs. To verify the linearity range, a Mandel fitting test (P = 99 %) was additionally performed (Mandel, 1964). Linear calibration curves fit reasonably ($r^2 > 0.990$) in a six-point calibration curve with a concentration scale of two or three orders of magnitude, depending on the compound (0.25–25 ng/g). Six replicates at four concentrations of standards (LOQs, 1.0, 5.0 and 10 ng/g) added to six blank samples, extracted as described above, were analysed on the same day for the determination of the precision (RSD lower than 20 %). The accuracy of the assay, expressed as mean recovery, was calculated as follows: blank and plasma samples were fortified at three levels, added to six blank samples, extracted as described above, over three days. The results obtained were in the range of 63–117 % (Table S5).

LODs and LOQs were evaluated based on the signal-to-noise ratio of 3 and 10 obtained by analysis of unfortified plasma samples (n = 4) (MacDougall et al., 1980) and were then tested experimentally by spiking five blank sample replicates at such levels (Table S5). The compound stability in tested biological matrices was evaluated by the analysis (n = 3) of spiked liver and hair samples after three freeze/thaw cycles (storage at −20 °C). No significant degradation was observed.

2.3. Data analysis and statistics

Preliminary descriptive analysis revealed skewness in some data and they were log-transformed in order to improve normality in the residuals. OP levels included in data analyses were above Limit of Quantification (LOQ). For statistical calculations, results below the LOD were imputed as the LOD divided by the square root of 2.

A significant level of 0.050 was used for all significance test. Multi-Way ANOVA was used to find associations between the log of the detected OPs in the sampled samples and the epidemiologic factors: age (<12 months (pre-adult), between 12 and 72 months (adult), and >72 (senior)); sex (males and females); size (large-medium and small-toy sized breeds); housing (indoor and outdoor); diet (dry and homemade) and smoker owner (yes or no) (Table 1).

Cluster analysis, principal component analysis (PCA), factor analysis mixed data (FAMD) and Spearman's rank correlation were applied in an attempt to reveal latent structures in the dataset. R software version 3.6.1 (R Core Team, 2019) with R Commander package programs (Fox, 2005) for statistical analyses with FactoMineR version 1.7 (Lê et al., 2008) for PCA and FAMD, and EZR version 1.6.1 (Kanda, 2013) for regression procedures were used.

3. Results and discussion

3.1. Levels of target OPs in plasma samples

OP concentrations (expressed in ng/g ww and ng/g lw) are summarized in Table 2.

3.1.1. PAHs

Unmetabolized PAHs in blood could reflect a recent exposure to these chemicals (Santos et al., 2019). Several studies have shown that inhalation exposure to PAHs produce temporary contamination of the blood in humans and experimental animals, including DNA and protein adducts involved in cancer risk (Moorthy et al., 2015).

Total PAH levels (ΣPAHs) ranged from <LOQs to 14 ng/g ww (equivalent to <LOQ–1744 ng/g lw), with a mean concentration of 1.4 ± 2.5 ng/g ww (equivalent to 175 ± 319 ng/g lw). Pyrene (P) and fluoranthene (F) were detected in >97 % of samples (mean concentration of 136 ± 253 and 22 ± 16 ng/g lw, respectively). Chrysene (Chr), benzo(*a*)anthracene (BaA), benzo(*a*)pyrene (BaP) and benzo(*g,h,i*)perylene (BghiP) were detected in <15 % of samples. To the best of our knowledge, only few studies have evaluated levels of PAHs in plasma dog samples. Henríquez-Hernández et al. (2016) detected 16 PAHs of non-parasitized dogs from Canary Islands (Spain) with phenanthrene, P, F and naphthalene as the most frequently detected in plasma (mean concentration of 3.1, 0.54,

Table 2
OP detection frequencies (DF, %), mean, median, concentration range and percentiles 25 and 75 in plasma samples in ng/g ww (ng/g lw).

OPs	DF (%)	Mean	Median	Min	Max	P25	P75
a-HCH	10	0.0086 (1.8)	0.0071 (0.87)	0.0071 (0.87)	0.098 (12)	0.0071 (0.87)	0.0071 (0.87)
HCB	100	0.26 (3)	0.26 (32)	0.026 (3.2)	0.59 (73)	0.15 (19)	0.32 (39)
ΣOCPs		***0.27 (33)***	***0.27 (34)***	***0.026 (4.0)***	***0.59 (73)***	***0.15 (19)***	***0.37 (46)***
PCB 77	41	0.035 (5.1)	0.011 (1.3)	0.011 (1.3)	0.20 (25)	0.011 (1.3)	0.067 (8.3)
PCB 118	69	0.11 (14)	0.094 (12)	0.011 (1.3)	0.43 (53)	0.011 (1.3)	0.15 (19)
PCB123	59	0.046 (6.3)	0.034 (4.2)	0.011 (1.3)	0.37 (46)	0.011 (1.3)	0.069 (8.5)
ΣDLPCBs	*89*	*0.19 (25)*	*0.19 (25)*	*0.032 (3.9)*	*0.57 (72)*	*0.068 (10)*	*0.27 (34)*
PCB 28	74	0.57 (71)	0.58 (71)	0.011 (1.3)	2.9 (355)	0.011 (4.0)	0.88 (108)
PCB 101	100	0.066 (8.2)	0.052 (6.4)	0.019 (2.4)	0.25 (31)	0.033 (4.0)	0.093 (12)
PCB 138	48	0.038 (8.2)	0.011 (7.1)	0.011 (1.3)	0.33 (24)	0.011 (5.1)	0.041 (12)
PCB 153	82	0.065 (5.4)	0.058 (1.3)	0.011 (0.36)	0.19 (40)	0.042 (1.3)	0.099 (9.3)
PCB 180	74	0.057 (7.4)	0.039 (4.8)	0.011 (1.3)	0.31 (38)	0.011 (1.3)	0.076 (9.3)
ΣNDLPCBs	*100*	*0.79 (100)*	*0.80 (115)*	*0.069 (13)*	*3.7 (454)*	*0.15 (21)*	*1.2 (147)*
ΣPCBs	***100***	***0.99 (125)***	***0.89 (115)***	***0.147 (25)***	***4.3 (526)***	***0.36 (47)***	***1.5 (184)***
Chlorpyr	26	0.017 (2.1)	0.012 (1.5)	0.012 (1.4)	0.11 (14)	0.012 (1.4)	0.022 (3.5)
ΣOPPs		***0.017 (2.1)***	***0.012 (1.5)***	***0.012 (1.4)***	***0.11 (14)***	***0.012 (1.4)***	***0.022 (3.5)***
F	97	0.18 (22)	0.13 (16)	0.021 (2.6)	1.1 (132)	0.081 (9.9)	0.23 (28)
P	100	1.1 (136)	0.59 (73)	0.021 (2.6)	11 (1354)	0.36 (45)	0.89 (109)
BaA	8	0.039 (7.3)	0.021 (2.6)	0.021 (2.6)	0.73 (90)	0.021 (2.6)	0.021 (2.6)
BaP	5	0.032 (6.5)	0.021 (2.6)	0.021 (2.6)	0.99 (123)	0.021 (2.6)	0.021 (2.6)
BghiP	2	0.0087 (1.1)	0.021 (2.6)	0.021 (2.6)	0.34 (42)	0.021 (2.6)	0.021 (2.6)
Chry	15	0.058 (9.4)	0.021 (2.6)	0.021 (2.6)	1.1 (134)	0.021 (2.6)	0.021 (2.6)
ΣPAHs		***1.4 (181)***	***0.84 (111)***	***0.11 (24)***	***14 (1745)***	***0.45 (64)***	***1.1 (145)***
PBDE 47	41	0.082 (14)	0.053 (6.6)	0.053 (6.6)	0.36 (44)	0.053 (6.6)	0.16 (20)
PBDE 99	12	0.028 (9.1)	0.053 (6.6)	0.053 (6.6)	0.25 (31)	0.053 (6.6)	0.053 (6.6)
PBDE 100	28	0.041 (9.8)	0.053 (6.6)	0.053 (6.6)	0.20 (25)	0.053 (6.6)	0.12 (43)
ΣPBDEs	***41***	***0.15 (33)***	***0.15 (32)***	***0.16 (20)***	***0.45 (58)***	***0.16 (20)***	***0.27 (43)***
c-,t-perm	23	1.1 (132)	0.071 (8.7)	0.071 (8.7)	12 (1447)	0.071 (8.7)	0.071 (8.7)
ΣPYRs	**23**	**1.1 (132)**	**0.071 (8.7)**	**0.071 (8.7)**	**12 (1447)**	**0.071 (8.7)**	**0.071 (8.7)**

