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An evaluation of dogs' exposure to benzophenones through hair sample analysis

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Abstract

Introduction: Benzophenones (BPs) are used in various branches of industry as ultraviolet radiation filters, but they pollute the natural environment, penetrate living organisms, and disrupt endocrine balance. Knowledge of the exposure of domestic animals to these substances is extremely scant. The aim of the study was to investigate long-term exposure of companion dogs to BPs and relate this to environmental factors. **Material and Methods:** Hair samples taken from 50 dogs and 50 bitches from under 2 to over 10 years old were analysed for BP content with liquid chromatography–tandem mass spectrometry. **Results:** The results revealed that dogs are most often exposed to 2-hydroxy-4-methoxybenzophenone (BP-3) and 4-dihydroxybenzophenone (BP-1). Concentration levels of BP-3 above the method quantification limit (MQL) were noted in 100% of the samples and fluctuated from 4.75 ng/g to 1,765 ng/g. In turn, concentration levels of BP-1 above the MQL were noted in 37% of the samples and ranged from <0.50 ng/g to 666 ng/g. Various factors (such as the use of hygiene and care products and the dog's diet) were found to affect BP concentration levels. Higher levels of BP-3 were observed in castrated/spayed animals and in animals that required veterinary intervention more often. **Conclusion:** The results obtained show that the analysis of hair samples may be a useful matrix for biomonitoring BPs in dogs, and that these substances may be toxic to them.

Keywords: endocrine disruptors, pets, dogs, benzophenones, exposure.

Introduction

The 300 and more benzophenones (BPs) are organic compounds that contain two phenyl groups linked to a carbonyl group in their molecule (20, 26). In nature, BPs are produced by some species of fungi and plants (75). Benzophenones are also synthesised by humans because of their ability to absorb ultraviolet (UV) radiation, for which these substances are exploited in various branches of industry (28). Benzophenones are used in the production of sunscreens, cosmetics, personal care products, inks, paints and other products that need to resist sunlight degradation (21, 44, 45, 64). They are also present in food containers, plastic bottles, clothes and perfumes. It is estimated that over 110,000 tons of BPs are produced annually (66). The most commonly used ones in industry are benzophenone 1 (BP-1, 4-dihydroxybenzophenone), benzophenone 2 (BP-2, 2,2',4,4'-tetrahydroxybenzophenone), benzophenone 3 (BP-3, 2-hydroxy-4-methoxybenzophenone, oxybenzone) and benzophenone 8 (BP-8, dioxybenzone) (28).

Benzophenones have the ability to penetrate into compartments of the natural environment, and the widespread use of these substances in industry has made them some of the most serious environmental pollutants. To date, the presence of BPs has been described in surface water, soil, air, household dust and plants (3, 8, 60, 67). Soil enriched with fertilisers from sewage sludge is particularly prone to having high levels of BPs (7, 8, 39). Previous studies have also reported the presence of BPs in food (5, 8, 10).

Human and animal organisms may be affected by BPs' endocrine-disrupting properties (26, 29, 30). Benzophenones enter the body through the gastrointestinal tract, skin and/or respiratory system and negatively influence primarily (but not exclusively) the endocrine, reproductive and nervous systems (26, 30, 45, 63). It has

been shown that BPs have mutagenic, genotoxic and cytotoxic activity, and that exposure to these substances in the prenatal period through transplacental transport may result in disturbances in the development of the nervous system (25, 29, 73). Furthermore, previous studies have described the participation of BPs in carcinogenic and neurodegenerative processes, as well as their obesogenic activity (41, 45).

These multidirectional negative effects of BPs have raised the importance in modern toxicology of monitoring of these substances in living organisms. Human exposure to BPs is the subject of many previous studies. These substances have not only been described in urine and blood serum, but also in hair, nails, amniotic fluid, breast milk, seminal plasma and various tissues (34, 45, 61). The concentration levels of BPs in the human body vary greatly in different parts of the world and observably depend on the particular environmental factors and degree of urbanisation and industrialisation of a given society, and the extent of use by its members of cosmetic and personal care products (34, 45, 65).

