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Biomonitoring parabens in dogs using fur sample analysis – Preliminary studies

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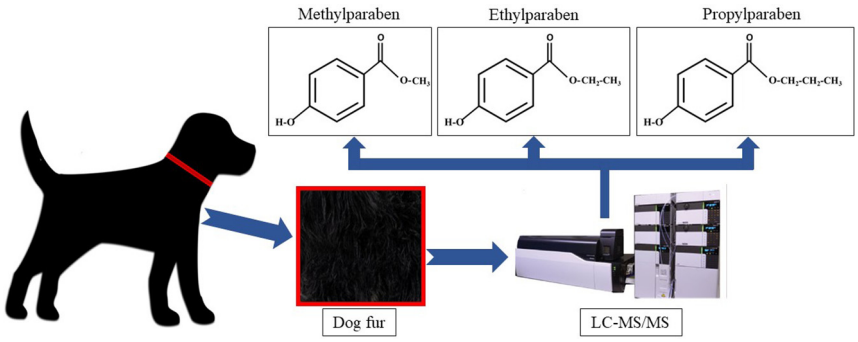
HIGHLIGHTS

- The first use of canine fur for a biomonitoring study of parabens
- MeP, EtP and/or PrP were detected in at least 90% of the samples.
- Clear differences in concentration levels were noted between particular animals.
- MeP and EtP concentrations were correlated with animal gender.

GRAPHICAL ABSTRACT



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ABSTRACT

Parabens are widely used in the food, cosmetics and pharmaceutical industry and are widespread in the environment. As endocrine disruptors, parabens have adverse effects on living organisms. However, knowledge of the exposure of domestic animals to parabens is extremely scarce.

Therefore, this study assessed the exposure level of dogs to three parabens commonly used in industry (i.e. methylparaben - MeP, ethylparaben - EtP and propylparaben - PrP) using fur sample analysis in liquid chromatography-tandem mass spectrometry.

The presence of parabens has been noted in the samples collected from all dogs included in the study ($n = 30$). Mean concentrations of MeP, EtP and PrP in the fur of dogs were 176 (relative standard deviation – RSD = 127.48%) ng/g dry weight (dw), 48.4 (RSD = 163.64%) ng/g dw and 79.8 ng/g dw (RSD = 151.89%), respectively. The highest concentrations were found for MeP (up to 1023 ng/g dw). Concentrations of MeP and EtP in males were statistically higher than those in females ($p < 0.05$). Statistically significantly higher concentration levels of PrP in young animals (up to three years old) were also found.

This is the first study concerning the use of fur samples to evaluate the exposure of domestic animals to parabens. The results indicate that an analysis of the fur may be a useful tool of paraben biomonitoring in dogs. The presence of parabens in the canine fur also suggests that these substances may play a role in veterinary toxicology. However, many aspects connected with this issue are not clear and require further study.

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1. Introduction

Parabens are a group of organic substances, i.e. alkyl esters of parahydroxybenzoic acid (pHBA), which have been widely used in the food, chemical, pharmaceutical and cosmetics industries since the 1930s (Kirchhof and de Gannes, 2013).

Among parabens, methylparaben (MeP), ethylparaben (EtP) and propylparaben (PrP) (Fig. S1) are the substances most frequently used in cosmetics and food processing (Jackson, 1996; Jiménez-Díaz et al., 2011) and, as a consequence, are the ones reported at the highest concentration levels in other biological and environmental matrices (Martín et al., 2019; Raza et al., 2018; Rodríguez-Gómez et al., 2014).

Parabens, which are primarily present in personal care products (Guo and Kannan, 2013), have also been detected in human and animal food (Liao et al., 2013; Chiesa et al., 2018; Karthikraj et al., 2018), surface and tap water (Feng et al., 2019; Malvar et al., 2019), soil (Arachchige Chamila Samarasinghe et al., 2021) and house dust (Ramirez et al., 2011).

Parabens penetrate to living organisms through the digestive system, skin, lungs and placental transfer (Kolatorova et al., 2018; Song et al., 2020). In humans, parabens have been observed, among others, in urine (Honda et al., 2018), blood (Zhang et al., 2020), breast milk (Park et al., 2019), hair (Martín et al., 2019) and breast tissue (Charles and Darbre, 2013). Parabens have also been noted in wild animals (Jeong et al., 2019).