0.53 and 0.40 ng/mL, respectively). Ruiz-Suárez et al. (2015 and 2016) also assessed PAH levels of 447 ± 190 (1.8 ± 2.5 PAH8) and 782 ± 324 ng/g lw in plasma samples of pet dogs from Canary Islands (Spain). They showed that 3-ring PAHs (not determined in the current study) were predominant followed by 4-ring PAH such as P (mean concentration of 7.6 ng/g lw) and F (mean concentration of 6.6 ng/g lw). They also determined PAHs and their metabolites in human plasma, concluding that dogs could not be good indicators of human exposure due to their different enzymatic capabilities to humans. P and F were also the PAHs most abundant detected in human biological samples (placenta and breast milk) collected in the same region by our research group (Fernández-Cruz et al., 2020; López Sanguos et al., 2023).

3.1.2. PYRs

PYRs are commonly used both in outdoor and indoor environments to prevent or control insect damage. Their high persistence in indoor environments has been observed (Nakagawa et al., 2020). Permethrin is mainly used in flea shampoos, insecticidal collars and spot-on formulations for pets. In our study, 61 % of dogs were treated with antiparasitic drugs (80 % permethrin as the main component of the commercial formulation). Many of these products contain synergists (e.g. piperonyl butoxide) that delay the active degradation substance, allowing the pesticide to remain for a long time in the body (Dymond and Swift, 2008).

In the present work, only permethrin was detected in 23 % plasma samples with a concentration range of <LOQ–12 ng/g ww (equivalent to <LOQ–1447 ng/g fat) and with a mean concentration of 1.1 ng/g ww (equivalent to 132 ± 352 ng/g lw). To the best of our knowledge, this is the first work that assess PYRs in the blood of pets. Few studies involving laboratory animals focused mainly on determining PYR metabolites after controlled exposure to parent compounds (Appenzeller et al., 2017; Lestremau et al., 2014). PYRs were also detected by our research team in human biological samples in the same region. Permethrin, cypermethrin and deltamethrin were the most common PYRs found in placenta, while only deltamethrin was the dominant in meconium (Fernández-Cruz et al., 2020). Permethrin, deltamethrin, cyfluthrin and cypermethrin were detected in human milk (López Sanguos et al., 2023). Although they are reported as non-toxic chemicals to mammals due to their easy metabolization, some researchers have discussed their potential bioaccumulation since they are suspected to produce alterations and neurotoxical effects in several animals including humans (Alonso et al., 2012; Chang et al., 2016; Corcellas et al., 2017; Etterson et al., 2017; Lestremau et al., 2014).

3.1.3. PCBs

Total PCB plasma concentrations (ΣPCBs) ranged from 0.21 to 4.3 ng/g (equivalent to 18–525 ng/g lw), with a mean concentration of 0.99 ± 0.78 ng/g ww (equivalent to 122 ng/g lw). Mean NDLPCB levels were 0.80 ± 0.72 (equivalent to 99 ± 89) whereas 0.19 ± 0.15 ng/g ww (equivalent to 23 ± 19 ng/g lw) were detected for DLPCBs. PCB 28 was the major PCB contributor (0.57 ± 0.62 ng/g ww) followed by PCB 118 (0.11 ± 0.11 ng/g ww) and PCB 101 (0.070 ± 0.050 ng/g ww). The congeners PCB 11, 52, 81, 105, 114, 126, 156, 157, 167, 169, 189, and 209 were not detected in any of the dog plasma samples. Marker PCBs (PCB 28, 52, 101, 138, 153 and 180) were the major congeners detected in human biological samples in the same region (Fernández-Cruz et al., 2020; López Sanguos et al., 2023). Our results obtained in plasma dog samples agree with those previous in Spain where PCB 28 dominated NDLPCB pattern (Ruiz-Suárez et al., 2015). PCB concentrations of 73 ± 69 and 24 ± 26 ng/g lw were found by Ruiz-Suárez et al. (2015) and (2016) in dog plasma samples from the Canary Islands, respectively. Mizukawa et al. (2013) and (2016) detected about 56 PCBs in blood samples of Japanese pet dogs. PCB levels ranged from <7.4–320 pg/g whole blood wet in 2013, and <7.4–120 pg/g pg/g whole blood wet in 2016. PCB 180 was predominant whereas PCB 118 was the majority DLPCB in both researches. PCB levels were also detected in Turkish dog plasma samples (10 ng/g lw, Yavuz et al., 2018) and in dog serum samples from Pakistan (18 ng/g lw, Ali et al., 2013). PCB 118 was also the majority compound whereas PCB 138 and 101 were the predominant NDLPCBs in the cities of Faisalabad, Islamabad and Lahore (Pakistan). Lau et al. (2017) found PCB 118 as the higher DLPCB (91 ng/g lw) and PCB 66 and 101 dominated NDLPCB pattern (184 and 77 ng/g lw, respectively) in euthyroid dog serum samples from North America (PCB mean level of 37 ng/g lw).

3.1.4. OCPs

OCP mean levels of 0.27 ± 0.017 ng/g ww (equivalent to 33 ± 2.1 ng/g lw) were found in dog plasma. Only HCB was detected in all plasma samples (17 ± 32 ng/g lw) followed by α-HCH with a detection frequency of 10 % (1.0 ± 3.2 ng/g lw). HCB and HCH were also detected in our previous studies in human biological samples (Fernández-Cruz et al., 2020; López Sanguos et al., 2023). OCPs represent one of the most persistent chemicals with high bioaccumulation potential in food webs because it is still being generated unintentionally as by-product in several chemical processes (Yavuz et al., 2018).

Similar OCP concentrations were found by Ruiz-Suárez et al. (2015) in plasma of Spanish dogs (32 ± 30 ng/g lw) detecting cyclodienes as majority compounds (27 ± 25 ng/g lw). The same authors found higher OCP concentrations of 76 ng/g lw in 2016 (Ruiz-Suárez et al., 2016) with HCB as also the major contributor (22 ± 35 ng/g lw). Ali et al. (2013) also detected similar levels of 32 ± 23 ng/g lw in serum dog samples from Pakistan where pentachlorophenol was the predominant compound (25 ± 20 ng/g lw) followed by HCB (3.8 ng/g lw). Henríquez-Hernández et al. (2016, 2017) found OCP median plasma levels of 1.2 and 1.5 ng/mL, respectively, in non-parasitized dogs from Spain where dieldrin and methoxychlor dominated OCP pattern.

