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ANALYSIS OF NICOTINE IN DOG HAIR: SIGNIFICANT DOSE-RESPONSE TO PASSIVE SMOKE

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Abstract

To study the effects of passive indoor smoke on house pets, hair samples were collected from indoor dogs living with owners who were non-smokers (non), moderate smokers (modt), and heavy smokers (hvy). Nicotine was extracted from the hair, and analyzed by gas chromatography-mass spectrometry. Nicotine contents in ppm (median ± standard error) were 0.0 ± 1.0 (non), 5 ± 3 (modt), and 26 ± 5 (hvy), and the differences were statistically significant (P << 0.05). We conclude that dogs process the nicotine in passive indoor smoke as humans do, so hair analysis is a reliable indicator of long-term exposure to passive smoke. Furthermore, based on our results and those of others, pets are quite likely to be at risk from deleterious health effects stemming from the inhalation of passive smoke.

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Introduction

Cigarette smoking kills nearly a half million Americans per year,[1] and has been shown to cause numerous diseases, including heart attacks and strokes, peripheral arterial disease, chronic obstructive pulmonary disease, high blood pressure, and several cancers (e.g., lung, pancreas, bladder, larynx/mouth). Breathing environmental tobacco smoke (a.k.a. passive smoke) has also been shown to be hazardous to the health of non-smokers. Health problems stemming from inhalation of passive smoke include cancer, stroke, high blood pressure, pulmonary dysfunction, and small-for-gestational-age births.[2]

Exposure to passive smoke is not limited to humans, however, as many smokers have pets that spend some or all of their time indoors. Experiments on fetal rats and guinea pigs have shown that nicotine (administered by injection into the bloodstream) interferes with brain development and inhibits melatonin synthesis.[3-5] Thus, prolonged exposure to passive smoke can also be hazardous to animals, as it is to humans. Passive smoke has been shown, for example, to increase the incidence of several illnesses in pets, including lung and oral/nasal cavity cancer[6-8] (but see [9]), dermatitis[10], and respiratory disease.[11-14] For a recent review, see reference 15.

Nicotine, the major pharmacologically active component of cigarettes, is a toxic liquid alkaloid that readily permeates epithelial tissue. An ingested or absorbed dose of 60 mg is fatal to a human adult, causing fever, trembling, nausea, convulsions, and ultimately, death.[16] From a single unfiltered cigarette, an individual may absorb a dose of 1 – 2.4 mg nicotine.[16] Nicotine is an acetylcholine agonist, activating acetylcholine receptors in the plasma membrane of excitable cells (e.g., neuron, heart), causing the opening of the receptor ion channel. It is a potent and addictive stimulant of nerve and muscle that can also be used as an insecticide.

Analysis for nicotine, or its metabolite cotinine, in blood, urine, or saliva gives a reliable indication of exposure to passive smoke within the preceding 1 – 3 days.,[15,17-19] Additionally, a simple new exposure test using silicone wristbands has been described recently.[20-22] The best test for long-term exposure is to analyze nicotine incorporated into hair[15,17,19,23-28] as this is a primary site of nicotine excretion/accumulation. Analyses using high pressure liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS) have demonstrated a strong correlation between the concentration of nicotine extracted from human hair and level of exposure to passive smoke.[2,17,26,29-31] Here we have expanded upon the results of previous studies in pets[32,33], showing a dose-dependent correlation in the hair of indoor pet dogs whose owners were non-, moderate, and heavy smokers.

Experimental Procedures

Collection of hair samples

Hair samples were collected from local (Salem, OR) dog grooming establishments. After the dogs were shampooed and rinsed, samples were cut with scissors as close to the scalp as possible, behind the ear. Each sample was placed in a paper envelope, which was sealed and labeled with the breed of dog and the smoking classification of the owner, which was ascertained by verbal query: non-smoker (non), moderate smoker (modt, 10 – 20 cigarettes/day), or heavy smoker (hvy, > 20 cigarettes/day).

Preparation of hair samples

All glassware was washed with technical grade acetone to minimize background contamination with nicotine. Hair samples removed from the envelope with tweezers were cut into 1 – 2 cm length, placed on a watch glass, rinsed for at least 15 min with technical grade acetone, then dried overnight. Care was taken to avoid direct contact with the experimenter's hands.