Knowledge of domestic animals' exposure to BPs is relatively scant, in contrast with the large canon of human exposure knowledge (32). It is known, nevertheless, that companion dogs and cats in particular are highly exposed to the same harmful substances polluting the environment as humans through living in close proximity to them (16, 36, 42, 43, 76). The main sources of exposure of companion animals to these substances are food, water, household dust, pet grooming products, their owners' cosmetics and household cleaning agents, pet toys and the furnishings of the houses in which they live (17, 32, 35). Although previous studies have reported correlations between the degree of exposure to environmental organic pollutants and diseases in companion animals (16, 56, 68, 76), the problem of such substances as a toxic and pathogenic factor in veterinary medicine is often marginalised. This problem is described in only one study, in which concentration levels of BP-1, BP-3 and BP-8 were evaluated in urine samples collected from dogs and cats living in the Albany area of New York State, USA (32).

It should be emphasised that, in addition to classic matrices such as urine or blood serum, other matrices are becoming increasingly important in contemporary environmental toxicology. One of them is hair samples (42, 43, 47, 72). This is because such samples can be collected easily and completely non-invasively, which is particularly important in animals, especially in the case of aggressive and timid individuals. Such samples are also easy to store and send even over long distances. Previous studies have shown that hair sample analysis is an appropriate method for determining exposure to organic compounds polluting the environment, and its results show similar sensitivity and reliability to those of urine or serum analysis (2, 12, 70). Moreover, pollutants' propensity to accumulate in the hair means that the analysis of hair samples better reflects the body's long-term environmental exposure than the analysis of urine and/or blood serum, in which the levels of substances fluctuate frequently (18, 27).

The aim of the present study was to amass data in this insufficiently researched area using this matrix which has recently come to be regarded as highly useful. The research determined the long-term exposure of dogs to selected BPs (BP-1, BP-2, BP-3 and BP-8) by analysis of hair samples, and found the relationships between various environmental factors and the level of exposure to these substances.

Material and Methods

Reagents. Formic acid, ammonium acetate and sodium dodecyl sulphate (SDS) were purchased from Panreac (Barcelona, Spain), C18 disperser sorbent was acquired from Scharlab (Barcelona, Spain), HPLC-grade methanol and water were sourced from Romil (Barcelona, Spain) and high purity standards of BP-1, BP-2, BP-3 and BP-8 were supplied by Sigma-Aldrich (Steinheim, Germany). Isotopically labelled BP used as an internal standard (BP-d_{10}) was obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Individual stock standard solutions were prepared at 1,000 mg/L in methanol and kept at −18°C. The working solutions were prepared weekly by dilution of the stock standard solution in methanol and stored at 4°C.

Sampling. Hair samples were collected from 100 male and female dogs of various breeds and ages living in Olsztyn (a city in north-eastern Poland) and its vicinity. All dogs included in the study were clinically healthy and had a normal body condition score according to the international canine body condition score system (11). The dogs were randomly selected from among animals the owners of which agreed to participate in the study. The characteristics of the animals included in the study are shown in supplementary Table S1. All the dogs' owners were asked to complete a questionnaire on their pet's feeding, the use of animal hygiene products, their use of hand cream and the frequency of visits to a veterinary clinic. The data obtained in these surveys are also presented in Table S1. The dogs could be classified in seven ways: by sex, as male (n = 50) or female (n = 50); by age, as young (under 2 years old, n = 18), middle-aged (from 2 to 10 years old, n = 54) or old (over 10 years old, n = 28); by diet, as pets which were mainly fed commercial dry food (n = 34), commercial canned food (n = 33) or food prepared at home (n = 33); by care regime, as dogs on which animal hygiene products were used (n = 75) or those on which none were used (n = 25); by owner practice, as dogs owned by people using hand cream at least once a week (n = 63) or those owned by people using it less often or not at all (n = 37); by healthcare need, as dogs that visited a veterinary clinic more than three times a year with various diseases (upper respiratory tract infections, diarrhoea or allergies) (n = 50) or dogs that visited a veterinary clinic less often (n = 50); and by reproductive status, as dogs which