3.1.5. PBDEs

PBDEs were detected in 41 % of the plasma samples with a mean concentration of 0.15 ± 0.14 ng/g ww (equivalent to 19 ± 18 ng/g lw). PBDE 47, 99 and 100 accounted for the total PBDE concentrations (10 ± 13, 5.0 ± 8.0 and 3.4 ± 9.1 ng/g lw, respectively). PBDE 47 was also the PBDE detected in highest concentration in human placenta and meconium samples of the same region (Fernández-Cruz et al., 2020), whereas PBDE 100 was detected as major contributor in human breast milk (López Sanguos et al., 2023). Lau et al. (2017) found the highest PBDE concentrations in hypothyroxinemic and euthyroid dogs from the United States (660 ± 457 and 523 ± 547 ng/g lw, respectively) with a detection frequency of 100 %. Nevertheless, Srebočan et al. (2019) showed levels of 0.019 ± 0.032 and 0.011 ± 0.0091 ng/g serum in obese and normal dogs from Croatia. Ali et al. (2013) found mean levels of 1.9 ± 1.1 ng/g lw in dogs from Pakistan while Venier and Hites (2011) showed 1.8 ± 0.40 ng/g ww in American dogs. PBDE 47 was the most frequent compounds in serum samples whereas PBDE 209 in whole blood samples (Mizukawa et al., 2013, 2016; Nomiyama et al., 2014). Mizukawa et al. (2013) and (2016) suggested that PBDE 209 presence in blood might be caused by the consumption of commercial dry food and also, by the country restrictions on the use of tetra- and octa-BDEs formulations. Andersen et al. (2015) showed that PBDE contamination trends in Artic fox samples were decreasing in accordance with other Artic mammals since PBDE formulations were banned.

3.1.6. OPPs

In the last decades, OPPs have been used in several activities involving food production or animal health. Despite their moderate persistence in the environment, residues of chlorpyrifos have been reported worldwide in different non-target organisms (European Food Safety Authority (EFSA) et al., 2017). In 2019 The European Commission decided to remove chlorpyrifos from all the EU Member States because of its genotoxic and neurotoxic potencial (EFSA, 2019).

Only chlorpyrifos was detected in plasma with a detection frequency of 26 % (0.017 ± 0.020 ng/g ww equivalen to 2.1 ± 2.7 ng/g lw). To the best of our knowledge, this is also the first work that assess OPPs in the blood of pets. Researches carried out in humans showed different OPP range

concentrations. Our previous studies with human biological samples showed that diazinon, parathion, fenthion and chlorpyrifos were detected in placenta, meconium and breast milk samples of the same region (Fernández-Cruz et al., 2020; López Sanguos et al., 2023). Simaremare et al. (2020) found chlorpyrifos levels of 73 μg/L in a pilot study with women blood in Taiwan. Lower levels were detected in human plasma from Thailand ranged from not detected to 0.66 ng/mL (Naksen et al., 2016) and from India with mean levels of 0.8 ng/mL (Banday et al., 2012). Berman et al. (2011) showed chlorpyrifos plasma median levels of 0.010 ng/mL with a detection frequency of 42 % in Israelite women. In a controlled study with plasma rat samples, Appenzeller et al. (2017) showed that chlorpyrifos was the only organophosphorus compound present even in the control samples with a mean levels of 0.15 ng/mL.

3.2. Factors contributing to burden of OPs in plasma samples of pet dogs

Several statistical analyses were performed in order to find significant correlation between the detected OP levels and the variables included in the epidemiologic questionnaire (Table 1). Multiple analysis of variance (MANOVA) revealed significant outcomes ($p < 0.05$) for selected epidemiological factors as is shown in Table 3.

Sex was the only significant factor ($p < 0.05$) for total PAHs and an important factor ($p > 0.050$) for total PBDE levels in plasma samples. Nevertheless, sex (PBDE 100), size (PCB 118), place (PCB 180) and age (Permethrin) were also significant factors ($p < 0.05$) when the individual OPs were evaluated. Fig. 1 shows plots of mean concentrations of individual P, PBDE 99 PCB 101, PCB 118 and permethrin compounds according to the sex and age of the pet dogs, in order to see if the mean levels varied between the results obtained for sex and age. Of the age factor, the highest P and PCB 101 levels detected in pre-adult (lower than 1 year old) and adult age (between 1 and 8 years old) could be explained by the OPs acquired during suckling (fat-rich diet of milk). Age has been previously reported as a one of the influence factors in OCP and PCB levels in tissues of hedgehogs (Arıkan et al., 2018). These results are in agreement with those obtained in our previous work in hair of pet animals (González-Gómez et al., 2018), in biological samples of wild boars (González-Gómez et al., 2020a), in feather samples of feral pigeons (González-Gómez et al., 2020b), and with those reported by Haave et al. (2003) in plasma samples of Bears at Svalbard. Females can transfer pollutants to their offspring through breast milk (Naso et al., 2004; González-Gómez et al., 2020a, 2020b). Opposite trend was observed by PBDEs (PBDE 99 and PBDE 100).

Fig. 2 shows plots of mean concentrations of individual P, Chlorpyr, PBDE 99, PCB 180, PCB 118 and permethrin compounds according to the sex and size of the pet dogs. Significant interactions were observed between sex and size for P, chlorpyrifos, PBDE 99, PCB 180 and Permethrin (Table 3). As can be seen in Fig. 2, chlorpyrifos levels detected in small pets were higher than the detected in pets of normal size. Similar trend was observed in our previous work where significant differences ($p < 0.0923$) were found for hair OPP levels in toy pets (González-Gómez et al., 2018). Although, permethrin levels followed the same trend in females, no differences were detected between small pets and normal pets in males (Fig. 2). The presence of OPPs and PYRs at home was in the past associated with the use of home sprays to prevent or control insect damage (Ostrea et al., 2006) and therefore, toy pets could be more exposed to OPPs and PYRs than normal size pets (Fig. 3). Similar trend was observed for PBDE 99 with toy pets (Fig. 2). They are suitable for small living spaces, where PBDE concentrations could be higher than houses in the countryside where normal size dogs are ubiquitous in country houses.

For better understanding of the P and PCB 180 levels in plasma samples, we have showed the interaction plots between sex and housing, and between housing and size (Fig. S1). As can be seen in Fig. 2, P and PCB 180 levels detected in toy pets of females were higher than in toy pets of males, the same trend was observed for pets living indoor than outdoor. Nevertheless, it should be pointed out the potential confounding effect of parameters such as age in females due to the lactation period (Fig. 1). There are only 18 plasma samples of females (2 pre-adult, 6 adult and 8 senior). Plasma P and PCB 180 levels detected in toy dogs in the cities were higher than the detected in rural dogs with normal size as it was previously obtained in our previous work in hair of pet animals (González-Gómez et al., 2018). We had concluded that small pets would be more exposed to urban pollution from exhaust pipes of cars.

Table 3
Summary of the statistically significant factors (age, sex, size and place) and their interactions (***$p < 0.0010$, **$p < 0.010$, *$p < 0.050$ and $^1p < 0.10$) affecting OP levels in plasma samples. Multi-Way ANOVA was used.