Each replicate comprised 20 – 30 mg of the rinsed hair segments weighed directly into separate 15 mL vials. The protein matrix of the hair samples was broken down by incubation in 2.00 mL of 5 M NaOH for 30 minutes, after which the following was added to each sample vial: 3 mL of diethyl ether containing 0.1 mg/mL of the internal standard 2,6-di-*t*-butyl-4-methylphenol, and 100 µL of *n*-butyl acetate.[30,34] The vials were closed with Teflon-lined screw caps and vortexed at medium speed for five minutes.

Each sample was then quantitatively transferred to a conical glass tube and centrifuged for 30 seconds to separate the aqueous and organic layers. Using a pipettor, precisely 2.00 mL of the organic layer was transferred to another conical glass tube, and evap-

orated to dryness under a stream of air. Then, precisely 200 µL of ether was added (by pipettor) to redissolve the organic residue. This extract was transferred quantitatively to GC/MC autosampler vials with low-volume glass inserts.

Instrumentation

Nicotine in the ether extracts of hair was determined by GC/MS on a Hewlett-Packard HP 5890 Series II instrument. The carrier gas was helium, with a linear velocity of 36.6 cm/s at 250 °C. Nicotine and the internal standard 2,6-di-*t*-butyl-4-methylphenol were separated on an HP-5 methyl silicone column (30 m x 0.25 mm) with a programmed temperature ramp of 60 to 250 °C (2 min initially at 60 °C, followed by an increase of 20 °C/min, with a final 3 min at 250 °C). The total run time was 16 min per sample. Retention times for nicotine and the internal standard were 7.86 and 9.14 min, respectively (Figure 1).

Calibration Curve

A series of standards from 0 to 20.00 ppm of nicotine in aqueous 0.1 M NaOH was prepared. A 2.00 mL aliquot of these standard solutions was ether-extracted following the protocol described above for the NaOH-digested hair samples. Triplicate samples were run for each standard concentration, and the entire process was carried out four times, with all of the resulting data averaged to make a single calibration curve (Figure 2). Linearity was good (slope = 0.0213 ± 0.0010 ppm^{-1}, R^2 = 0.992), given that the non-zero intercept (0.111 ± 0.011) was statistically significant (P = 0.0005). This non-zero intercept was probably due to background contamination of adsorbed nicotine. The full linear equation was used to determine nicotine concentrations in the hair sample GC/MS ether extracts: peak area ratio = 0.02126 ppm^{-1}·[nicotine] + 0.11137.

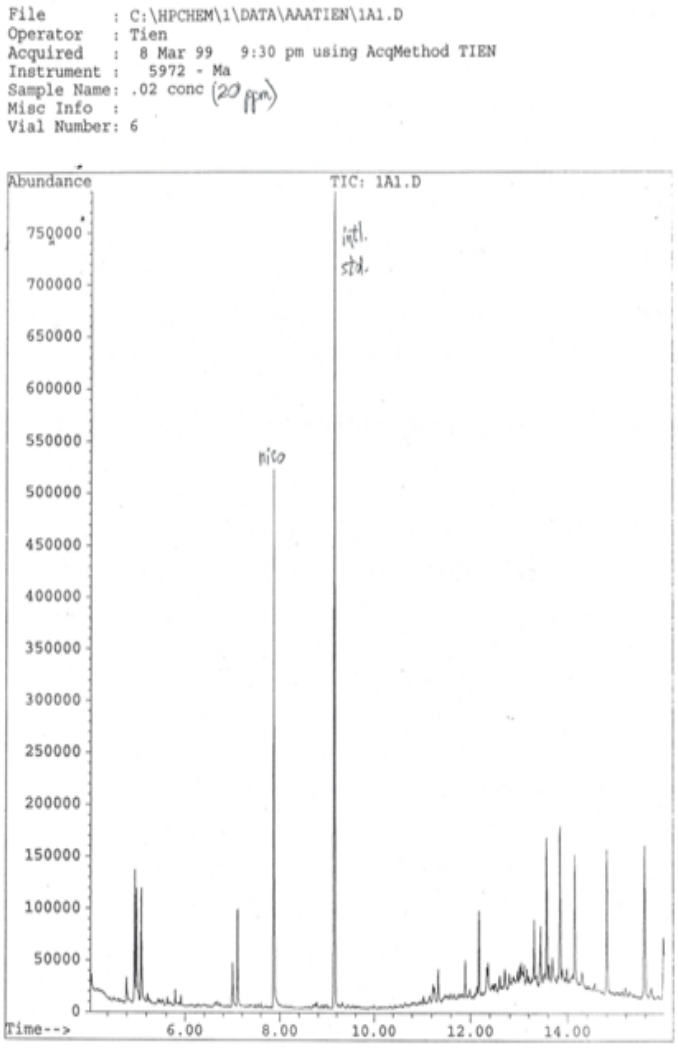

Figure 1: GC/MS of 20 ppm nicotine standard

The nicotine concentration in each GC/MS ether extract was converted to mass by multiplying by the mass of 200 µL of ether, 0.141(2) g:

µg of nicotine per GC/MS sample = ppm nico (= µg nico/g ether) x 0.141(2) g ether.