had been castrated/spayed (n = 54) or those which had not been subjected to these procedures (n = 46). Hair samples were collected in the period from November 2022 to March 2023. Approximately 2 g of hair was taken in the same way from the same body part of all dogs. The sample was cut from the abdomen right next to the skin. Immediately after collection, it was wrapped in aluminium foil and stored at room temperature in the dark until further analysis. The sampling was performed during care or veterinary treatment and was completely painless and stress-free. Therefore, according to the Act for the Protection of Animals for Scientific or Educational Purposes of 15 January 2015 (Official Journal of Laws of the Republic of Poland 2015, item 266), the present study did not require approval from the ethics committee.

Before analysis, surface contamination was removed from the hair samples. For this purpose, the samples were first washed in ultrapure water, then in SDS (0.1%, w/v) and finally twice more in ultrapure water. Between each step of washing and the next, the hair was subjected to sonication for 5 min. After washing the hair was cut into pieces of 2–3 mm length with stainless-steel scissors and dried at room temperature.

Hair sample treatment and analysis. Analysis was carried out according to the method previously described by Rodríguez-Gómez *et al.* (61), with slight modification. Washed hair samples (100 mg) spiked with the BP-d_{10} internal standard (125 ng/g) were incubated overnight at 38°C with a mixture of methanol and acetic acid (2 mL, 85:15, v/v) in 10 mL glass centrifuge tubes. The samples were then cooled to room temperature, treated with 3 mL of acetone for the extraction of analytes, sonicated for 15 min, and centrifuged for 10 min at 2,900 × *g*. The liquid phase containing the analytes was transferred to 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. After this, a 10 µL aliquot of the extract was injected into the liquid chromatography–tandem mass spectrometry instrument (LC-MS/MS).

Analysis was performed using an Agilent 1290 Infinity II ultra-high performance liquid chromatography system (Agilent, Santa Clara, CA, USA). Separation was carried out using a HALO C-18 Rapid Resolution column with 50 × 4.6 mm i.d. and 2.7 µm particle size (Advanced Materials Technology, Wilmington, DE, USA). The mobile phase was composed of a 10 mM ammonium acetate solution (solvent A) and methanol (solvent B). The elution gradient was linear: from 23% to 70% solvent B from 0 to 14 min, from 70% to 80% of solvent B from 14 to 19 min, an increase to 100% from 19 to 25 min, retention at 100% from 25 to 27 min, reduction to initial conditions from 27 to 29 min and retention at these conditions for equilibration from 29 min to 34 min. The column temperature was maintained at 30°C and the flow rate was 0.6 mL/min.

The LC system was coupled to a 6495 triple quadrupole mass spectrometer (Agilent) for mass spectrometry with an electrospray ionisation source operated in both positive and negative modes. The analyses were carried out using dynamic multiple-reaction-monitoring mode and two transitions were selected for each analyte for quantification and confirmation purposes. The MS/MS setting parameters and transitions for each BP are given in supplementary Table S2.

Quality assurance and quality control. To achieve precise and reliable results, a quality assurance/quality control (QA/QC) protocol was established. It described the use of control spiked samples, solvent (methanol) injections, standards containing a mixture of the target compounds (20 ng/mL) and procedural blanks (processed in the same way as the samples) in each analytical batch (15 samples). Blank samples were processed in the same manner as hair samples.

In-house reference probe materials (prepared by spiking real samples at 10, 50 and 100 ng/g levels) were utilised to check the accuracy during validation and QA/QC. The analytical features of the method are described in supplementary Table S3.

