

ACCUMULATION OF EXTRAORDINARILY HIGH CONCENTRATIONS OF EPISODIC PCB CONGENERS IN HORSES FROM VARIOUS FORAGES

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Introduction

Although PCBs are of relatively low volatility, the atmosphere is the major global transport medium¹ and vegetation accumulates the more volatile PCB congeners². Market produce³ and pasture forage⁴ accumulate especially *ortho*-rich PCB congeners with lower numbers of chlorine atoms. The *para*-poor nature of the more volatile congeners also renders them more readily metabolized and episodic in mammals⁵. Cows consuming these forages pass mainly low levels of the more persistent PCB congeners through the milk so that the exposure to episodic congeners would not necessarily be detected^{6,7}. Other animals and humans may also be exposed to high levels of these episodic congeners. Equine species are good candidates because of the mass of food consumed and the duration of foraging. In the single paper reporting PCB residues in horse meat, it was the highest of the commodities tested⁸. This paper reports initial findings in determining PCB residues in horses and their forage.

Methods

Blood samples were obtained from four geldings of sound quarter horse breeding with a range in age from 2 years to 19 years. They had all been on the same farm for more than one year and consumed essentially the same forage and feed. The samples were collected in early March prior to general grazing, anticipating an increasing accumulation of episodic congeners as the season progressed. Samples of oats, grass hay, alfalfa hay, dormant intended forage and a control dormant native warm season grass were also collected.

One mL of serum was used for gravimetric lipid determinations and 2 mL of serum were extracted with 4 X 10 mL of hexane:acetone (1:1) for PCB determinations. The extracts were combined and volume reduced to 1 ml, dried with sodium sulfate and cleaned by alumina (2% deactivated) column chromatography, eluting with 80 mL hexane. After collecting the hexane eluate, an additional equal volume of hexane was passed through the column to insure complete PCB collection.

Moisture content of the plant material was determined by drying 10 g to constant weight at 35° C. For the grass hay, 20 g was hydrated with 1.0 ml ultra-pure water and extracted with hexane:acetone (4:1) in 2 x 200 ml aliquots. This was excessive, and, for the remaining plant material, only 5.0 g was extracted with 2 x 100 ml hexane:acetone. The extracts were dried with anhydrous sodium sulfate, volume was reduced, solvent exchanged to hexane, and extracts were cleaned by alumina column chromatography as with the serum.

For all samples, the hexane eluate was concentrated and exchanged to isoctane (final volume of 1 mL). Samples were analyzed for 141 PCB congeners on a Hewlett Packard Gas Chromatography 6890N system equipped with a 60 m x 0.25 mm x 0.25 μ m DB-5 capillary column, a micro electron capture detector, and Agilent Chemstation software. Five standard congener mixtures (Accustandard[®], Inc., New Haven, CT) were used to establish multilevel calibration curves. The limits of quantification and detection were 5.0 and 2.5 ng/mL respectively.

Results and Discussion

The geldings had exceptionally high total PCB levels (Table 1). Likewise, the forages and oats appear to be some of the highest vegetation PCB concentrations reported (Table 1). Switch grass (*Panicum virgatum*) is a native prairie warm season grass with a relatively short growing season. The stand that was sampled was planted 3-5 years previously and was subjected to a controlled burn in the Spring of 2002. The low level of PCBs in the switch grass further confirms the lack of process or field contamination since all samples were collected in the same trip, stored in the same refrigerator and extracted/analyzed at the same time.

Table 1. Characteristics and Total PCB (ng/g) in Horses, Feed and Forages.

Sample	Age	Percent		Total PCB	
		moisture	lipid	(ng/g wet)	(ng/g lipid)
Oats (commercial)	2002 season	10.6	na	28.3	-
Alfalfa	2002 season	14.7	na	69.6	-
Grass Hay	2002 season	4.9	na	35.6	-
Pasture (ungrazed)	2002 overwintered	47.5	na	60.6	-
Switch grass	2002 overwintered	18.6	na	1.0	-
Horse 1	18 years	na	0.50	42	8486
Horse 2	19 years	na	3.72	98	2627
Horse 3	2 years	na	2.41	45	1868
Horse 4	14 years	na	2.12	5	247

Total PCB is total of quantifiable congeners.

Even at these very high concentrations, there were very few of the persistent congeners usually associated with even ambient PCB exposures and body burdens (Table 2). The proportions of the more volatile episodic congeners are consistent with other vegetation sources^{2,3,4} and strongly suggest recent exposure⁹. The lack of a definite age trend further suggests recent/current exposures and the unexpectedly high concentrations in the oats and hay offer a ready source.

Table 2. PCB congener analysis results.

