

4. Research report

Due to delay in the approval of the study “**ARTEMIS: Assessing Risk from exposure To Environmental contaminants in Portuguese Mother-Infant pairS**”, it was not possible to collect the urine samples that were supposed to be analyzed during this period at CMES. As a contingency plan, urine samples from pets, that are considered good sentinels for human exposure to indoor contaminants were studied.

4.1. Title of research project

Levels of organohalogen and per- and polyfluoroalkyl contaminants in cats' and dogs' serum from Portugal

4.2. Project members

Ana Catarina Sousa, Department of Biology and Comprehensive Health Research Centre (CHRC), University of Évora, Évora, Portugal

M. Ramiro Pastorinho, Department of Medical Sciences and Comprehensive Health Research Centre (CHRC), University of Évora, Évora, Portugal

Hugo Vilhena, Department of Veterinary Medicine, Vasco da Gama University School, Coimbra, Portugal

Aika Sato, Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Japan

Hazuki Mizukawa, Department of Science and Technology for Biological Resources and Environment, Graduate School of Agriculture, Ehime University, Matsuyama, Japan

Rumi Tanoue, Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Japan

Key Nomiya, Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Japan

Tatsuya Kunisue, Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Japan

4.3. Purposes

To access, for the first time in Portugal, the exposure of pet cats and dogs to organohalogen compounds and per- and polyfluoroalkyl substances (PFAS).

4.4. Methods

Sampling

This study was developed under the umbrella of the PETTO Project, approved by the Portuguese Veterinary Authority (*Direção Geral de Agricultura e Veterinária-DGAV* 0421/000/2013). Blood samples were collected from the jugular or cephalic veins of pets' cats (n=12) and dogs (n=8) by a certified veterinarian at the veterinary hospital after the owner signed an informed consent form. Samples were immediately centrifugated and serum was collected. Whenever possible two aliquots of 1.5ml were prepared and immediately frozen. Serum samples were transported to CMES in cool conditions and preserved at -20°C until analysis.

Chemical analysis

Levels of organohalogen compounds including PCBs and hydroxylated metabolites (OH-PCBs); PBDEs and metabolites (OH-PBDEs, MeO-PBDEs), and BPhs were analyzed in 12 pet cats' samples and 8 pet dogs' samples following the protocol fully described in Nomiya, Yamamoto et al. (2022).

Levels of per- and polyfluoroalkyl substances (PFAS), including PFOS, PFHxS, PFDoA, PFUDA, PFDA, PFNA, PFOA, PFHpA and PFBA were analyzed following the protocol recently implemented at CMES and not yet published. Briefly, for PFAS analysis, 100 μL of serum was treated with acetonitrile containing 5% formic acid to induce protein precipitation. The resulting supernatant was subjected to solid phase extraction using an Oasis WAX cartridge, followed by concentration under a nitrogen stream. Qualitative and quantitative analysis of the 34 PFASs was then performed using a liquid chromatograph-quadrupole time-of-flight mass spectrometer (LC-QToF/MS; SCIEX X500R) (Sato et al., 2023, Joint Conference on Environmental Chemicals).

4.5. Results

Organohalogen compounds

Levels of PCBs, OH-PCBs, PBDEs, OH-PBDEs, MeO-PBDEs, and BPhs in both cat and dog serum samples are described in Figure 1. Significantly higher concentrations of BPhs (mean: 1700 pg.g^{-1}) were found in cat serum, followed by PBDEs (430 pg.g^{-1}), PCBs (median: 380 pg.g^{-1}), OH-PCBs (median: 280 pg.g^{-1}), and OH-PBDEs (median: 99 pg.g^{-1}). The lowest levels were registered for MeO-PBDEs (median: 2.4 pg.g^{-1}).

A different profile was observed for dogs' serum samples, with significantly higher concentrations of OH-PCBs (median: 530 pg.g^{-1}), followed by PCBs (130 pg.g^{-1}), PBDEs (median: 87 pg.g^{-1}), BPhs (41 pg.g^{-1}) and MeO-PBDEs ($<1.0 \text{ pg.g}^{-1}$). Such results underline that dogs have a stronger capacity to metabolize PCBs than cats and suggests that the low exposure to naturally occurring halogenated compounds might be a consequence of lower exposures from pet food.