OP group	OPs	Significant factors	Significant interactions
PAHs	P	Sex*	Sex:Size[1] (0.08653); Age:Sex[1] (0.06983)
	Chry	Place[1] (0.09796)	
OPPs	Chlorpyr		Sex:Size*
PBDEs	PBDE 100	Sex*	
	PBDE 99		Age:Sex[1] (0.05992); Sex:Size[1] (0.08992)
PCBs	PCB 101	Sex[1] (0.06013)	
	PCB 118	Size*	
	PCB 180	Place*	Sex:size*
PYRs	Permethrin	Age**	Sex:size**; Sex:Place[1] (0.085221)

3.3. Correlations between hair and plasma samples of the selected pet dogs

In our previous work, we have also determined OPs in hair pet samples (González-Gómez et al., 2018). Data are also shown in Table S6. Distribution pattern of ΣPAHs >ΣPBDEs >ΣOPPs >ΣOCPs >ΣPCBs was observed in hair, whereas ΣPAHs > ΣPYRs > ΣPCBs > ΣOCPs > ΣPBDEs >ΣOPPs was detected in plasma. We found similar detection trend for some OPs (PAHs, NDLPCBs, OCPs, OPPs and PBDEs).

Nevertheless, only OP plasma levels of 9 of the studied dogs were correlated with those obtained from hair from the same pet. Cluster analysis was performed to check the unsupervised distribution of the common data obtained in hair and plasma samples by using Ward method (Fig. S2). Choosing a relatively large and safe cutting value at the linkage distance of 7.0, the samples have been grouped in four clusters (coloured in black, red, green and blue) in the hierarchical clustering on the factor map showed in Fig. S2A. Using the same colour code, a PCA is showed in Fig. 2C chosen as supplementary factors age, sex, housing and size. Otherwise, the PCA outputs obtained were used to explore relationships between OPs in the selected samples. Statistic models (the explained validation variance) suggested that four PCs (describing 31.75 %, 26.57 %, 19.79 % and 12.47 % of the variance) contained significant information for the selected samples. The graph of variables (correlation circle) shows the relationship between variables (Fig. S2B) and the dimensions. Moreover, FAMD was also performed to handle the mixed type of data and to confirm the results obtained. FAMD does the analysis with a combination of PCA and MCA techniques. MCA stands for Multiple Correspondence Analysis which is suitable for multiple categorical factors specifically (place, sex, age and size). Fig. S3 shows the correlation between variables both -OP levels detected (quantitative variables) and epidemiological factors (qualitative variables)- and the principal dimensions, as well as, the contribution of variables to the dimensions 1 and 2. From the Fig. S3, age and size factors contributed most of the first dimension, and size and sex factors of the second dimension. Moreover, correlation between P levels in plasma and size, and between PCB 180 levels and housing are shown. These correlations agree with the significant factors obtained by MANOVA (Table 3).

Spearman's rank correlation test was used to find correlations between OPs detected in plasma samples with those obtained previously in hair samples. Correlations were found between PCB101 and PCB 180 (0.309; $p < 0.050$), and between PBDE 99 and 100 (0.78; $p < 0.00010$) in plasma samples, concluding that similar organobromine and organochlorine sources of compounds could be responsible for the detected concentrations in plasma samples. Moreover, correlations between P (0.798; $p < 0.050$), Chry (0.683; $p < 0.0621$) and PBDE 100 (-0.7135; $p > 0.050$) levels in both biological samples using Pearson's correlation coefficient.

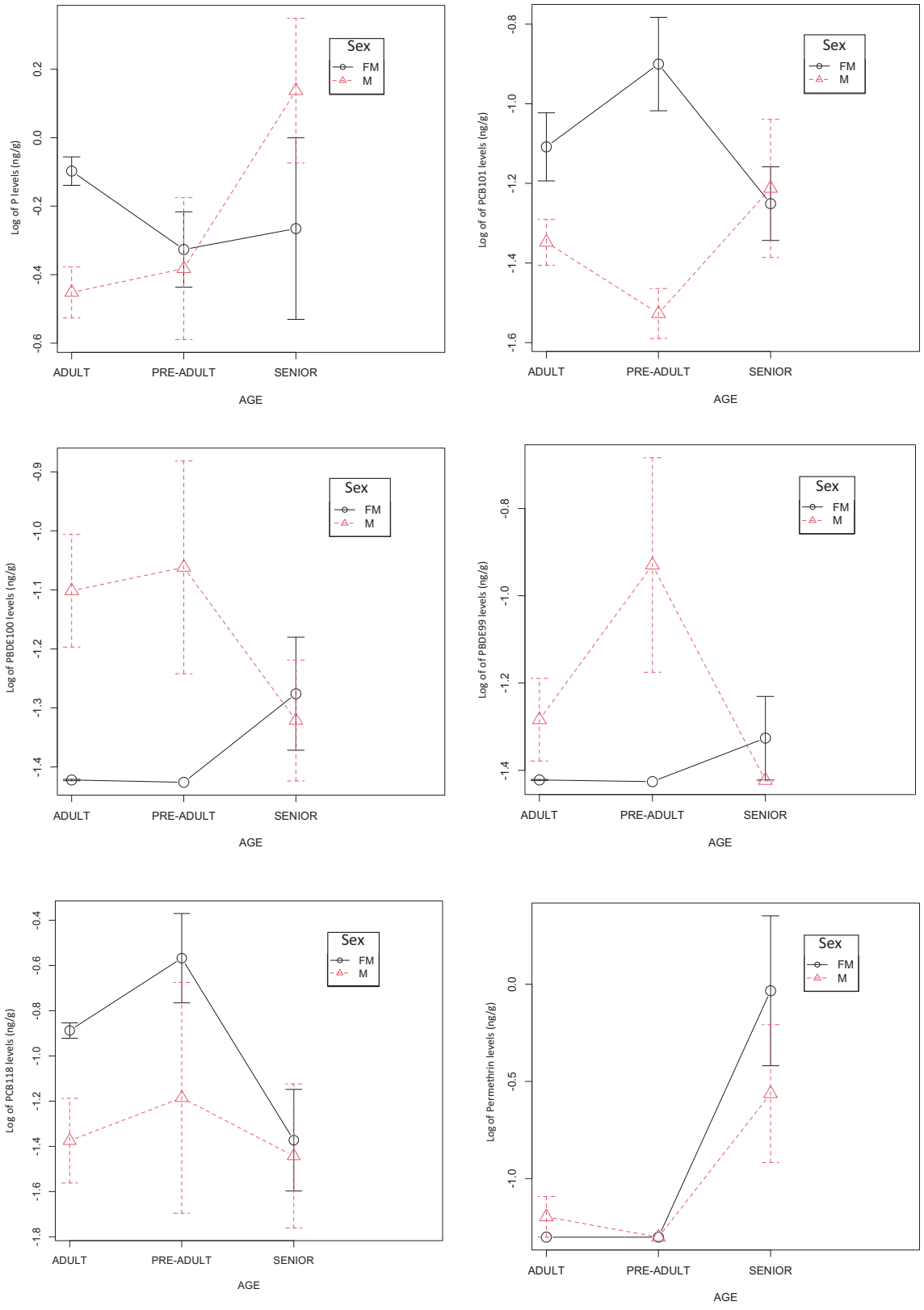


Fig. 1. Mean plots of the log concentration levels of P, PCB 101, PBDE 100, PBDE 99, PCB 118 and permethrin detected in plasma samples for age (pre-adult, adult and senior) and sex (females (FM) and males (M)).