Knowledge of the exposure of domestic animals to parabens is limited (according to the best information of the authors) to two studies concerning the presence of these substances in dog and cat food and urine (Dzięcioł et al., 2014; Karthikraj et al., 2018). However, it is relatively well established that pets living in close proximity to people are highly exposed to the various environmental pollutants characteristic of the human environment (Bost et al., 2016; Makowska et al., 2021).

Knowledge of the correlations between exposure to endocrine disruptors and specific diseases of domestic animals is extremely scarce. It is only known that a higher degree of exposure to perfluoroalkyl substances increases the risk of kidney diseases, respiratory disturbances and/or hyperthyroidism in cats (Bost et al., 2016; Wang et al., 2018). However, several studies have shown that environmental endocrine disruptors (bisphenol A, perfluoroalkyl substances and parabens) influence many internal organs and systems in pet and farm animals (Szymanska et al., 2018; Jeong et al., 2020; Tekin et al., 2020). Therefore, determination of the degree of exposure of pets to parabens seems to be an important issue in veterinary toxicology.

For many years, parabens were regarded as non-toxic substances. It is now known that parabens show estrogenic effects and long-term exposure to these substances leads to various disturbances in the internal organs and systems (Kirchhof and de Gannes, 2013; Petric et al., 2021), including the reproductive, immunological and endocrine systems (Nowak et al., 2019; Petric et al., 2021). Moreover, parabens show genotoxic, carcinogenic and cytotoxic effects (Güzel Bayülken and Ayaz, 2019; Petric et al., 2021).

In recent years, hair analysis has played an increasingly important role in the assessment of the exposure of living organisms to environmental pollutants and replaces traditional biomonitoring based on blood sample analysis (Tzatzarakis et al., 2015; Martín et al., 2016; Makowska et al., 2021). This is because the collection of hair is easy and completely non-invasive and probes are easy to store and transport. Simultaneously, previous studies have shown that hair analysis may be a good alternative in toxicological studies since the results obtained in such investigations are similar in terms of sensitivity and reliability to those obtained during the evaluation of blood or urine samples (Alves et al., 2015).

Therefore, the present study aimed to establish the degree of exposure of dogs to common industry parabens, such as MeP, EtP and PrP. The innovation of this study is that it is the first time fur sample analysis was used to achieve this goal.

2. Materials and methods

2.1. Chemical and reagents

Paraben standards of MeP (≥99.0%), EtP (≥99.0%) and PrP (≥99.0%) were purchased from Sigma-Aldrich (Steinheim, Germany). The internal standard (IS) ethylparaben-d_5 (EtP-d_5) was supplied by Cambridge Isotope Laboratories (MA, USA). Analytical grade acetic acid (HAc), ammonium acetate and sodium dodecyl sulfate (SDS) were acquired from Panreac (Barcelona, Spain). Acetone, methanol and water (all chromatographic analysis grade) were supplied by Romil (Barcelona, Spain). Stock solutions of each compound, at a concentration of 1000 mg/L, were prepared in methanol and stored at −18 °C. Working solutions were prepared by diluting the standard stock solutions in methanol. These solutions were stored at 4 °C and prepared fresh weekly.

2.2. Fur sample collection and preparation

Fur samples were collected in Olsztyn – a city in northeastern Poland. Samples were collected from 30 clinically healthy dogs of both genders aged from 1.5 to 16 years. The animals for the experiment were selected randomly from clients of veterinary clinics and dog groomers. All dog owners agreed to sample collection. The dogs were of different breeds and had various body condition scores (BCS). All dogs included in the study were indoor dogs, which went out to walk with their owners several (from four to seven) times a day. All dogs also had a diet that included both dry and canned commercial food. Details of the animals included in the study are presented in supplementary material - Table S1. Sample collection was performed according to the Act for the Protection of Animals for Scientific or Educational Purposes of 15 January 2015 (Official Gazette 2015, No. 266), applicable in the Republic of Poland. Due to the fact that fur was collected during veterinary and/or beauty treatment and such procedure is not invasive or stressful, these activities did not require additional agreement from the Bioethical Committee. The dogs included in the experiment were divided by gender to male ($n = 9$) and female ($n = 21$), by age into young (under the age of 3 years, n = 9), middle-aged (from 3 to 10 years, $n = 13$) and old (over 10 years, $n = 8$) and by BCS. The latter classification was made in accordance with the international canine body condition score system (Chun et al., 2019) and dogs included in the experiment were divided into skinnier animals (BCS points 1–3, $n = 5$), animals with normal weight (BCS points 4–5, $n = 12$) and obese dogs (BCS points 6–9, $n = 13$).