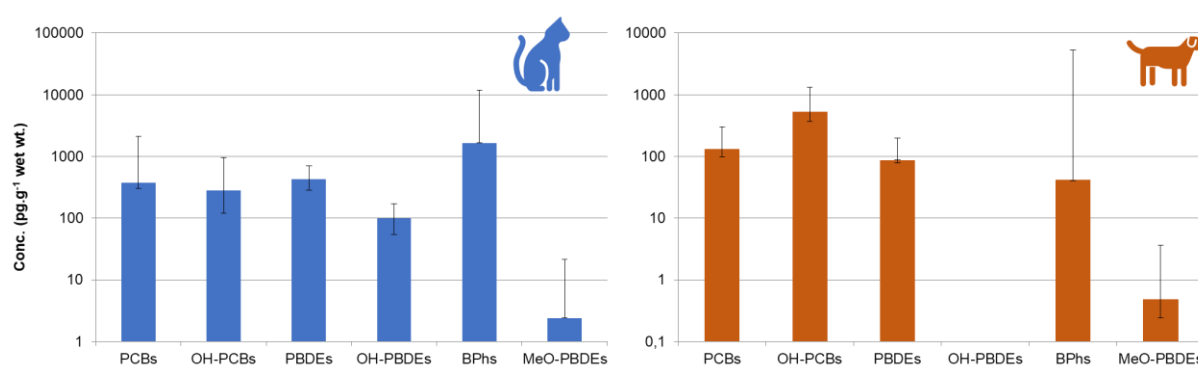


Figure 1. Median (min-max) concentrations (pg g^{-1} wet wt.) of organohalogen contaminants in cat ($n=12$) and dog ($n=8$) serum samples.

Per- and polyfluoroalkyl substances (PFAS)

For PFAS only cat serum samples were analyzed (n= 11). The main contaminants are PFOS and PFOA (Figure 2), yet long-chain PFCAs (C9~C12) have also been detected which suggests that cats may be less likely to excrete long-chain PFCAs. Overall, the PFAS levels are relatively low (Figure 3) with Σ PFAS varying between 1.4 and 4.5 ng.mL⁻¹ (median: 2.6 ng.mL⁻¹).

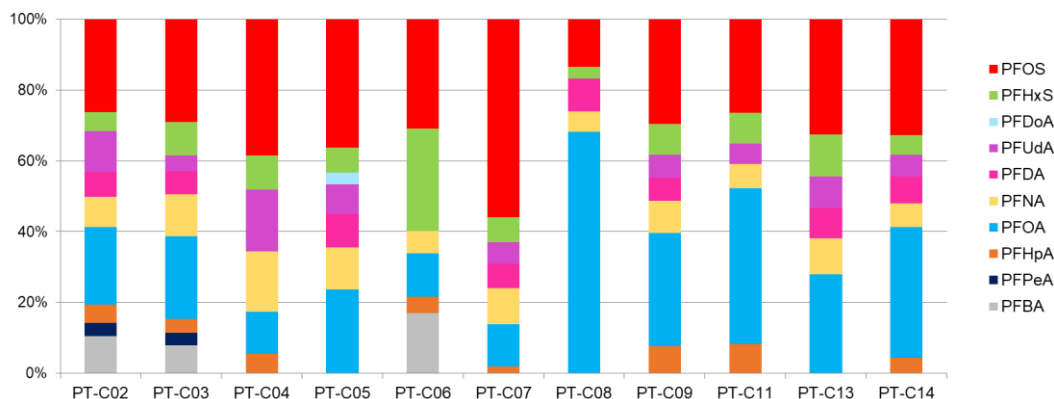


Figure 2. Profiles of per- and polyfluoroalkyl substances analyzed in the 11 cat serum samples.

