



# The assessment of daily dietary intake reveals the existence of a different pattern of bioaccumulation of chlorinated pollutants between domestic dogs and cats



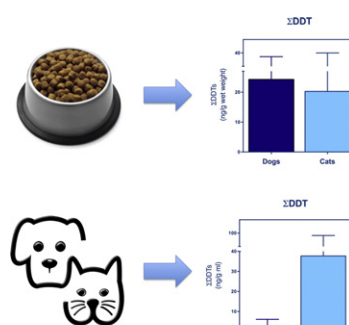
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## HIGHLIGHTS

- First assessment of the dietary intake of POPs in pet animals.
- Intake levels of pollutants are more than double in dogs than in cats.
- Proportionality between intake of PAHs and their plasma levels in both species.
- Lower levels of organochlorines in dog plasma, although their intake was higher.
- Dogs seem to be able of eliminating certain recalcitrant contaminants.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Pet dogs and cats have been proposed as sentinel species to assess environmental contamination and human exposure to a variety of pollutants, including POPs. However, some authors have reported that dogs but not cats exhibit intriguingly low levels of some of the most commonly detected POPs, such as DDT and its metabolites. This research was designed to explore these differences between dogs and cats. Thus, we first determined the concentrations of 53 persistent and semi-persistent pollutants (16 polycyclic aromatic hydrocarbons (PAHs), 18 polychlorinated biphenyls (PCBs) and 19 organochlorine pesticides (OCPs)) in samples of the most consumed brands of commercial feed for dogs and cats, and we calculated the daily dietary intake of these pollutants in both species. Higher levels of pollutants were found in dog food and our results showed that the median values of intake were about twice higher in dogs than in cats for all the three groups of pollutants ( $\Sigma$ PAHs: 274.8 vs. 141.8;  $\Sigma$ OCPs: 233.1 vs. 83;  $\Sigma$ PCBs: 101.8 vs. 43.8 (ng/kg bw/day); respectively). Additionally, we determined the plasma levels of the same pollutants in 42 and 35 pet dogs and cats, respectively. All these animals lived indoors and were fed on the commercial brands of feed analyzed. As expected (considering the intake), the plasma levels of PAHs were higher in dogs than in cats. However, for organochlorines (OCPs and PCBs) the plasma levels were much higher in cats than in dogs (as much as 23 times higher for DDTs), in spite of the higher intake in dogs. This reveals a lower capacity of bioaccumulation of some pollutants in dogs, which is probably related with higher metabolizing capabilities in this species.

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## 1. Introduction

Certain environmental contaminants, including organochlorine pesticides (OCPs), and industrial products such as polychlorinated biphenyls (PCBs), are known for their toxicity and their resistance to degradation in the environment and biota. For these reasons they are included within the group of chemicals known as persistent organic pollutants (POPs) (El-Shahawi et al., 2010). Other compounds, such as polycyclic aromatic hydrocarbons (PAHs), strictly speaking cannot be considered as POPs because of their efficient metabolism. However, due to their high prevalence in the environment and their toxicity, they are frequently considered as such, and therefore are studied together (Lammel et al., 2013). It has been established that the ingestion of contaminated food contributes more than 90% to the total exposure to these compounds, and foodstuffs of animal origin are recognized as one of the main contributors (Almeida-González et al., 2012; Boada et al., 2014; Formigaro et al., 2014; Luzardo et al., 2012, 2013a; Malisch and Kotz, 2014; Rodríguez-Hernández et al., 2014; Schwarz et al., 2014; Zhou et al., 2012). As all these compounds are highly soluble in fat, their ingestion usually leads to bioaccumulation throughout the life and to biomagnification in the food chain (El-Shahawi et al., 2010; Safe, 1995). Numerous studies have revealed that many POPs, individually and in combination, may contribute to the development of severe health problems such as immune suppression, genotoxic effects, cancer, or endocrine disruption (Bergman et al., 2012; Boada et al., 2012; Kortenkamp et al., 2011; Lauby-Secretan et al., 2013; Valerón et al., 2009). For these reasons the majority of POPs have been banned or severely restricted (El-Shahawi et al., 2010).

Despite the time that has elapsed since the ban of many of these chemicals, today still relevant concentrations of many of them are detected, as witnessed by very recent studies (Boada et al., 2015; Henríquez-Hernández et al., 2011; Luzardo et al., 2014b; Storelli and Zizzo, 2014). Indeed, in some regions of the planet it has been reported that the levels of some compounds, such as PCBs, are even increasing (García-Alvarez et al., 2014; Luzardo et al., 2014a). So, the monitoring of their levels in the environment remains a priority, especially, as regards to exposure of human populations (Diamond et al., 2015). This assessment of exposure to POPs can be done by directly measuring levels in biological samples donated by human volunteers. However, the assessment can be also performed by indirect estimates. Among these, calculations of the intake of pollutants in a given population to assess the exposure, or the employment of sentinel species are usually considered. Firstly, dietary intake estimations are made by combining food consumption data with the concentrations of contaminants found in food samples (Kesse-Guyot et al., 2013; Llobet et al., 2003; Luzardo et al., 2012; Zhou et al., 2012). These are studies that are usually linked to surveillance systems of human diseases in order to obtain quick and reliable information on the prevalence and occurrence of foodborne diseases and risks associated to food (Riviere et al., 2014; Veyrand et al., 2013). Additionally, this methodology has been also used to assess the exposure of animal species to pollutants (Formigaro et al., 2014). Secondly, all kinds of animals, which are convenient to sample, have been used to act as sentinels that allow the assessment of the environmental contamination status, and the estimation of the exposure of other species, including humans (Reif, 2011).