Statistical analysis. The statistical analysis was performed using GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, CA, USA) with a non-parametric Mann–Whitney test for a comparison of two groups of animals and a Kruskal–Wallis test for the comparison of three groups. In the statistical analysis, only values above the method quantification limit (MQL) were taken into account. Differences were considered statistically significant at P-value < 0.05.

Results

The QA/QC outcomes were satisfactory: no quantifiable amounts of the compounds studied were noted in the blank samples, the recoveries were in the range of 98–113% and the relative standard deviation was <12%.

During the study, all the BPs included in the investigation were found in the dog hair samples. The concentration levels of these substances in particular animals varied considerably (Table 1 and Table S4).

The only BP observed in all samples at levels above the MQL was BP-3. Its concentration levels fluctuated from 4.75 ng/g to 1,765.00 ng/g with a mean value of 73.77 ng/g. The second most frequently observed BP was BP-1, found in 37% of the samples. Interestingly, the mean concentration level of BP-1 was higher than that of BP-3 and amounted to 88.86 ng/g, the levels in particular animals ranging from below the method detection limit (MDL) to 666 ng/g. The remaining two BPs, namely BP-2 and BP-8, were found in only 5% of the study samples. The concentration levels of BP2 in

particular animals fluctuated from below the MDL to 8.86 ng/g (mean 7.48 ng/g), and those of BP-8 from below the MDL to 14.20 (mean 6.36 ng/g).

The mean concentration levels of BP-1 (± standard deviation) in male dogs amounted to 109.70 ± 171.70 ng/g, and in female dogs to 69.11 ± 71.50 ng/g. In the case of BP-3 these values were 100.30 ± 251.10 ng/g and 47.29 ± 68.35 ng/g. The concentration levels of both BP-1 and BP-3 were higher in male dogs, but sex differences in BP concentration levels were not statistically significant.

Some differences in the mean values of BP-1 and BP-3 were also observed between different age groups of dogs. The mean concentration level of BP-1 amounted to 123.80 ± 113.10 ng/g in animals aged up to 2 years, 50.18 ± 59.23 ng/g in dogs which were 2–10 years old and 75.65 ± 108.80 ng/g in those aged over 10 years. In the case of BP-3 the mean values amounted to 61.38 ± 87.89 ng/g, 77.91 ± 240.90 ng/g and 73.89 ± 91.67 ng/g, respectively. However, there were no statistically significant differences in BP-1 and BP-3 levels between particular age groups of dogs.

Regarding the comparison of BP-1 and BP-3 concentration levels between animals having different diets, the mean concentration level of BP-1 was 67.53 ± 95.18 ng/g in dogs eating primarily commercial dry food, 91.93 ± 85.95 ng/g in animals receiving mainly commercial canned food and 150.30 ± 257.90 ng/g in dogs provided food prepared at home. In this case there were no statistically significant differences between particular diet groups. The BP-3 values in turn amounted to 52.08 ± 47.48 ng/g, 137.30 ± 307.80 ng/g and 32.57 ± 49.01 ng/g, respectively. In contrast to the diet group differences of BP-1 concentration, differences in BP-3 concentration levels between dogs receiving food prepared at home and dogs fed commercial canned food were statistically significant.

Statistically significant differences were also noted in BP-3 concentration levels between dogs which had been castrated/spayed (for which the mean amounted to 91.25 ± 224.30 ng/g) and intact dogs (for which the mean was 41.31 ± 57.27 ng/g). In the case of BP-1 these values were 72.85 ± 75.99 ng/g in castrated/spayed dogs and 126.70 ± 210.10 in intact dogs, but the differences between the groups were not statistically significant.

During the present study, clear correlations were observed between BP-1 and BP-3 concentration levels and the use of both animal hygiene products and owners' hand cream. When comparing dogs on which these products were used with dogs on which they were not used, it was found that the mean concentration levels in the first group amounted to 96.07 ± 133.30 ng/g for BP-1 and 91.27 ± 210.90 for BP-3 and in the second group to 7.15 ± 9.42 ng/g and 21.29 ± 15.83 ng/g, respectively. The product-exposed dogs' hair samples contained statistically significantly higher concentrations of both BPs.