To date, there is only one study where OCP, PCB and PBDE levels have been reported in dog serum and hair (Ali et al., 2013). They did not find significant correlations between POPs detected in both matrices ($p > 0.050$), attributing such results to the limited number of paired samples available ($n = 10$). Altshul et al. (2004) described a significant Spearman's correlation (0.80; $p < 0.050$) for *p'p*-DDE in human hair and blood samples. Zheng et al. (2016) observed positive correlations ($p < 0.0010$) in both matrices (blood and hair) for PCBs analysed (including NDLPCBs) in residents

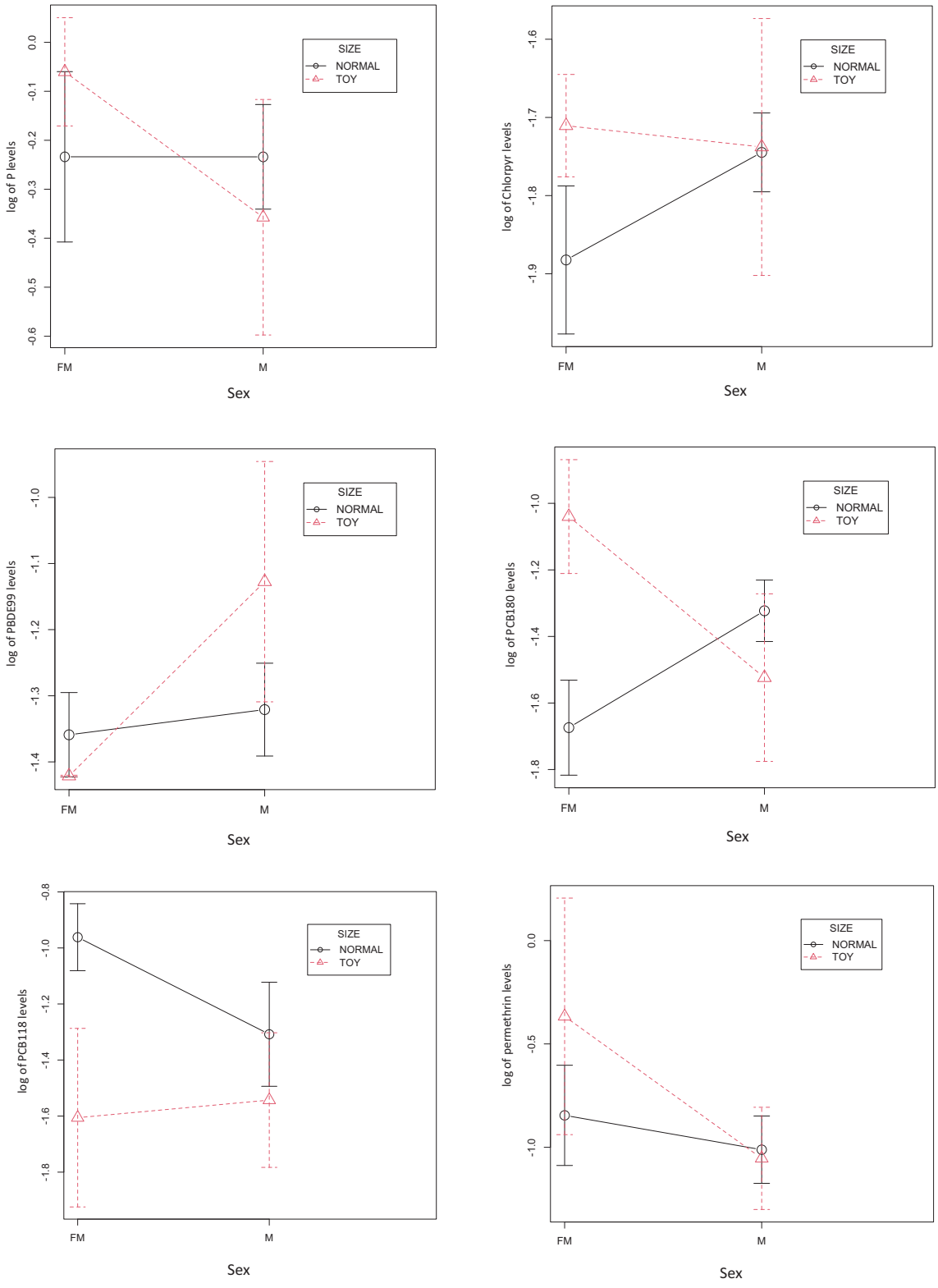


Fig. 2. Mean plots of the log concentration levels of P, Chlorpyr, PBDE 99, PCB 180, PCB 118 and permethrin detected in plasma samples for sex (female (FM) and male (M)) and size (normal and toy).

of China. Barmpas et al. (2020) observed statistically significant Spearman's correlations only for PCB 101 (0.280; $p = 0.022$) between both matrices in pregnant women from Greece. In controlled studies with rats chronically exposed to OPs, it was found a Spearman correlation coefficient of 0.94 for DDT ($p < 0.0001$) (Chata et al., 2016) (Fig. 3).

4. Conclusions

Most target pollutants were detected in blood plasma samples of dog pets from Galicia (north-western Spain), mean concentrations were in the following order: ΣPAHs > ΣPYRs > ΣPCBs > ΣOCPs > ΣPBDEs >ΣOPPs.

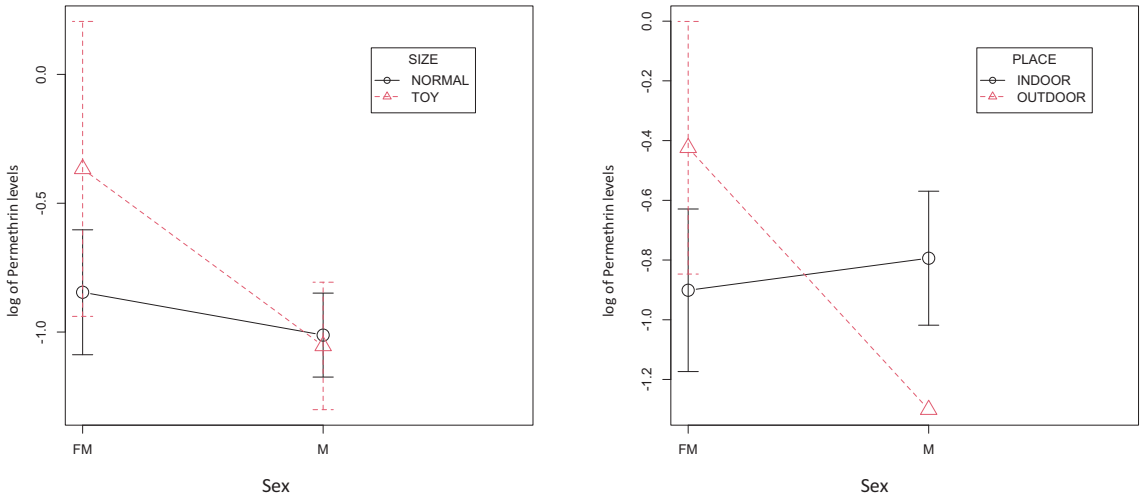


Fig. 3. Mean plots of the log concentration levels of Permethrin detected in plasma samples for sex(female (FM) and male (F)) and size (normal and toy), and for sex (female (FM) and male (F)) and place of living (indoor and outdoor).

Age, housing, size and sex factors were set as the factors with statistical significance for some of the target OPs in plasma samples, whereas size and sex showed statistical differences for some of the target OPs in hair in our previous study (González-Gómez et al., 2018).

