Supplementary Information

Mass balance study of brominated flame retardants in female captive peregrine falcons

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Table S1. Information about the three captive female peregrine falcons studied.

Female ID number	*Body weight (g)*	*Age at sampling (year hatched)*	*Condition*	*Egg contents weight (g)*
223	700	16 (1990)	Somewhat lean	33.45
398	1052	11 (1995)	Good	33.97
466	971	4 (2002)	Good	37.72

Table S2. Absolute recovery of surrogate standards in the mass balance samples.

Surrogate standard	Samples: mean recovery ± STD	Blanks: mean recovery ± STD
BDE-138	69 ± 7% (n = 20)	89 ± 5% (n = 7)
$^{13}C_{12}$-BDE-209	77 ± 7% (n = 17)	89 ± 6% (n = 7)
Dechlorane 603	63 ± 5% (n = 20)	88 ± 7% (n = 7)

Determination of relative recoveries

The relative recoveries of the analytes were calculated from the results from a previous recovery exercise with egg samples spiked with HBCDD and all the native BDE congeners analysed for in this study[1]. Only Dechlorane was added as surrogate standard in that study (absolute recovery 88 ± 3%) and a native PCB congener (CB-189, not present in the samples) was added as recovery standard.

With the assumption that the absolute recoveries of all hexaBDEs are equal, the relative recoveries of the tri-heptaBDEs versus the surrogate standard BDE-138 used in this study, were estimated by means of BDE-153 (absolute recovery 93 ± 16%, that study). This assumption seems reasonable considering that the relative recovery of BDE-154 was 101 ± 0.7% (Table S2). For the other tri-heptaBDEs, the relative recoveries were between 88 and 105%. Octa-decaBDEs were quantified versus the labeled BDE-209 surrogate standard in this study. With the assumption that native and labeled compounds are recovered equally, the relative recoveries of octa-nonaBDEs were calculated versus the native BDE-209 (absolute recovery 96 ± 18%, that study) and were 92-109% (Table S2).

Table S3. Relative recoveries of analytes from spiked eggs. BDE-28 to BDE-191 were quantified versus BDE-153 (as a surrogate for BDE-138), and BDE-196 to BDE-207 were quantified versus BDE-209. HBCDD was quantified versus Dechlorane.

Analyte	Relative recovery ± STD
BDE-28	95 ± 3.2% (n = 6)
BDE-35	88 ± 2.5% (n = 6)
BDE-47	96 ± 3.7% (n = 8)
BDE-49	96 ± 3.3% (n = 6)
BDE-66	98 ± 2.8% (n = 6)
BDE-77	100 ± 2.5% (n = 6)
BDE-85	100 ± 1.4% (n = 6)
BDE-99	105 ± 5.1% (n = 8)
BDE-100	101 ± 2.1% (n = 6)
BDE-154	101 ± 0.68% (n = 6)
BDE-173	90 ± 8.9% (n = 5)
BDE-182	92 ± 8.6% (n = 6)
BDE-183	93 ± 7.8% (n = 6)
BDE-184	94 ± 6.4% (n = 6)
BDE-185	91 ±10% (n = 6)
BDE-191	91 ± 11% (n = 6)
BDE-196	101 ± 9.3% (n = 5)
BDE-197	109 ± 16% (n = 5)
BDE-203	100 ± 9.3% (n = 5)
BDE-205	102 ± 9.0% (n = 5)
BDE-206	97 ± 3.1% (n = 5)
BDE-207	92 ± 6.8% (n = 5)
HBCDD	121 ± 11% (n = 6)

Table S4. Concentrations of BFRs in captive peregrine falcon food, plasma, eggs (pg/g ww) and feces (pg/g dw). Concentrations below the LOQ but above the LOD are given as a *range (in italics)*. Concentrations below the LOD are given as < *values in italics*.