A similar situation was observed when the use of hand cream by dogs' owners was considered. In dogs with owners using hand cream more than once a week, mean concentration levels of BP-1 and BP-3 reached 118.60 ± 151.70 ng/g and 100.21 ± 228.61 ng/g, respectively. These values were statistically significantly higher than those in dogs owned by infrequent hand cream users, where mean concentration levels were only 34.01 ± 39.21 ng/g and 28.76 ± 28.67 ng/g for BP-1 and BP-3, respectively.

During the present study, clear connections between BP-3 concentration levels and frequency of visits to a veterinary clinic were observed. In samples collected from dogs which visited the veterinary clinic three times or more a year, the mean BP-3 concentration level was 115.50 ± 253.30 ng/g and was statistically significantly higher than the 31.82 ± 38.21 ng/g observed in dogs who visited the veterinary clinic less than three times a year. The mean level of BP-1 amounted to 118.60 ± 161.30 ng/g and 49.80 ± 54.72 ng/g in the frequent patients and less frequent patients, respectively. In contrast to the BP-3 concentration differences, those in BP-1 concentrations were not statistically significant.

A summary of the results containing the mean concentration levels of BP-1 and BP-3 obtained in particular groups of dogs is provided in Table 2. The presence of BP-2 and BP-8 was found in too few samples to permit comparisons between particular groups of dogs.

Table 1. Concentration values (ng/g) and frequency of detection (%) of benzophenones as cumulative data from the analysed dog hair samples (n = 100)

Compound	Range (ng/g)	Arithmetic mean	Geometric mean	Median	Frequency of detection above MQL (%)
BP-1	<MDL–666	88.86	34.00	39.90	37
BP-2	<MDL–8.86	7.48	7.38	7.65	5
BP-3	4.75–1,765.00	73.77	33.96	30.65	100
BP-8	<MDL–14.2	6.36	5.28	4.77	5

BP-1 – benzophenone 1; BP-2 – benzophenone 2; BP-3 – benzophenone 3; BP-8 – benzophenone 8; MDL – method detection limit (0.50 ng/g for BP-1, 0.30 ng/g for BP-2, 0.50 ng/g for BP-3 and 0.90 ng/g for BP-8); MQL – method quantification limit (2.00 ng/g for BP-1, 1.00 ng/g for BP-2, 2.00 ng/g for BP-3 and 3.00 ng/g for BP-8)

Table 2. The correlation with health, pet care and owner factors of benzophenone 1 (BP-1) and benzophenone 3 (BP-3) mean concentration levels in ng/g (±standard deviation) in canine hair samples

Sex						
Compound	Female		Male			P-value
BP-1	69.11 ± 71.50		109.70 ± 171.70			0.7242
BP-3	47.29 ± 68.35		100.30 ± 251.10			0.1365
Age						
Compound	Young (Y)	Middle-aged (M)	Old (O)	P-value Y/M	P-value M/O	P-value Y/O
BP-1	123.80 ± 113.10	50.18 ± 59.23	75.65 ± 108.8	0.1843	>0.9999	>0.9999
BP-3	61.38 ± 87.89	77.91 ± 240.9	73.89 ± 91.67	>0.9999	>0.9999	>0.9999
Diet						
Compound	Commercial dry food (D)	Commercial canned food (C)	Food prepared at home (H)	P-value D/C	P-value C/H	P-value D/H
BP-1	67.53±95.18	91.93 ± 85.95	150.30 ± 257.90	0.9319	>0.9999	>0.9999
BP-3	52.08±47.48	137.30 ± 307.80*	32.57 ± 49.01*	0.7265	0.0038*	0.1123
Castration/sterilisation						
Compound	Castrated/spayed dogs		Intact dogs			P-value
BP-1	72.85 ± 75.99		126.70 ± 210.10			0.6290
BP-3	91.25 ± 224.30*		41.31 ± 57.27*			0.0197*
Use of animal hygiene products						
Compound	Yes		No			P-value
BP-1	96.07 ± 133.30*		7.15 ± 9.42*			0.0255*
BP-3	91.27 ± 210.90*		21.29 ± 15.83*			<0.0001*
Frequency of hand cream application by dog owners						
Compound	More than once a week		Less than once a week			P-value
BP-1	118.60 ± 151.70*		34.01 ± 39.21*			0.0210*
BP-3	100.21 ± 228.61*		28.76 ± 28.67*			0.0005*
Frequency of visits to veterinary clinics						
Compound	More than three times a year		Less than three times a year			P-value
BP-1	118.60 ± 161.30		49.80±54.72			0.1447
BP-3	115.50 ± 253.3*		31.82 ± 38.31*			<0.0001*