It seems obvious that the more suitable species to act as sentinels of human exposure are the pets, because they closely share the habitat with their owners. So, there are numerous authors that have explored the potential of dogs and cats in this sense (Andrade et al., 2010; Baker et al., 2005; Calderón-Garciduenas et al., 2001; Heyder and Takenaka, 1996; Rabinowitz et al., 2008; Reif, 2011). However, in the case of exposure to POPs the results have been variable, because although some authors have suggested that cats seem to be adequate sentinels of human exposure to these contaminants (Ali et al., 2013; Dirtu et al., 2013; Guo et al., 2012), the role of dogs as such does not seem so clear (Ruiz-Suárez et al., 2015; Sévère et al., 2015). One reason is

that several authors have reported that, intriguingly, dogs and other canines exhibit extremely low levels of some of the more abundant POPs in most mammals (including cats and humans), such as DDE and DDT, which suggests a higher metabolic capacity of these animals (Georgii et al., 1994; Kunisue et al., 2005; Ruiz-Suárez et al., 2015; Sévère et al., 2015; Shore et al., 2001; Storelli et al., 2009). This is what led us to design the present investigation, to explore these differences between dogs and cats.

In light of the above, the objectives of the present study were the following: (1) To determine the levels of selected POPs (OCPs, PCBs, and PAHs) in commercial feed for dogs and cats; (2) to estimate the daily dietary intake of these POPs by dogs and cats on the basis of the recommended consumption of these feeds; (3) To analyze the plasma samples collected from two groups of domestic dogs and cats fed on these commercial feeds; and (4) to evaluate the potential differences in contaminant levels between both species in relation with their respective intakes.

## 2. Material and methods

### 2.1. Sampling

Blood samples of pet dogs ( $n = 42$ , 24 females and 18 males) and cats ( $n = 35$ , 19 females and 16 males) were collected during 2013–2014 through cephalic vein puncture. All samples were collected in the Veterinary Hospital of the University of Las Palmas de Gran Canaria (ULPGC, Canary Islands, Spain) during a routine care. Only clinically normal animals were included in the study after owner's consent. All the dogs and cats were adults. The mean age of dogs was 5.2 y.o. (range = 2–14), and the mean age of cats was 4.8 y.o. (range = 2–11). No statistically significant differences in age were observed between males and females. All the animals included in this study were healthy, lived inside the houses with their owners, and were fed with commercial feed. Samples of blood were collected in heparinized tubes and maintained at 4 °C. Plasma was separated after centrifugation and kept frozen at –20 °C in the Laboratory of Toxicology of the ULPGC until sample preparation for chemical analysis.

In addition, we made a random purchase of different brands of commercial feed for dogs and cats in supermarkets and specialty stores from Gran Canaria (Canary Islands, Spain). The feed brands were chosen having into account their composition, and were matched between species according to raw materials they contain. Samples were acquired in triplicate, and chosen among the top selling brands (7 brands of dog feed, and 9 brands of cat feed). All the samples were individually processed as described below, and we used the mean values of the triplicate samples of each brand in the calculations of dietary intake.

### 2.2. Chemicals, reagents and analytes of interest

All the organic solvents (dichloromethane, hexane, ethyl acetate, and cyclohexane) were of mass spectrometry grade (VWR International, PA, USA). Ultrapure (UP) water was produced in the laboratory using a Milli-Q Gradient A10 apparatus (Millipore, Molsheim, France). The inert desiccant (Celite® 545) was purchased from Sigma-Aldrich (St. Louis, USA). Bio-Beads SX-3 were purchased from BioRad Laboratories (Hercules, USA). Standards of OCPs, PCB congeners, and internal standards (ISs, PCB 202, tetrachloro-*m*-xylene, *p,p'*-DDE-d8, heptachloro epoxide *cis*, and phenanthrene-d10), were purchased from Dr Ehrenstorfer, Reference Materials (Augsburg, Germany). Standards of PAHs were purchased from Absolute Standards, Inc. (Connecticut, USA). All standards were neat compounds. Stock solutions of each compound at 1 mg/ml were prepared in cyclohexane and stored at –20 °C. Diluted solutions from 0.05 ng/ml to 40 ng/ml were used for calibration curves (9 points).

We determined the levels of 53 organic compounds in plasma samples and commercial feed for dogs and cats: (a) 19 OCPs: methoxychlor;

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