Similar organobromine and organochlorine sources of compounds could be responsible for the detected concentrations in plasma, since statistically significant correlations were found between PCB101 and PCB 180 as well as between PBDE 99 and 100. Correlations for each OP in both plasma and hair samples were also explored. Positive correlations were found between P and Chry, whereas PBDE 100 presented negative correlation. Therefore, the usefulness of hair as a biomonitoring tool of PAH and organobromine compounds should be explored in greater depth in the future as an useful non-invasive matrix to asses body burdens.

CRediT authorship contribution statement

González-Gómez: Sample Analysis, Validation, Writing - Original draft preparation. Figueiredo-González: Experimental Design Supervision, Sample collection, Reviewing- Original draft. Villar-López: Experimental Design Supervision, Reviewing - Original draft, Martínez-Carballo: Experimental Design Supervision, Data Analysis, Writing - Reviewing and Editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded by the GRC-ED431C 2022/35 (Ministry of Culture, Education and University of Xunta de Galicia) contract. Funding for open access charge: Universidade de Vigo/CISUG is also acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.161462.

References

Ali, N., Malik, R.N., Mehdi, T., Eqani, S.A.M.A.S., Javeed, A., Neels, H., Covaci, A., 2013. Organohalogenated contaminants (OHCs) in the serum and hair of pet cats and dogs: biosentinels of indoor pollution. Sci. Total Environ. 449, 29–36. https://doi.org/10.1016/j.scitotenv.2013.01.037.

Alonso, M.B., Feo, M.L., Corcellas, C., Vidal, L.G., Bertozzi, C.P., Marigo, J., Torres, J.P.M., 2012. Pyrethroids: a new threat to marine mammals? Environ. Int. 47, 99–106. https://doi.org/10.1016/j.envint.2012.06.010.

Altshul, L., Covaci, A., Hauser, R., 2004. The relationship between levels of PCBs and pesticides in human hair and blood: preliminary results. Environ. Health. Perspect. 112 (11), 1193–1199. https://doi.org/10.1289/ehp.6916.

Andersen, M.S., Fuglei, E., König, M., Lipasti, I., Pedersen, Å.Ø., Polder, A., Routti, H., 2015. Levels and temporal trends of persistent organic pollutants (POPs) in arctic foxes (Vulpes lagopus) from Svalbard in relation to dietary habits and food availability. Sci. Total Environ. 511, 112–122. https://doi.org/10.1016/j.scitotenv.2014.12.039.

Appenzeller, B.M., Hardy, E.M., Grova, N., Chata, C., Faÿs, F., Briand, O., Duca, R.C., 2017. Hair analysis for the biomonitoring of pesticide exposure: comparison with blood and urine in a rat model. Arch. Toxicol. 91 (8), 2813–2825. https://doi.org/10.1007/s00204-016-1910-9.

Arıkan, K., Özkan, L., Arıkan, Z.Y., Turan, S.L., 2018b. The association between reproductive success with persistent organochlorine pollutants residue in feathers of spur-winged lapwing (Vanellus spinosus L.). Environ. Sci. Pollut. Res. 25 (26), 26423–26432. https://doi.org/10.1007/s11356-018-2687-6.

Banday, M., Dhar, J.K., Aslam, S., Qureshi, S., Jan, T., Gupta, B., 2012. Determination of pesticide residues in blood serum samples from inhabitants of 'Dal Lake' hamlets in J&K, India (2008–2010). Int. J. Pharm. Pharm. Sci. 4 (5), 389–395.

Barmpas, M., Vakonaki, E., Tzatzarakis, M., Sifakis, S., Alegakis, A., Grigoriadis, T., Tsatsakis, A., 2020. Organochlorine pollutants' levels in hair, amniotic fluid and serum samples of pregnant women in Greece.A cohort study. Environ. Toxicol. Pharmacol. 73, 103279. https://doi.org/10.1016/j.etap.2019.103279.

Berman, T., Hochner-Celnikier, D., Barr, D.B., Needham, L.L., Amitai, Y., Wormser, U., Richter, E., 2011. Pesticide exposure among pregnant women in Jerusalem, Israel: results of a pilot study. Environ. Int. 37 (1), 198–203. https://doi.org/10.1016/j.envint.2010.09.002.

Bernert, J.T., Turner, W.E., Patterson Jr., D.G., Needham, L.L., 2007. Calculation of serum "total lipid" concentrations for the adjustment of persistent organohalogen toxicant measurements in human samples. Chemosphere 68 (5), 824–831.

Boada, L.D., Henríquez-Hernández, L.A., Navarro, P., Zumbado, M., Almeida-González, M., Camacho, M., Luzardo, O.P., 2015. Exposure to polycyclic aromatic hydrocarbons (PAHs) and bladder cancer: evaluation from a gene-environment perspective in a hospital-based case-control study in the Canary Islands (Spain). Int. J. Occup. Environ. Health 21 (1), 23–30. https://doi.org/10.1179/2049396714Y.0000000085.

Braouezec, C., Enriquez, B., Blanchard, M., Chevreuil, M., Teil, M.J., 2016. Cat serum contamination by phthalates, PCBs, and PBDEs versus food and indoor air. Environ. Sci. Pollut. Res. 23 (10), 9574–9584. https://doi.org/10.1007/s11356-016-6063-0.

Chang, J., Wang, Y., Wang, H., Li, J., Xu, P., 2016. Bioaccumulation and enantioselectivity of type I and type II pyrethroid pesticides in earthworm. Chemosphere 144, 1351–1357. https://doi.org/10.1016/j.chemosphere.2015.10.011.

Chata, C., Hardy, E.M., Grova, N., Appenzeller, B.M., 2016. Influence of pesticide physicochemical properties on the association between plasma and hair concentration. Anal. Bioanal. Chem. 408 (13), 3601–3612. https://doi.org/10.1007/s00216-016-9442-y.

Corcellas, C., Andreu, A., Máñez, M., Sergio, F., Hiraldo, F., Eljarrat, E., Barceló, D., 2017. Pyrethroid insecticides in wild bird eggs from a World Heritage Listed Park: a case study in Doñana National Park (Spain). Environ. Pollut. 228, 321–330. https://doi.org/10.1016/j.envpol.2017.05.035.

Dirtu, A.C., Niessen, S.J., Jorens, P.G., Covaci, A., 2013. Organohalogenated contaminants in domestic cats' plasma in relation to spontaneous acromegaly and type 2 diabetes mellitus: a clue for endocrine disruption in humans? Environ. Int. 57, 60–67. https://doi.org/10.1016/j.envint.2013.04.004.

Dymond, N.L., Swift, I.M., 2008. Permethrin toxicity in cats: a retrospective study of 20 cases. Aus. Vet. J. 86 (6), 219–223. https://doi.org/10.1111/j.1751-0813.2008.00308.x.

EFSA, 2019. Statement on the available outcomes of the human health assessment in the context of the pesticides peer review of the active substance chlorpyrifos. EFSA J. 17 (8), 05809. https://doi.org/10.2903/j.efsa.2019.5809.

EFSABrancato, A., Brocca, D., De Lentdecker, C., Erdos, Z., Ferreira, L., Greco, L., Jarrah, S., Kardassi, D., 2017. Brancato, A., Brocca, D., De Lentdecker, C., Erdos, Z., Ferreira, L., Greco, L., Jarrah, S., Kardassi, D., 2017. Review of the existing maximum residue levels for chlorpyrifos according to Article 12 of Regulation (EC) No 396/2005. EFSA J. 15, 1–70. https://doi.org/10.2903/j.efsa.2017.4734.