BP – benzophenone; * – statistically significant difference (P-value < 0.05)

Table 3. Selected previous studies on concentration levels of benzophenone 1 (BP-1), benzophenone 2 (BP-2), benzophenone 3 (BP-3) and benzophenone 8 (BP-8) in humans and animals. Benzophenone concentration levels are shown in ng/g (in solid matrices) or ng/mL (in liquid matrices)

Species	Country	Matrix	n	BP-1	BP-2	BP-3	BP-8	Ref.
Human	Brazil	urine	300	0.01–1,910.00	0.25–8.25	0.70–9.83	0.01–2.69	59
	China	amniotic fluid	15	<0.02–3.22		<0.01–0.38	<0.01–0.60	66
	China	urine	100	<0.07–14.60		<0.11–46.10		78
		blood	75	<0.06–0.15		<0.41–3.88		
	Denmark	urine	309	<LOD–129.00	<LOD–9.84	<LOD–644.00		23
		blood serum	306	<LOD–0.86	<LOD–0.10	<LOD–5.18		
		seminal plasma	297	<LOD–10.90	<LOD	<LOD–5.94		
	Spain	breast milk	10	ND–0.51		ND–15.70	ND–0.73	61
		placenta	79	<LOD–3.32	0.38–1.93	0.65–3.09	<LOD	19
		nails	22	6.30–194.00	ND	25.00–245.00	ND–3.90	48
	USA	urine	2517			0.40–21,700.00		6
Dog	USA	urine	50	<LOQ–9.20		<LOQ–46.00	LOQ–0.70	32
Cat	USA	urine	50	<LOQ–13.50		<LOQ–61.60	<LOQ–8.40	
Wild bat	Poland	guano	5	10.50–34.80	<LOD	<LOQ–15.50	<LOD	46
Beluga whale	Canada	liver	80			<LOD–1,320.00		4
Wild bird	Spain	eggs		<LOQ–677.00		16.90–49.30		50
Turtle	Italy	blood	32			<LOD–28,430.00		13
Fish	Brazil	body	11	<LOQ		3.50–15.40		51
	Greece	body	8	ND	11.80–41.90	<LOQ–1.80		14
	Spain	body	193			ND–2.20		25

LOD – limit of detection; LOQ – limit of quantification; ND – not detected

Discussion

The results obtained have confirmed that dogs are exposed to benzophenones. It is related to the common use of BPs in various branches of industry and the resultant extensive pollution of the environment (26, 34, 44, 45), and to dogs' exposure to the same toxic factors as their owners, being companion animals living in close proximity to humans. The present study has shown that dogs are exposed to BP-3 to the greatest extent, which was found in all samples included in the study. It agrees with previous studies, which have shown that BP-3 is the benzophenone that pollutes the natural environment most often and in the largest quantities and one which occurs in living organisms (26, 44, 45). The present study has also revealed the second most common BP in dogs to be BP-1. Its presence in hair samples results not only from the presence of this substance in the environment and food (26), but potentially also from its derivation from BP-3 as a metabolite produced in the bodies of humans and animals (54).