	Sample type	**Lipid %**	**BDE 47**	**BDE 49**	**BDE 99**	**BDE 100**	**BDE 153**	**BDE 154[a)]**	**BDE 183**	**BDE 196**	**BDE 197**	**BDE 203**	**BDE 206**	**BDE 207**	**BDE 208 [b)]**	**BDE 209**	**DBDPE**	**HBCDD**
Chicken mean (n=3)	food	8.3	5.8	0.26	13	3.7	9.7	*<0.4*	14	2.1	5.1	*<3*	1.1	2.3	0.88	22	230	<8
range		7.45-9.79	5.4-6.1	*<0.2*-0.26	13-14	3.6-3.7	9.5-9.9	*<0.4*-*<0.4*	13-15	2.0-2.3	4.7-5.5	*<3*-*<3*	*<0.3*-1.1	1.8-2.8	0.63-1.1	18-26	190-280	*<5*-<8
Quail mean (n=3)	food	10.4	1.5	0.43	2.7	0.50	*<1*	*<0.5*	0.90	<0.7	0.25	*<3*	0.45	1.5	0.44	33	61	21
range		10.3-10.5	1.4-1.7	0.36-0.47	2.6-2.8	0.40-0.58	*<1*-*<1*	*<0.4*-*<0.5*	<0.6-1.3	*<0.4*-<0.8	0.16-0.39	*<3*-*<3*	<0.3-0.69	1.4-1.7	<0.3-0.54	14-68	<30-130	21-21
Falcon identification code																		
223	plasma	1.01	*<7*	*<1*	<7	*<24*	30	8.0	11	*2-5*	3.0	*<15*	*0.2-0.5*	5.6	3.9	63	*<20*	*<10*
398	plasma	1.17	*<6*	*<1*	*<4*	*<23*	28	6.1	12	*2-5*	3.3	*<10*	*0.1-0.3*	3.8	2.8	48	*<20*	*<10*
466	plasma	1.41	*<7*	*<1*	10	*<25*	18	5.8	10	*2-5*	3.2	*<15*	*0.1-0.4*	5.7	4.0	80	*<20*	*<10*
223	egg	5.64	5.1	*<0.4*	59	23	350	81	97	26	32	12	4.1	40	26	350	*<40*	840
398	egg	4.95	*1-4*	*<0.4*	21	8.0	180	35	73	21	24	8.5	2.3	27	20	260	*20-60*	290
466	egg	5.59	7.8	*<0.3*	57	20	130	37	47	18	20	*3-10*	3.1	38	21	360	*<40*	650
223	feces	0.983	*1-4*	*<0.4*	*1-3*	0.7	4.4	*0.3-1*	2.1	1.3	0.46	*<5*	1.8	2.4	1.3	53	nd*	*<9*
398	feces	5.64	*4-11*	*<1*	13	4.4	20	*1-4*	12	18	*<1*	*<20*	50	69	52	1200	nd*	*<30*
466	feces	4.27	*<17*	4.7	*<17*	3.0	14	*3-8*	14	*3-9*	*<2*	*<40*	30	40	24	800	*100-300*	140
466	feces	2.19	*<7*	*<0.8*	<7	1.6	6.9	*0.7-2*	3.2	3.5	0.95	*<10*	3.1	5.8	3.9	100	nd*	*<20*

[a)] Quantified on peak height. Not baseline separated from other compound.

[b)] Estimated with BDE-206 and relative response factors.

* Not detected due to high background concentrations from sampling trays.

Food ingestion calculations

The average daily food intake for the 3 month period before blood sampling (November 2005 - January 2006) was estimated to be 130 g wet weight/d based on a previous one year feeding study of a captive female falcon.[2] Data on the number and average body weight of cockerels (39 g) and quail (144 g) consumed daily by each falcon pair over these 3 months prior to blood sampling was obtained from food diaries. When compiling the data from the diaries it was discovered that the falcons had also been given white mice (average body weight 30 g)as part of the diet. Half of each food type given was assumed to be consumed by the female. The average proportion of the amount of cockerels, quail and mice given to each falcon pair was determined and these were 75-76% for cockerels, 15.8-16.3% for quail and 8-9% for mice. The mice were included in the diet primarily during late November and the beginning of December.