Esteban, M., Castaño, A., 2009. Non-invasive matrices in human biomonitoring: a review. Environ. Int. 35 (2), 438–449. https://doi.org/10.1016/j.envint.2008.09.003.

Etterson, M., Garber, K., Odenkirchen, E., 2017. Mechanistic modeling of insecticide risks to breeding birds in North American agroecosystems. PloS One 12 (5). https://doi.org/10.1371/journal.pone.0176998.

Fernández-Cruz, T., Álvarez-Silvares, E., Domínguez-Vigo, P., Simal-Gándara, J., Martínez-Carballo, E., 2020. Prenatal exposure to organic pollutants in northwestern Spain using noninvasive matrices (placenta and meconium). Sci. Total Environ. 731, 138341. https://doi.org/10.1016/j.scitotenv.2020.138341.

Fernández-Cruz, T., Martínez-Carballo, E., Simal-Gándara, J., 2017. Optimization of selective pressurized liquid extraction of organic pollutants in placenta to evaluate prenatal exposure. J. Chromatogr. A 1495, 1–11. https://doi.org/10.1016/j.chroma.2017.03.010.

Ferré, D.M., Ludueña, H.R., Romano, R.R., Gorla, N.B.M., 2020. Evaluation of the genotoxic potential of cypermethrin, chlorpyrifos and their subsequent mixture, on cultured bovine lymphocytes. Chemosphere 243, 125341. https://doi.org/10.1016/j.chemosphere.2019.125341.

Fox, J., 2005. The R Commander: a basic-statistics graphical user interface to R. J. Stat. Softw. 14 (9), 1–42. https://doi.org/10.18637/jss.v014.i09.

González-Gómez, X., Cambeiro-Pérez, N., Martínez-Carballo, E., Simal-Gándara, J., 2018. Screening of organic pollutants in pet hair samples and the significance of environmental factors. Sci. Total Environ. 625, 311–319. https://doi.org/10.1016/j.scitotenv.2017.12.270.

González-Gómez, X., Cambeiro-Pérez, N., Figueiredo-González, M., Martínez-Carballo, 2020. Wild boar (Sus scrofa) as bioindicator for environmental exposure of organic pollutants. Chemosphere 128848. https://doi.org/10.1016/j.chemosphere.2020.128848.

González-Gómez, X., Simal-Gándara, J., Alvarez, L.E.F., López-Beceiro, A.M., Pérez-López, M., Martínez-Carballo, E., 2020b. Non-invasive biomonitoring of organic pollutants using feather samples in feral pigeons (Columba livia domestica). Environ. Pollut. 115672. https://doi.org/10.1016/j.envpol.2020.115672.

Haave, M., Ropstad, E., Derocher, A.E., Lie, E., Dahl, E., Wiig, Ø., Jenssen, B.M., 2003. Polychlorinated biphenyls and reproductive hormones in female polar bears at Svalbard. Environ. Health Perspect. 111 (4), 431–436. https://doi.org/10.1289/ehp.5553.

Henríquez-Hernández, L.A., Carretón, E., Camacho, M., Montoya-Alonso, J.A., Boada, L.D., Valerón, P.F., Luzardo, O.P., 2016. Influence of parasitism in dogs on their serum levels of persistent organochlorine compounds and polycyclic aromatic hydrocarbons. Sci. Total Environ. 562, 128–135. https://doi.org/10.1016/j.scitotenv.2016.03.204.

Henríquez-Hernández, L.A., Carretón, E., Camacho, M., Montoya-Alonso, J.A., Boada, L.D., Valerón, P.F., Luzardo, O.P., 2017. The heartworm (Dirofilaria immitis) seems to be able to metabolize organochlorine pesticides and polychlorinated biphenyls: a case–control study in dogs. Sci. Total Environ. 575, 1445–1452. https://doi.org/10.1016/j.scitotenv.2016.09.236.

Kanda, Y., 2013. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 48, 452–458. https://doi.org/10.1038/bmt.2012.244.

Lau, G., Walter, K., Kass, P., Puschner, B., 2017. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in the serum of hypothyroxinemic and euthyroid dogs. PeerJ 5, e3780. https://doi.org/10.7717/peerj.3780.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. 25, 1–18. https://doi.org/10.18637/jss.v025.i01.

Lestremau, F., Willemin, M.E., Chatellier, C., Desmots, S., Brochot, C., 2014. Determination of cis-permethrin, trans-permethrin and associated metabolites in rat blood and organs by gas chromatography–ion trap mass spectrometry. Anal. Bioanal. Chem. 406 (14), 3477–3487. https://doi.org/10.1007/s00216-014-7774z.

López Sanguos, C., López Suárez, O., Martínez-Carballo, E., Couce, M.L., 2023. Postnatal exposure to organic pollutants in maternal milk in north-western Spain. Environ. Pollut. 318, 120903. https://doi.org/10.1016/j.envpol.2022.120903.

MacDougall, D., Crummett, W.B., et al., 1980. Guidelines for data acquisition and dataquality evaluation in environmental chemistry. Anal. Chem. 52, 2242. https://doi.org/10.1021/ac50064a004.

Mandel, J., 1964. The Statistical Analysis of Experimental Data. Interscience Publishers. John Wiley & Sons, New York.

Mizukawa, H., Nomiyama, K., Nakatsu, S., Yachimori, S., Hayashi, T., Tashiro, Y., Tanabe, S., 2013. Species-specific differences in the accumulation features of organohalogen contaminants and their metabolites in the blood of Japanese terrestrial mammals. Environ. Pollut. 174, 28–37. https://doi.org/10.1016/j.envpol.2012.11.004.

Mizukawa, H., Nomiyama, K., Nakatsu, S., Iwata, H., Yoo, J., Kubota, A., Kunisue, T., 2016. Organohalogen compounds in pet dog and cat: do pets biotransform natural brominated products in food to harmful hydroxlated substances? Environ. Sci. Technol. 50 (1), 444–452. https://doi.org/10.1021/acs.est.5b04216.

Moorthy, B., Chun, C., Carlin, D.J., 2015. Polycyclic aromatic hydrocarbons: from metabolism to lung cancer. Toxicol. Sci. 145 (1), 5–15. https://doi.org/10.1093/toxsci/kfv040.

Nakagawa, L.E., do Nascimento, C.M., Costa, A.R., Polatto, R., Papini, S., 2020. Persistence of indoor permethrin and estimation of dermal and non-dietary exposure. J. Expo. Sci. Environ. Epidemiol. 30, 547–553. https://doi.org/10.1038/s41370-019-0132-7.

Naksen, W., Prapamontol, T., Mangklabruks, A., Chantara, S., Thavornyutikarn, P., Robson, M.G., Panuwet, P., 2016. A single method for detecting 11 organophosphate pesticides in human plasma and breastmilk using GC-FPD. J. Chromatogr. B 1025, 92–104. https://doi.org/10.1016/j.jchromb.2016.04.045.

Naso, B., Zaccaroni, A., Perrone, D., Ferrante, M.C., Severino, L., Stracciari, G.L., Lucisano, A., 2004. Organochlorine pesticides and polychlorinated biphenyls in European roe deer Capreolus capreolus resident in a protected area in Northern Italy. Sci. Total Environ. 328 (1–3), 83–93. https://doi.org/10.1016/j.scitotenv.2004.02.006.