PCB congener Bold = major congener	Cl position (major congener)	% of Total PCB							
		Hay	Alfalfa	Pasture grass	Oats	Horse #			
						1	2	3	4
4	2-2'			5.35					
6	2-3'		NQ	NQ					
8	2-4'	4.72	5.17	5.34	5.49	2.55	3.53	3.20	
19	2,6-2'	2.60	2.74	2.46	2.61				
18	2,5-2'	10.50	11.78	10.82	11.28	12.42	12.04	13.41	13.07
17/15	2,4-2'	5.74	5.95	5.80	6.14	10.09	10.07	9.66	26.96
27/24	2,6-3'	NQ	NQ	NQ	NQ				
16/32	2,3-2'	4.49	4.43	4.46	4.58	4.75	5.07	4.42	3.19
26	2,5-3'	2.07	1.95	1.89	2.09	7.54	4.07	6.67	NQ
25	2,4-3'	ND	NQ	ND	ND	NQ	NQ	NQ	
31	2,5-4'	7.26	7.84	7.41	8.21	8.65	8.93	10.11	
28	2,4-4'	7.37	7.76	7.51	8.02	7.85	8.15	9.10	1.53
33/20	3,4-2'	5.03	4.96	4.82	4.68	4.07	3.97	3.56	11.09
53	2,5-2',6'					NQ	1.19	NQ	NQ
22	2,3-4'	3.22	3.27	3.08	3.49	1.84	2.09	2.22	10.87
45	2,3,6-2'	1.78	1.96	NQ	NQ	1.36	1.07	1.81	7.02
52	2,5-2',5'	5.73	6.72	6.11	6.84	7.33	7.91	7.63	
49	2,4-2',5'	3.45	3.42	3.08	3.49	5.30	4.37	5.48	
47	2,4-2',4'	2.04	2.28	1.95	2.27	2.98	2.59	2.81	NQ
75	2,4,6-4'	NQ	1.51	NQ					
48	2,4,5-2'	2.30	2.73	2.17	2.96	1.60	2.07	0.10	0.83
44	2,3-2',5'	5.17	5.62	5.37	5.37	4.60	4.18	4.16	0.83
59	2,3,6-3'	ND	ND	ND	ND	ND	ND	ND	ND
42	2,3-2',4'	3.21	2.89	2.69	2.98	1.52	1.65	1.14	2.92
71	2,6-3',4'	1.62	1.83	1.72	1.94	1.37	1.03	1.60	3.08
64/41	2,3,6-4'	3.30	2.69	2.59	2.50	1.67	1.83	1.41	0.32
40	2,3-2',3'	NQ	NQ	NQ	NQ	NQ	0.76	NQ	NQ
74	2,4,5-4'	1.67	1.91	1.60	2.19	NQ	2.52	2.95	NQ
70	2,5-3',4'	3.73	4.34	4.10	4.64	4.58	4.27	5.40	6.76
66	2,4-3',4'	2.37	2.42	2.29	2.44	2.33	2.28	1.80	9.08
95	2,3,6-2',5'	ND	ND	ND	ND	0.63	0.61	0.49	2.45
60	2,3,4-4'	2.81	2.44	2.65	2.86	0.71	1.14	0.86	
84	2,3,6-2',3'	1.55	NQ	NQ	NQ	1.78	NQ	NQ	NQ
101/90	2,4,5-2',5'	2.17	2.39	2.12	2.41	2.48	2.61	NQ	NQ
99	2,4,5-2',4'	1.74	NQ	2.12	NQ	ND	NQ	ND	ND
97	2,4,5-2',3'	ND	ND	ND	ND	ND	ND	ND	ND
115	2,3,4,6-4'	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
87/85	2,3,4-2',5'	NQ	NQ	NQ	NQ	ND	ND	ND	ND
77	3,4-3',4'	ND	ND	ND	ND	ND	ND	ND	ND
110	2,3,6-3',4'	1.88	NQ	NQ	NQ	NQ	NQ	NQ	NQ
149	2,3,6-2',4',5'	NQ	NQ	NQ	NQ	ND	NQ	ND	NQ
118	2,4,5-3',4'	NQ	NQ	NQ	NQ	ND	NQ	NQ	NQ
146	2,3,5-2',4',5'			ND	ND				
153	2,4,5-2',4',5'	ND	ND	ND	ND				
105	2,3,4-3',4'	ND	ND						
138	2,3,4-2',4',5'	NQ		ND	ND				
Total PCB (ng/g, wet)		17.80	69.56	60.58	28.31	42.43	97.71	45.01	5.24

Additional farms in a 50 mile radius with similar horses and age ranges have been contacted and have agreed to provide serum and forage samples for a more comprehensive survey. Sampling will be conducted as the grazing season progresses on a limited number of subjects and forages.

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