To get the total mass of nicotine per hair sample, µg of nicotine per GC/MS sample was multiplied by 1.55 (= 3.1/2) to account for the volume of organic layer left behind in the initial two-phase mixture. Finally, hair sample nicotine content was calculated by dividing the total µg nicotine by the initial mass of hair in each weighed sample: ppm nicotine = corrected µg nicotine/g of hair.

Results and Discussion

A total of 83 dog hair samples were tested, representing a number of different breeds: *n* = 29, 17, and 37 for non-, moderate, and heavy smokers. A histogram of all results is presented in Figure 3. In each of the three categories, a single sample was inordinately high (more than three standard deviations above the average), and was deemed to be an outlier after Q-testing (Table 2S, Appendix). Statistical results are tabulated below (Table 1); raw data can be found in Table 1S, and additional statistical analyses in Table 2S, in the Appendix.

Standard deviations for the average nicotine content in the three groups are rather large because each group included at least a few samples with no nicotine as well as two or more samples that exceeded the average value by more than two standard deviations. For this reason, we believe that median values ± standard error (bolded in Table 1) are more instructive than average values ± standard deviation. In any case, it is clear that dog hair nicotine

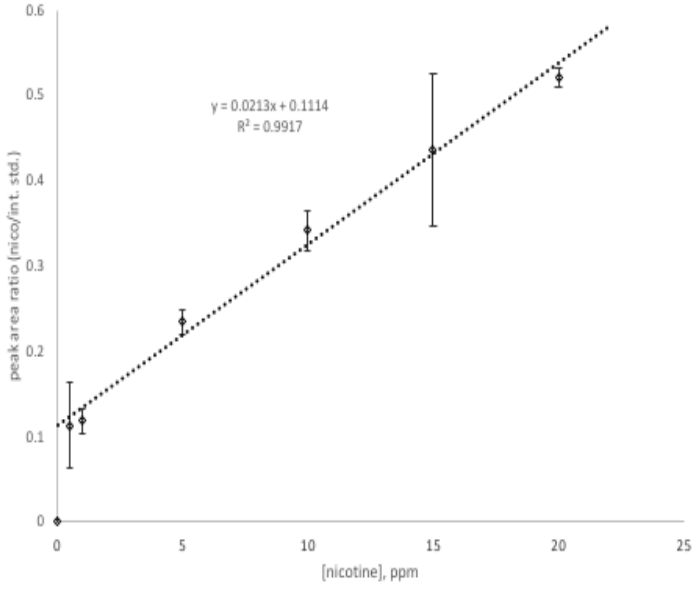

Figure 2: Nicotine calibration curve

content increased in the order non < modt < hvy. This can be seen in the maximum values (20.5 < 33 < 120 ppm) and in the number of samples lacking nicotine (82% > 35% > 8%). Finally, P-values were much less than 0.05 for the two comparisons: modt > non (P = 0.0002), and hvy > modt (P = 0.003).

Given that some of the wide range of experimental results was undoubtedly due to metabolic disparities in the different dog breeds sampled[6,15], it was fortunate that we had enough samples from a single breed, Cocker Spaniels, to obtain statistically significant results. Cocker Spaniel nicotine content results (Table 1) mirrored those from the entire data set: hvy (≈ 40 ppm) > modt > non (≈ 0 ppm), with the differences being statistically significant.

Conclusions

Our results on indoor pet dogs agree with previous studies on human hair from participants exposed to passive smoke: hvy > modt > non, with the differences being statistically significant.