The daily input flux from cockerels was then calculated using the proportion of cockerels consumed in the diet multiplied by the average daily food intake and the wet weight concentration of an analyte in cockerels. The input flux for quail was calculated using the proportion of quail consumed multiplied by the average daily food intake and the wet weight concentrations of an analyte in quail. Because no concentration data were available for mice, the input flux from mice was calculated using the proportion of mice consumed multiplied by the average daily food intake and a proportional mix of cockerel and quail wet weight concentrations. The total input flux is the sum of these three input fluxes.

Fecal output calculations

After feeding, peregrine falcons produce feces and egest a pellet containing bones, hair and feathers. Duke et al. [3] studied digestion time in several raptors including peregrine falcon. They determined that fecal excretion after feeding was fairly complete after 24 h and that peregrine falcon egested one pellet at the end of this period. The average food intake for peregrine falcon was determined to be 33.7 g dry weight (dw)/kg body weight (BW) and day. 4.7% of the food was egested as a pellet leading to an estimated pellet egestion of 1.6 g dw/kg BW and day.[3]

Barton and Houston[4-6] determined the digestive efficiency (DE) in several raptors, including peregrine falcons, using the following equation:

$$DE\ (\%) = \left[1 - \left(\frac{feces\ output\ (g\ dw) + pellet\ output\ (g\ dw)}{food\ intake\ (g\ dw)}\right)\right] \times 100 \qquad (1)$$

Although they measured feces production and pellet egestion, they did not report values. They did report the digestive efficiencies, which were 78.8 % (n=3)[5] and 75.2 % (n=9)[6]. Using the values for pellet egestion and dry weight food intake from Duke et al.[3] in the digestive efficiency equation on a kg BW and day basis, and the digestive efficiencies calculated by Barton and Houston[5, 6], fecal output was estimated to be 5.7 and 6.8 g dw/kg BW and day for peregrine falcons. For the purposes of the mass balance, we used the weighted mean of these values, 6.5 g dw/kg BW and day.

1. A. K. Johansson, U. Sellstrom, P. Lindberg, A. Bignert and C. A. de Wit, Polybrominated Diphenyl Ether Congener Patterns, Hexabromocyclododecane, and Brominated Biphenyl 153 in Eggs of Peregrine Falcons (Falco Peregrinus) Breeding in Sweden, *Environ. Toxicol. Chem.*, 2009, **28**, 9-17.
2. P. Lindberg, Relations between the diet of Fennoscandian peregrines Falco peregrinus and organochlorines and mercury in their eggs and feathers, with a comparison to the gyrfalcon Falco rusticolus, PhD dissertation, University of Gothenburg, 1983.
3. G. E. Duke, A. A. Jegers, G. Loff and O. A. Evanson, Gastric Digestion in Some Raptors, *Comp. Biochem. Physiol. A-Physiol.* 1975, **50**, 649-656.
4. N. W. H. Barton and D. C. Houston, The Use of Titanium-Dioxide As An Inert Marker for Digestion Studies in Raptors, *Comp. Biochem. Physiol. A-Physiol.* 1991, **100**, 1025-1029.
5. N. W. H. Barton and D. C. Houston, A Comparison of Digestive Efficiency in Birds of Prey, *Ibis*, 1993, **135**, 363-371.
6. N. W. H. Barton and D. C. Houston, The Influence of Gut Morphology on Digestion Time in Raptors, *Comp. Biochem. Physiol. A-Physiol.*, 1993, **105**, 571-578.