Many previous studies have shown human exposure to BPs (Table 3). Most of them described BPs in urine. This matrix is most often used for these types of analyses, because BPs are eliminated from the body mainly by the kidneys (53). However, BPs have also been described in human blood, breast milk, seminal plasma, amniotic fluid, nails and various tissues (45, 70). It should be emphasised that, until now, hair samples had not been used to study the exposure of living organisms to BPs.

In contrast to knowledge of human exposure to BPs, knowledge of animals' exposure to them is extremely sparse. Some studies have shown wild animal exposure to these substances (Table 3). However, to the best of the authors' knowledge, studies concerning domestic animals are limited to one study, in which levels of BP-1, BP-3 and BP-8 were described in the urine of dogs and cats in the USA (32).

It is difficult to compare the present results with those published by Karthikraj *et al.* (32) because the matrices used in the analyses were completely different. It is known that the levels of various substances in urine may undergo short-term changes and do not always correspond to the levels of the same substances in hair (18). Moreover, the study of Karthikraj *et al.* (32) was conducted in a completely different part of the world to the current research, which is relevant given that the levels of BPs both in the environment and in living organisms noticeably depend on the place where the observations were made. Benzophenone concentration levels in humans are related to the urbanisation and industrialisation of the investigated area; the lifestyle, ethnic group and financial status of its residents; and the frequency of their cosmetic use (6, 34, 45).

Although an exact comparison of BP concentration levels in such diverse matrices as urine and hair is practically impossible, it is illustrative that the values observed in dog hair during the present study appear to be higher than those observed in dog urine in a previous study (Table 3). The reason for this may be the probable accumulation of BPs in the hair, which does not take place in urine, in which these substances are excreted daily. Moreover, it should be remembered that substances present in hair samples may come from two sources: external, when compounds penetrate the hair directly from the environment; and internal, when compounds reach the hair through blood and the follicle and root (78). The double sources may also be the reason for the higher BP concentrations in hair compared to in urine or blood.

During the present study, no statistically significant differences in BP concentration levels between male and female dogs or between animals of one age and another were observed. This outcome differs from those of previous studies in humans. Such studies have shown that BP concentration levels are usually higher in women than in men (6, 9, 37, 71, 79), as well as in young adults than in children and elderly people (9, 22, 73, 79). The lack of statistically significant differences in BP concentration levels between dogs of different ages and sexes reported in this study strongly suggests that differences noted in studies in humans may be due to the greater likelihood of women and young adults using cosmetics and personal care products containing BPs (6, 45, 79). However, some studies have described differences in BP metabolism and the effects of exposure to these substances between males and females (55, 58). Therefore, it cannot be excluded that these differences also result in concentration levels of BPs differing between individuals purely through sex difference; however, the results of the present study do not confirm this.

Previous studies have proved that the use of cosmetics (especially makeup, skin lotions and lipstick), shampoos, personal care products and sunscreens is one of the most important ways by which people are exposed to BPs (especially BP-3) (40, 45). Concentration levels of BP-3 in these products may even reach 1,480,000 ng/g (40). It is also known that transdermal absorption is the major route of exposure to BP-3 (31, 45, 62). The degree of exposure to BP associated with personal care products and cosmetics clearly depends on the part of the world in which particular examples are used and the lifestyle of the citizens using them; it has been calculated that cosmetic products containing BPs are the reason for daily transdermal absorption by US American women of BP-3 at the level of 24.4 μg day^{-1} (40). In the present study notably higher concentration levels of BP-1 and BP-3 were found in hair samples collected from dogs in the care of which animal hygiene products were used, as well as those collected from dogs with owners often using hand cream. This confirms that cosmetics and hygiene products may be an important source of exposure to BPs not only for humans, but also for animals.