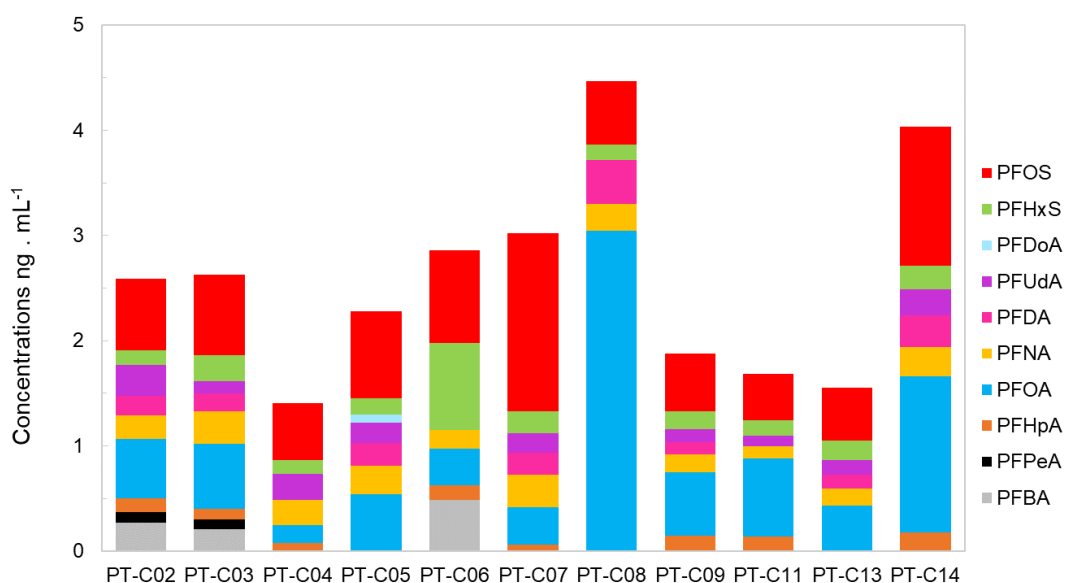


Figure 3. Concentrations (ng.mL⁻¹) of per- and polyfluoroalkyl substances in cat serum samples (n=11).

4.6. Future challenges

Due to time constraints, it was not possible to analyze PFAS in dog serum samples, however we intend to analyze these compounds in the future so that a manuscript comparing the levels of organohalogen compounds and per- and polyfluoroalkyl substances in both species can be prepared and submitted to an international journal (IF>3).

4.7. Achievements

The following papers were submitted and are currently under review (both with LaMer

acknowledgments)

Coelho SD, Maricoto t, Taborda-Barata L, Annesi-Maesano I, Isobe T, Sousa ACA (under review, 2nd round) Relationship between flame retardants and respiratory health– A Systematic review and meta-analysis of observational studies.

Environmental Pollution, *Journal IF 2023: 8.9*

Mascarenhas C, Sousa ACA, Rato L (under review, 2nd round) Effects of pharmaceutical substances with obesogenic activity on male reproductive health. International Journal of Molecular Sciences, Special Issue Endocrine Disruptors Exposure and Human Health. Preprints

<https://doi.org/10.20944/preprints202401.0324.v1>, *Journal IF 2023: 6.208*

References

Nomiyama, K., Y. Yamamoto, A. Eguchi, H. Nishikawa, H. Mizukawa, N. Yokoyama, O. Ichii, M. Takiguchi, S. M. M. Nakayama, Y. Ikenaka and M. Ishizuka (2022). "Health impact assessment of pet cats caused by organohalogen contaminants by serum metabolomics and thyroid hormone analysis." *Science of The Total Environment*: 156490.

佐藤愛佳, 佐藤楓夏, 水川葉月, 池中良徳, 田上瑠美, 横山望, 滝口満喜, 中津賞, 野見山桂 (2023) コンパニオンアニマルの PFAS 汚染：イヌ・ネコの種差要因と汚染の地域差. 環境化学物質 3 学会合同大会 (徳島 ; 2023 年)