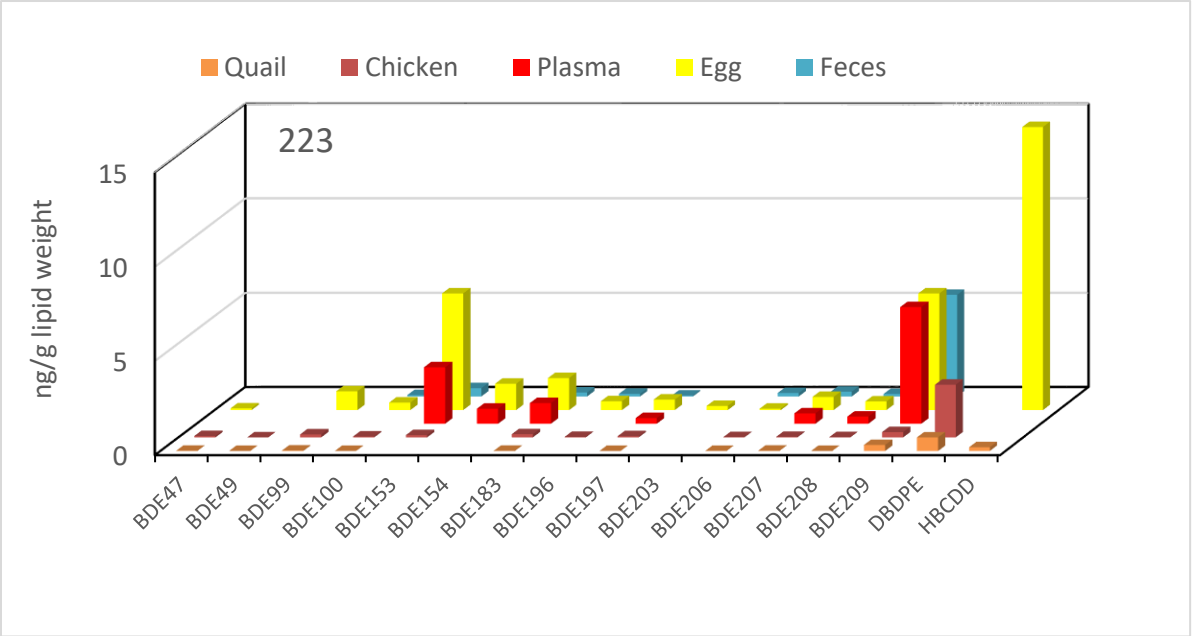

Figure S1. PBDE, DBDPE and HBCDD concentrations (ng/g lipid weight) in food (quail, chicken), plasma, eggs, and feces from peregrine falcon 223.