About 2 g of fur were collected from each dog. The fur was taken from the abdomen and was cut as close to the skin as possible. After cutting the fur, samples were wrapped in aluminum foil and kept at room temperature in the dark.

Exogenous substances absorbed on the fur surface were removed through several washing steps following a method previously described by Martín et al. (2016). This involved first washing in ultrapure water, followed by SDS (0.1%, *w*/*v*) and again twice with ultrapure water. In each washing step, the fur samples were sonicated for 5 min. The fur was then cut into pieces of about 2–3 mm in length, air-dried and stored in aluminum foil at room temperature until further analyses.

2.3. Fur sample treatment and analysis

Parabens were extracted and analyzed following a method previously described by Martín et al. (2016) with slight modifications. Washed fur samples (100 mg) containing EtP-d_5 (12.5 ng) were put into 10 mL screw-cap glass centrifuge tubes and incubated with a mixture of methanol and HAc (2 mL, 85:15, *v*/v). Incubation was performed at 38 °C for 12 h. After this period, samples were cooled and extracted with 3 mL of acetone in an ultrasonic bath for 15 min. The samples were then centrifuged for 10 min at 2900 ×*g*. The liquid

phase was separated into a clean tube and evaporated to dryness under a nitrogen stream at room temperature. The residue was reconstituted in 0.25 mL of methanol and filtered through a 0.22 μm nylon filter. Following this, 10 μL of the extract was injected into the LC-MS/MS apparatus.

Chromatographic measurements were performed using an Agilent 1260 Infinity II (Agilent, Santa Clara, CA, USA). Separation was carried out using a HALO C-18 Rapid Resolution (50 × 4.6 mm i.d., 2.7 μm particle size) column. The mobile phase was composed of a 10 mM ammonium acetate solution (solvent A) and MeOH (solvent B). The elution gradient was as follows: 0–14 min, linear gradient from 28% to 70% of solvent B, at a flow rate of 0.6 mL/min, from 70% to 80% of solvent B in 5 min, and then increased to 100% in 6 min and held for 2 min. The column temperature was maintained at 30 °C.

The LC system was coupled to a 6410 triple quadrupole mass spectrometer (MS/MS) with an electrospray ionization source operated in negative mode. Two multiple reaction monitoring (MRM) transitions were selected for each analyte for quantification and confirmation purposes. The MS/MS setting parameters and transitions for each paraben are given in the Supplementary material (Table S2).

2.4. Quality assurance and quality control

In order to achieve reliable results, a quality assurance/quality control protocol was formulated. It describes the use of control spiked samples, solvent (methanol) injections, standards containing a mixture of parabens (20 ng/mL) and procedural blanks, all by duplicates, into each analytical batch (15 samples).

All glassware was washed with abundant milli-Q water and sonicated with acetonitrile to remove any possible contamination. The material was then rinsed with Milli-Q water. Background contamination was evaluated by including procedural blanks processed in the same manner as the fur samples. No quantifiable amounts of parabens were noted in blank samples.

The matrix-matched calibration was applied for the quantification of parabens in the samples. For this, a pool of washed fur samples from different dogs was prepared containing the analytes at different concentration levels (from MQL to 1500 ng/g dry weight [dw] for MeP and to 1000 ng/g dw for EtP and PrP). The mixtures were vortexed for 2 min and then left to stand for 24 h at 4 °C in the dark before analysis. The areas of the compounds that are present in the blank extract have to be subtracted to the area obtained from the matrix-matched standards to construct the calibration curves.

Due to the lack of certified reference materials, in-house reference probes (prepared by spiking a pool of washed fur samples at the 10, 50 and 100 ng/g dw levels) were used to check the accuracy during validation and QA/QC (the recoveries were in the range 94–104%). The method's detection limit values were 0.75 ng/g dw for MeP and EtP and 0.60 ng/g dw for PrP. The analytical features of the method are described in Table S3.

2.5. Statistical analysis

The statistical analysis involved using Student's *t*-test for a comparison of two groups (males versus females) and a one-way Anova test for the comparison of three groups (young versus middle-aged versus old and skinnier versus normal-weight versus obese animals) (Statistica 13.3, StatSoft, Inc., Cracow, Poland). The differences were considered statistically significative at $p < 0.05$.

3. Results

During the study, at least one investigated paraben was observed in fur samples collected from each dog included in the study (Tables 1 and S4). The concentration of parabens varied considerably between the animals (Table S4).

Table 1
Concentration values (ng/g) and frequency of detection of methylparaben (MeP), ethylparaben (EtP) and propylparaben (PrP) in the canine fur samples ($n = 30$) – cumulative data.