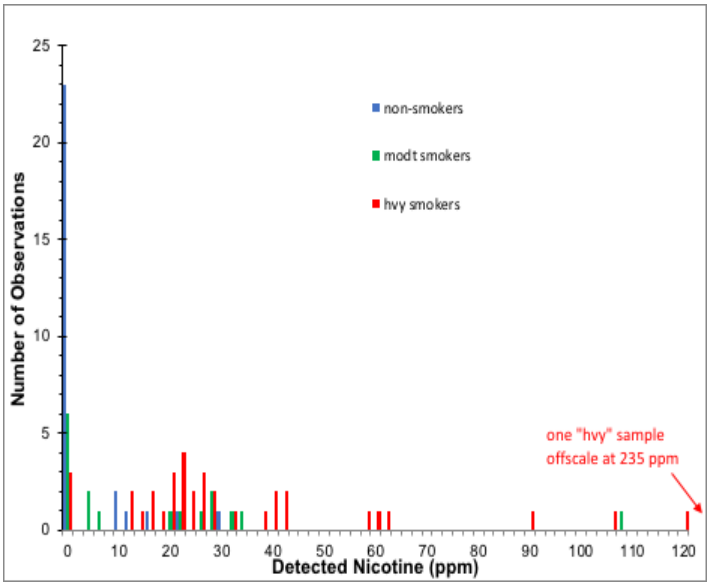

Figure 3: Dog hair nicotine content (ppm), histogram of all observations.

Table 1: Statistics for nicotine content (ppm) of hair samples from dogs belonging to non-, moderate, and heavy smokers.

	non	modt.	hvy
Number of samples:	28	17	37
Number of samples with no detected nicotine:	23 (82%)	6 (35%)	3 (8%)
Maximum (ppm):	20.5	33	120
Minimum (ppm):	0	0	0
Average (ppm):	2.3	13	36
Standard deviation (ppm):	5.5	14	31
Median (ppm):	**0.0**	**5**	**26**
Standard error (ppm):	**1.0**	**3**	**5**
P-value:		0.0002*	0.003**
Cocker Spaniel avg ppm:	3	30	43
Cocker Spaniel std. devn:	6 (n = 4)	2 (n = 3)	11 (n = 6)

*Moderate vs. non-smoker dogs; **heavy vs. moderate smoker dogs. Calculated P-values were from a one-tailed t-test assuming equal variances.

Many previous studies have used pet owner questionnaires to estimate exposure to passive smoke; our results (along with those of others[32,33,35]) have shown that, as suggested by Puzycki et al[15], analysis of nicotine in pet hair may be a more reliable indicator of exposure. Furthermore, the dog hair nicotine concentrations that we measured, especially in the heavy smokers group, are in the range where they can be expected to compromise pet health, as noted by quite a few recent studies. [6-8,10-15] It is thus quite likely that passive smoke inhalation harms pets as well as humans. Further study is called for to investigate whether breed and hair color differences affect hair nicotine content of indoor dogs exposed to passive smoke.[15,19]

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Appendix

Table 1S: Dog hair nicotine content (ppm), tabulated histogram of raw data.

Non-smokers		***Moderate Smokers***		***Heavy smokers***	
[nico], ppm	**# samples**	**[nico], ppm**	**# samples**	**[nico], ppm**	**# samples**
0	23	0	6	0	3
9	1	3	2	11	2
9.5	1	5	1	13	1
10.5	1	20	1	15	1
16	1	22	1	16	1
20.5	1	26	1	18	1
29.5	1	28	2	19	1
total:	29	31	1	20	2
		33	1	21	2
		108	1	22	2
		total:	17	24	2
				26	3
				27	1
				28	1
				32	1
				37	1
				40	2
				42	2
				58	1
				60	1
				61	1
				90	1
				100	1
				105	1
				120	1
				235	1
				total:	37

Table 2S: Dog hair nicotine content (ppm) statistics.

	Non-smokers	**Moderate smokers**	**Heavy smokers**
# samples (*n*):	29	17	37
Maximum (ppm)	29.5	108	235
2nd highest (ppm)	20.5	33	120
Minimum (ppm)	0	0	0
Q-test value	0.30*	0.45**	0.35**
Q-fraction***	0.31	0.69	0.49
Average (ppm)	2.(3)	13.	36.
Std. devn (ppm)	6.	14.	31.
Median (ppm)	0.0	5.	26.
Std. error (ppm):	1.0	3.	5.
# 0 ppm samples:	23	6	3
% of samples w/ 0 ppm:	82%	35%	8%

* Q-test value for 95% confidence; ** Q-test value for 99% confidence; *** Q-fraction = (maximum – 2nd highest)/(maximum – minimum); because Q-fraction > Q-test value for all three data sets, the highest sample can be discarded as a statistical outlier