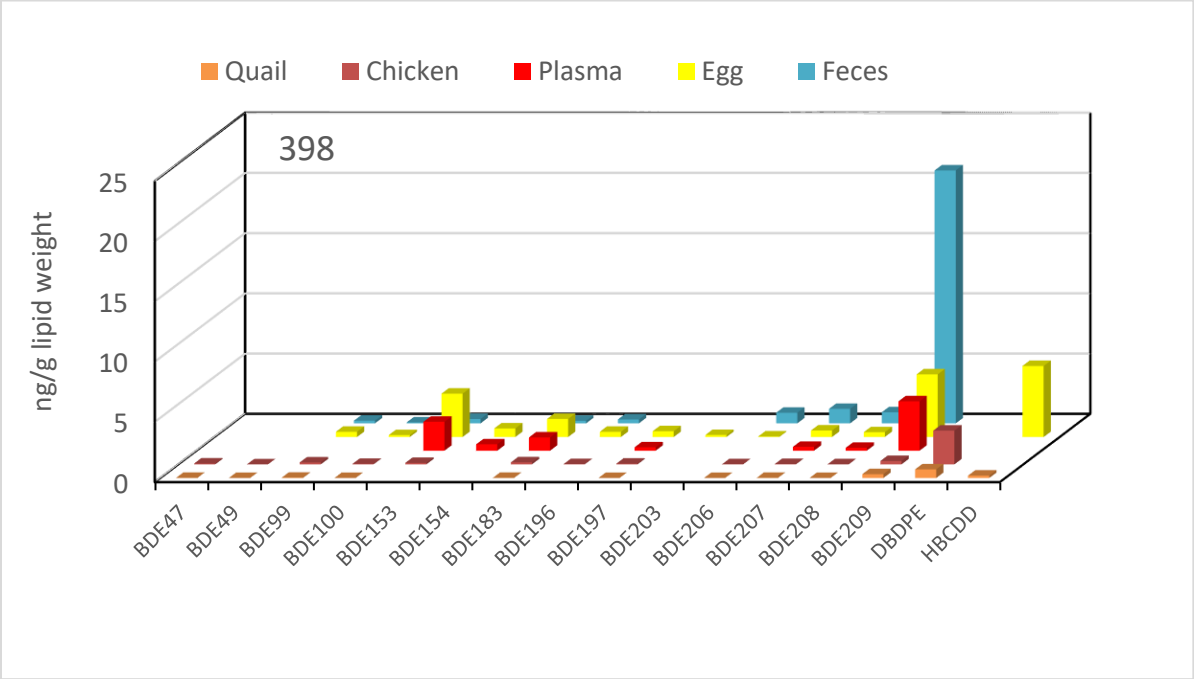

Figure S2. PBDE, DBDPE and HBCDD concentrations (ng/g lipid weight) in food (quail, chicken), plasma, eggs, and feces from peregrine falcon 398.

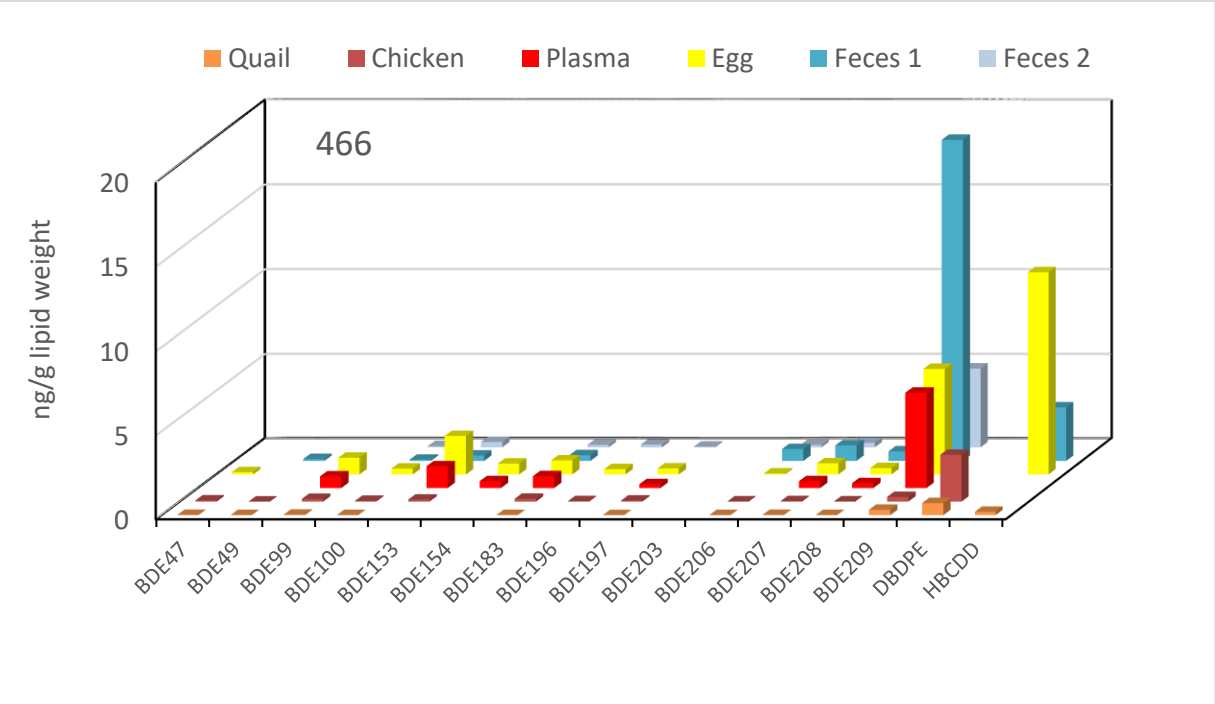

Figure S3. PBDE, DBDPE and HBCDD concentrations (ng/g lipid weight) in food (quail, chicken), plasma, eggs, and two feces samples from peregrine falcon 466.