Compounds	Range (ng/g)	Arithmetic mean (ng/g)[a]	Geometric mean (ng/g)[a]	Median (ng/g)[a]	Frequency of detection (%)
MeP	<MQL–1023	176	88.5	118	90
EtP	<MQL–382	48.4	21.3	17.9	97
PrP	8.12–527	79.8	41.9	39.4	100

[a] Samples with paraben levels <MQL are considered as MDL/square root of 2 (MQL: 2.50 ng/g for MeP and EtP and 2.00 ng/g for PrP).

PrP was the only paraben quantified in all samples and its levels fluctuated from 8.12 ± 0.25 ng/g dw to 527 ± 8.11 ng/g dw (Table 1). The presence of EtP at levels above the method quantification level (MQL - 2.50 ng/g dw) was noted in 29 out of the 30 dogs included in the study, ranging from 2.61 ± 0.15 to 382 ± 45.0 ng/g dw (Table 1). In turn, MeP in levels above MQL was observed in 27 dogs (Table S4). The concentration levels observed for MeP were particularly different between animals (much more than in the other analyzed parabens) and fluctuated from 6.42 ± 0.64 to 1023 ± 171 ng/g dw (Table 1). MeP was the only paraben with levels exceeding 1000 ng/g dw.

During the present study, statistically significant differences in the paraben levels in the fur between male and female dogs were noted (Table 2). The average level of MeP in males amounted to 291 ± 112 ng/g dw, which was higher than the average level observed in females (101 ± 24.5 ng/g dw). A similar situation was also noted for EtP, whose levels amounted to 98.4 ± 42.6 ng/g dw and 24.7 ± 5.68 ng/g dw in the fur of males and females, respectively. Contrary to MeP and EtP, inter-gender differences in the levels of PrP noted in the present study were not statistically significant (Table 2).

Nevertheless, age-dependent differences were not statistically significant (Table 2). The only exception was PrP, whose levels measured for young animals (160 ± 64.3 ng/g dw) were statistically significantly higher than ($p < 0.05$) the levels noted in middle-aged dogs (37.6 ± 9.36 ng/g dw).

Moreover, during the present study, no significant correlations between the weight of animals and concentration of parabens in the fur were observed and the levels of all substances studied in skinnier dogs, dogs with physiological weight and obese dogs did not show statistical differences (Table 2).

4. Discussion

The present study confirmed that dogs in Poland are exposed to parabens. It should be noted that knowledge concerning concentration

Table 2
Comparison of concentration levels (ng/g) of methylparaben (MeP), ethylparaben (EtP) and propylparaben (PrP) in particular groups of dogs included in the experiment: male ($n = 9$), female ($n = 21$), young (aged up to 3 years, n = 9), middle-aged (from 3 to 10 years, $n = 13$), old (aged over 10 years, $n = 8$), skinnier (BCS points 1–3, $n = 5$), with normal weight (BCS points 4–5, $n = 12$) and obese (BSC points 6–9, n = 13). Values are presented as mean ± standard error of the mean (SEM).

Groups of animals	Parabens		
	MeP	EtP	PrP
Male	291 ± 112*	98.4 ± 42.6*	123 ± 55.1
Female	101 ± 24.5*	24.7 ± 5.68*	61.1 ± 20.8
Young	224 ± 69.9	60.1 ± 23.5	160 ± 64.3*
Middle-aged	64.8 ± 18.0	20.5 ± 6.51	37.6 ± 9.36*
Old	235 ± 118	74.6 ± 45.3	58.2 ± 23.0
BCS pt. 1–3	236 ± 119	85.2 ± 40.3	153 ± 96.5
BCS pt. 4–5	77.6 ± 16.3	19.3 ± 4.01	43.6 ± 7.43
BCS pt. 6–9	202 ± 77.3	57.4 ± 28.4	85.1 ± 34.8

Statistical differences ($p \leq 0.05$) are marked with *.

Table 3
Overview of biomonitoring previous studies on parabens in the living organisms and environment in Poland.