Another route (apart from skin absorption) of penetration of BPs into living organisms is the digestive tract (45, 49, 73). It is established that BPs may often be present in tap and bottled drinking water and various types of food (8, 38, 67). Benzophenones have been

found in fish and other marine organisms at relatively high concentration levels (8, 25), which is associated with significant pollution of surface water with BPs (44).

The presence of BPs has also been described in other types of food, and an important source of them in food is the packaging (5, 8). It is common practice that BPs are added to plastic food containers as blockers of ultraviolet radiation, and because of this, food can have a longer expiration date in transparent boxes (45, 77). Furthermore, BPs may be a component of box coatings and food packaging printing ink, and from them may have a means of penetrating from boxes to food (5). Unfortunately, as yet animal food has not been tested for the presence of BPs. However, the results of the present study, in which dogs fed mainly wet food showed clearly higher levels of BP-3 concentration than dogs fed food prepared at home, strongly suggest that some kinds of dog food may carry a higher risk of exposure of dogs to this substance.

During the present study, it was found that BP-3 concentrations were statistically significantly higher in dogs which visited a veterinary clinic more often than others. This observation may be evidence of the negative impact of this substance on the health of dogs. It is relatively well researched that BPs (including BP-3) as endocrine disruptors act on oestrogen receptors (as agonists) and androgen receptors (as antagonists) (20, 41, 45). Because these receptors are present in many organs, BPs may have adverse effects on various internal systems, including the nervous, gastrointestinal cardiovascular and reproductive systems (41, 45, 52).

Previous studies have also reported that benzophenones show immunosuppressive activity that disturbs the balance between T helper 1 and T helper 2 lymphocytes, reducing the production of interferon gamma and increasing interleukin 10 synthesis (24, 33, 57). Other investigations have described allergic, carcinogenic and genotoxic processes induced by exposure to BPs (1, 15, 41, 45). The previously referred to multidirectional toxic activity of BPs probably causes that dogs that are more exposed to BPs are more susceptible to various diseases and therefore require more frequent visits to a veterinary clinic. The present results seem to confirm this theory.

Interestingly, during the present study statistically significantly higher concentration levels of BP-3 were noted in dogs which had been subjected to castration or spaying compared to intact animals. The reason for this difference is not known. Previous studies have reported that higher exposure to BPs is associated with lower levels of sex hormones (69), but it is connected with the endocrine-disrupting activity of BPs (41). While the results of the present study suggest that the levels of sex hormones may have an impact on the metabolism of BP, and the lower concentration of sex hormones observed in neutered dogs reduces the rate of metabolism of BP-3, an exact explanation of this problem requires further investigation.

The present study is the first evaluation of dog exposure to BPs through the analysis of hair samples. The study confirms that hair samples, as a matrix that can be collected easily and stress-free, are useful for assessing the exposure of domestic animals to BPs. The results obtained showed that dogs were exposed mainly to BP-3 and BP-1, whereas BP-2 and BP-8 were present in only a small percentage of the samples tested, which generally is in agreement with previous investigations regarding the presence of BPs in the environment and humans. The present results also indicated that BP concentration levels in hair samples depended on various factors, including a dog's diet, sex hormone levels and the use of cosmetics and hygiene products both for animals and their owners. In addition, higher exposure to BP-3 has been shown to be associated with the need for more frequent veterinary medical treatment, which strongly suggests the negative impact of BPs on the health of dogs.

Conclusion

To sum up, the present study has proved that dogs, as animals living in close proximity to humans, are exposed to health-detrimental BPs, and that the degree of this exposure depends on various environmental factors. In light of the present study, hair samples may be a good alternative to "classic" matrices such as urine or blood serum in which to analyse dog exposure to BPs. However, many aspects related to factors influencing dog exposure to BPs, the use of hair to assess this exposure, and exact correlations between BPs and the occurrence of specific diseases in dogs require further studies.

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