Nomiyama, K., Hirakawa, S., Eguchi, A., Kanbara, C., Imaeda, D., Yoo, J., Kunisue, T., Kim, E.Y., Iwata, H., Tanabe, S., 2014. Toxicological assessment of polychlorinated biphenyls and their metabolites in the liver of Baikal Seal (Pusa sibirica). Environ. Sci. Technol. 48, 13530–13539. https://doi.org/10.1021/es5043386.

Norrgran Engdahl, J., Bignert, A., Jones, B., Athanassiadis, I., Bergman, Å., Weiss, J.M., 2017. Cats'internal exposure to selected brominated flame retardants and organochlorines correlated to house dust and cat food. Environ. Sci. Technol. 51 (5), 3012–3020. https://doi.org/10.1021/acs.est.6b05025.

Ostrea, J.E.M., Villanueva-Uy, E., Bielawski, D.M., Posecion Jr., N.C., Corrion Jr., M.L., Jin Jr., Y., Ager Jr., J.W., 2006. Maternal hair-an appropriate matrix for detecting maternal exposure to pesticides during pregnancy. Environ. Res. 101 (3), 312–322. https://doi.org/10.1016/j.envres.2006.02.006.

Pleil, J.D., Stiegel, M.A., Sobus, J.R., Tabucchi, S., Ghio, A.J., Madden, M.C., 2010. Cumulative exposure assessment for trace-level polycyclic aromatic hydrocarbons (PAHs) using human blood and plasma analysis. J. Chromatogr. B 878 (21), 1753–1760. https://doi.org/10.1016/j.jchromb.2010.04.035.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ravindra, K., Sokhi, R., Van Grieken, R., 2008. Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. Atmos. Environ. 42 (13), 2895–2921. https://doi.org/10.1016/j.atmosenv.2007.12.010.

Ruiz-Suárez, N., Camacho, M., Boada, L.D., Henríquez-Hernández, L.A., Rial, C., Valerón, P.F., Luzardo, O.P., 2015. The assessment of daily dietary intake reveals the existence of a different pattern of bioaccumulation of chlorinated pollutants between domestic dogs and cats. Sci. Total Environ. 530, 45–52. https://doi.org/10.1016/j.scitotenv.2015.05.070.

Ruiz-Suárez, N., Rial, C., Boada, L.D., Henríquez-Hernández, L.A., Valeron, P.F., Camacho, M., Luzardo, O.P., 2016. Are pet dogs good sentinels of human exposure to environmental polycyclic aromatic hydrocarbons, organochlorine pesticides and polychlorinated biphenyls? J. Appl. Anim. Res. 44, 135–145. https://doi.org/10.1080/09712119.2015.1021808.

Santos, P.M., del Nogal Sánchez, M., Pavón, J.L.P., Cordero, B.M., 2019. Determination of polycyclic aromatic hydrocarbons in human biological samples: a critical review. TrAC-TrendsAnal. Chem. 113, 194–209. https://doi.org/10.1016/j.trac.2019.02.010.

Sévère, S., Marchand, P., Guiffard, I., Morio, F., Venisseau, A., Veyrand, B., Abadie, J., 2015. Pollutants in pet dogs: a model for environmental links to breast cancer. Springerplus 4 (1), 27. https://doi.org/10.1186/s40064-015-0790-4.

Simaremare, S.R.S., Hung, C.C., Hsieh, C.J., Yiin, L.M., 2020. Relationship between organophosphate and pyrethroid insecticides in blood and their metabolites in urine: a pilot study. Int. J. Environ. Res. PublicHeath 17 (1), 34. https://doi.org/10.3390/ijerph17010034.

Sinha, S.N., Pal, R., Dewan, A., Mansuri, M.M., Saiyed, H.N., 2006. Effect of dissociation energy on ion formation and sensitivity of an analytical method for determination of chlorpyrifos in human blood, using gas chromatography–mass spectrometer (GC–MS in MS/MS). Int. J. Mass Spectrom. 253 (1–2), 48–57. https://doi.org/10.1016/j.ijms.2006.02.020.

Sonne, C., Larsen, H.J.S., Kirkegaard, M., Letcher, R.J., Dietz, R., 2010. Trans-generational and neonatal humoral immune responses in West Greenland sledge dogs (Canis familiaris) exposed to organohalogenated environmental contaminants. Sci. Total Environ. 408 (23), 5801–5807. https://doi.org/10.1016/j.scitotenv.2010.07.076.

Sonne, C., Siebert, U., Gonnsen, K., Desforges, J.P., Eulaers, I., Persson, S., Harding, K.C., 2020. Health effects from contaminant exposure in Baltic Sea birds and marine mammals: a review. Environ. Int. 139, 105725. https://doi.org/10.1016/j.envint.2020.105725.

Srebočan, E., Barić Rafaj, R., Prevendar Crnić, A., Mrljak, V., 2019. Levels of polybrominated diphenyl ether congeners in the serum of dogs as a potential indicator of environmental pollution and human exposure-short communication. Vet. Arh. 89 (2), 247–255. https://doi.org/10.24099/vet.arhiv.0093.

Storelli, M.M., Storelli, A., Barone, G., Franchini, D., 2009. Accumulation of polychlorinated biphenyls and organochlorine pesticide in pet cats and dogs: assessment of toxicological status. Sci. Total Environ. 408 (1), 64–68. https://doi.org/10.1016/j.scitotenv.2009.09.018.

Takaguchi, K., Nishikawa, H., Mizukawa, H., Tanoue, R., Yokoyama, N., Ichii, O., Ishizuka, M., 2019. Effects of PCB exposure on serum thyroid hormone levels in dogs and cats. Sci. Total Environ. 688, 1172–1183. https://doi.org/10.1016/j.scitotenv.2019.06.300.

Venier, M., Hites, R.A., 2011. Flame retardants in the serum of pet dogs and in their food. Environ. Sci. Technol. 45 (10), 4602–4608. https://doi.org/10.1021/es1043529.

Verreault, J., Maisonneuve, F., Dietz, R., Sonne, C., Letcher, R.J., 2009. Comparative hepatic activity of xenobiotic-metabolizing enzymes and concentrations of organohalogens and their hydroxylated analogues in captive greenland sledge dogs (Canis familiaris). Environ. Toxicol. Chem. 28 (1), 162–172. https://doi.org/10.1897/08-176.1.

Walter, K.M., Lin, Y.P., Kass, P.H., Puschner, B., 2017. Association of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) with hyperthyroidism in domestic felines, sentinels for thyroid hormone disruption. BMC Vet. Res. 13 (1), 120. https://doi.org/10.1186/s12917-017-1031-6.

Yavuz, O., Arslan, H.H., Esin, C., Das, Y.K., Aksoy, A., 2018. Determination of plasma concentrations of organochlorine pesticides and polychlorinated biphenyls in pet cats and dogs. Toxicol. Ind. Health 34 (8), 541–553. https://doi.org/10.1177/0748233718773182.

Zheng, J., Yu, L.H., Chen, S.J., Hu, G.C., Chen, K.H., Yan, X., Mai, B.X., 2016. Polychlorinated biphenyls (PCBs) in human hair and serum from e-waste recycling workers in Southern China: concentrations, chiral signatures, correlations, and source identification. Environ. Sci. Technol. 50 (3), 1579–1586. https://doi.org/10.1021/acs.est.5b04955.