Matrix	MeP	EtP	PrP	BuP	iBP	References
Human urine						
Males (average concentration in μg/L)	47.6	8.6	22.3	1.4	1.1	Jurewicz et al., 2017a
	15.6	9.39	3.7	3.48	2.27	Jurewicz et al., 2017b
Females (average concentration in μg/L)	107.9	12.9	18.67	5.02	2.8	Jurewicz et al., 2020
Dog urine	ND					Dzięcioł et al., 2014
Surface water (ng/L)						
Water from rivers and lakes in Greater Poland Voivodeship (ng/L)	8.7–456.6	ND	ND–144.4	ND–19.6		Zgoła-Grześkowiak et al., 2016
	1.7–1578	0.8–27.5	0.5–93.9	0.6–22.6		Czarczyńska-Goślińska et al., 2017
Drwina River (ng/L)	10.6–616.9	0.9–349.0	1.0–165.7	2.7–23.6		Styszko et al., 2021
Vistula River (ng/L)	10.3–29.6	1.0–1.7	0.9–1.3	2.4–7.6		Styszko et al., 2021
Wasterwater (ng/L)	2235.0–40,898.6	791.2–8169.4	542.2–7803.3	68.8–710.7		Styszko et al., 2021

levels of parabens in the environment and living organisms in Poland is relatively scarce in comparison with other parts of the world (Table 3).

During the present study, the highest concentration level was noted for MeP. This is in agreement with previous studies on human urine, water and wastewater conducted in Poland, in which the levels of this substance were also higher than the concentration levels of other parabens (Table 3). Moreover, studies performed in various parts of the world have also found that MeP is a paraben whose concentration level is higher than other substances belonging to this group of chemical compounds in various types of environmental and biological samples (Haman et al., 2015; Karthikraj et al., 2018; Martín et al., 2019).

To the best of the authors' knowledge, there are only two previous studies which have focused on the presence of parabens in domestic animals and both of them were conducted on urine samples (Dzięcioł et al., 2014; Karthikraj et al., 2018). During the first study, including ten female dogs, the presence of MeP in urine was detected (Dzięcioł et al., 2014). The second study conducted on 30 dogs and 30 cats found the presence of parabens and their metabolites (Karthikraj et al., 2018). In dogs, the mean urine concentrations of MeP, EtP and PrP reached 128 ng/ml, 1 ng/ml and 0.5 ng/ml, respectively, while in cat urine, the mean concentration of total parabens was 30 times lower than that measured in dog samples (Karthikraj et al., 2018).

The comparison of the above-mentioned results with observations obtained during the present investigation is difficult due to various matrices. Parabens from urine are extracted daily, and probably may accumulate in the fur. Moreover, the fur analysis takes into account both external (from the environment, dust and fur care products) and internal (contact with blood at the fur follicle/root) exposure to parabens. Differences in the concentration levels of parabens may also be connected with various environmental factors in which the animals lived and differences in the diet. However, although all dogs included in this study had a similar lifestyle (indoor dogs) and diet, significant differences in the levels of parabens between particular dogs were observed. This strongly suggests that other factors, e.g. frequent use of cosmetics containing parabens by the pet owner or frequent house cleaning, may be relevant for pet animal exposure to parabens. Therefore, further studies on the exact correlations between environmental factors and the concentration levels of parabens in canines are needed.

It should be noted that analysis of the hair to evaluate the exposure to parabens is a relatively new method, which till now has been used only in humans (Table 4). However, this method has some limitations. Since fur analysis takes into account both external and internal exposure, the difficulty in separating externally deposited compounds from endogenously deposited compounds makes the interpretation of fur analysis difficult. Moreover, the distribution rate of parabens in the fur after exposure has not been studied till now. It is also known that parabens are short-living compounds and urine samples seem to be the best matrix to study exposure to these substances, and there is no clear evidence that parabens accumulate in hair/fur. However, previous works have shown that analysis of paraben concentration levels in the human hair (Table 4) is a good alternative to analyzing "traditional" samples. It has also been demonstrated that despite the large differences in the concentration of parabens in humans between particular studies, an analysis of the hair achieves similar results to urine analysis (Asimakopoulos et al., 2014; Hines et al., 2015; Martín et al., 2016, 2019).

It is difficult to compare exposure to parabens in humans and dogs in view of the major discrepancies in results between particular studies in humans (Table 4). However, the maximum concentration of particular parabens noted in the present study is clearly lower than the maximum concentrations of these substances observed in humans in some previous studies (Table 4). This may be connected with the fact that relatively high levels of parabens are present in cosmetics for humans, including creams, lipsticks and others (Fransway et al., 2019), which are not used in animals. For this reason, the gastrointestinal and respiratory systems in animals seem to be more important vectors of paraben penetration than transdermal penetration. This thesis is supported by the fact that relatively high concentrations of parabens have been found in both pet animal food (up to 1550 ng/g of fresh weight [Karthikraj et al., 2018]) and in the indoor air and house dust (up to 2400 ng/g [Kirchhof and de Gannes, 2013]).

In the present study, statistically higher concentrations of MeP and EtP were observed in male dogs. Previous studies on dogs have not described such intergender differences (Karthikraj et al., 2018). In turn, studies performed in humans have shown a higher concentration of parabens in women than in men, which is explained by the fact that women more often use large amounts of cosmetic products containing

Table 4
Overview of biomonitoring studies of parabens in human hair.

Country	Unit	MeP	EtP	PrP	BeP	BuP	References
Germany	ng/g		810–1980	400–1520	0.033–0.067	0.067–0.086	(Martín et al., 2015)
Greece	pg/mg	17.6–27,437.0	11.0–4224.5		2.1–66.6	1.8–2513.7	(Karzi et al., 2019)
Korea	ng/g	48.3–224.2	11.5–158.3	70.2–214.5			(Cho and Song, 2019)
Spain	ng/g	78–624	7.0–42	27–238			(Martín et al., 2016)
	ng/g	10.2–33	9	11.6–107			(Rodríguez-Gómez et al., 2017)
	ng/g	68.3–14,187	2.9–6565	12.5–9009			(Martín et al., 2019)

parabens (Ashrap et al., 2018). The reasons for intergender differences noted in the present study are not clear and may be connected with differences in hormonal activity and metabolism. Moreover, the higher concentration level of MeP in male dogs may result from the fact that this substance is considered by some authors to be an important pheromone in dogs. Previous initial investigations reported that MeP is present in the vaginal secretions of female dogs in the estrus and may stimulate male dogs to exhibit mating behavior (Goodwin et al., 1979). Although more recent studies have not confirmed these observations (Dzięcioł et al., 2014), the question of whether MeP is a pheromone physiologically synthesized in female dogs is still open. Nevertheless, the elucidation of intergender differences in the concentration of parabens in domestic animals requires further research.

During the present study, a statistically higher concentration of PrP was observed in young dogs in comparison with middle-aged dogs. Such differences between animals of various ages, which have also been noted in previous studies on dogs and cats (Karthikraj et al., 2018), are not clear but may be connected (like intergender differences) with various hormonal activities and age-dependent discrepancies in metabolic processes.

The exact impact of parabens on the health of dogs is not known. By analogy with experimental studies and observations performed on a human population, it can be assumed that parabens in dogs may have cytotoxic and genotoxic activities and affect the reproductive, endocrine and immune systems (Nowak et al., 2019; Petric et al., 2021). An important role in the mechanism of the harmful effects of parabens may be played by their lipophilicity. Thanks to this feature, parabens easily penetrate through the skin into the body as well as permeate the cell membranes in the internal organs, including the blood-brain barrier (van der Meer et al., 2017). Moreover, it is known that lipids are an important sorbent of parabens. Therefore, parabens may accumulate in the adipose tissue and change its endocrine activity, affecting energy balance as well as endocrine and metabolic homeostasis of living organisms (Artacho-Cordón et al., 2018).

However, considering the fact that issues connected with the adverse effects of parabens in humans are not quite clear and subject to debate (Kirchhof and de Gannes, 2013; Petric et al., 2021), further studies are needed to explain the pathological roles of parabens in domestic animals.

5. Conclusion

The results obtained in the present study clearly indicate that dogs are exposed to parabens and that fur analysis is a suitable tool to evaluate the degree of this exposure. It should be noted that fur analysis, despite some limitations, appears to be a good alternative to studies using "classical" blood or urine samples due to easy collection and storage. Moreover, the extreme differences in paraben concentrations between particular animals suggest that exposure to these substances depends on various factors in the immediate environment in which the animal lives, but a precise understanding of these factors requires further studies.

The fact that parabens were present in the fur of all dogs included in the study strongly suggests that parabens may play a role in veterinary toxicology and cause health disorders in domestic animals. However, because the adverse effects of parabens in humans are still not clear and are subject to debate, further toxicological and clinical studies are required to determine the influence of these substances on animal health.

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CRediT authorship contribution statement

Krystyna Makowska: Writing – original draft, Resources, Formal analysis. **Julia Martín:** Investigation, Validation. **Andrzej Rychlik:** Funding acquisition, Supervision. **Irene Aparicio:** Investigation. **Juan Luis Santos:** Investigation. **Esteban Alonso:** Investigation. **Sławomir Gonkowski:** Conceptualization, Writing – review & editing, Supervision.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150757.

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