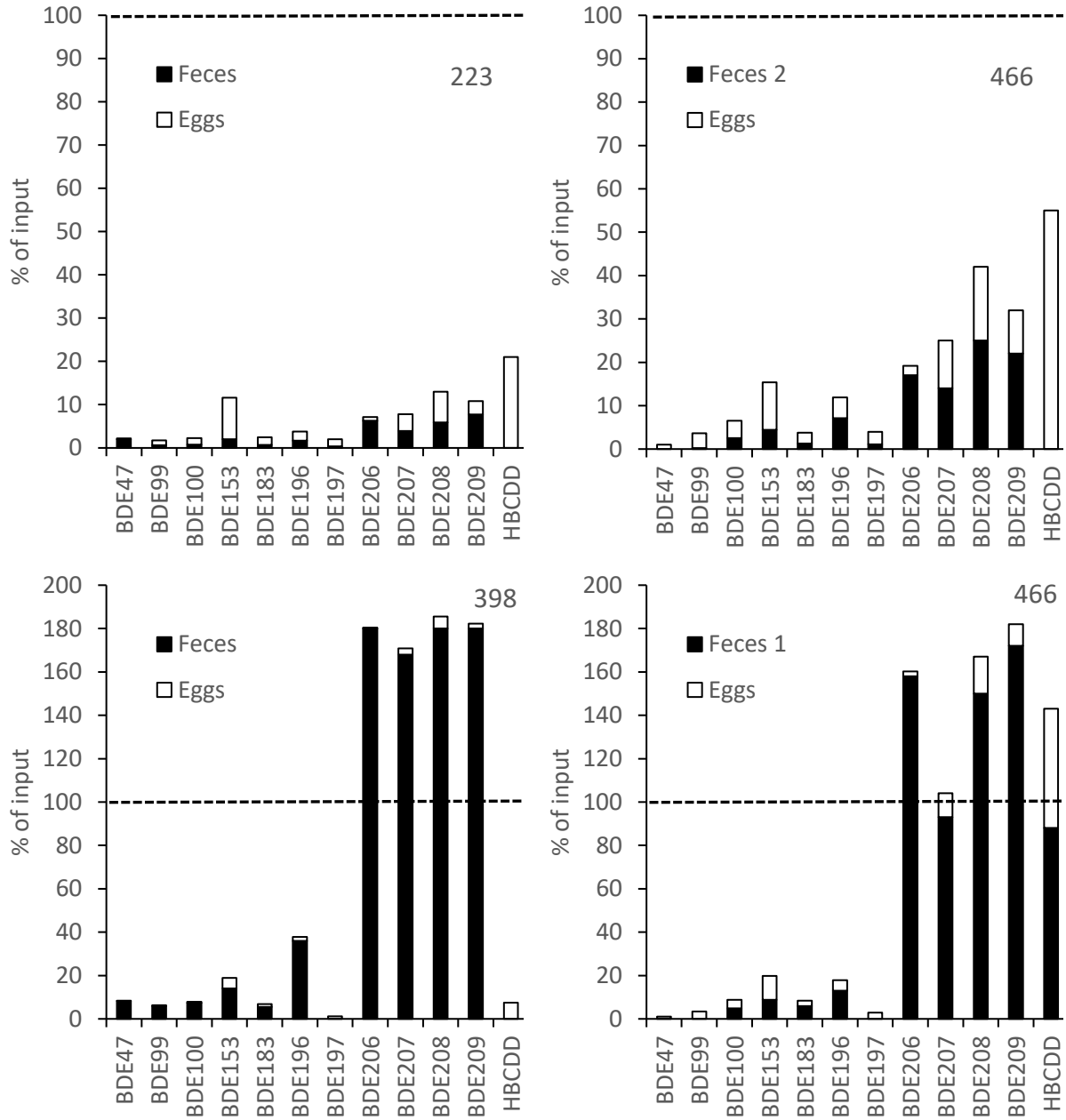

Figure S4. Results of the input-output mass balance for the three individual peregrine falcons (223, 398, 466 with both feces samples) with outputs from eggs and feces plotted as the percentage of total input from food. For falcon 398, BDE-207, -208 and -209 fecal outputs were 260, 360 and 270%, but are scaled to 180% for visual purposes here. The dotted horizontal line represents the total input from food (100%).

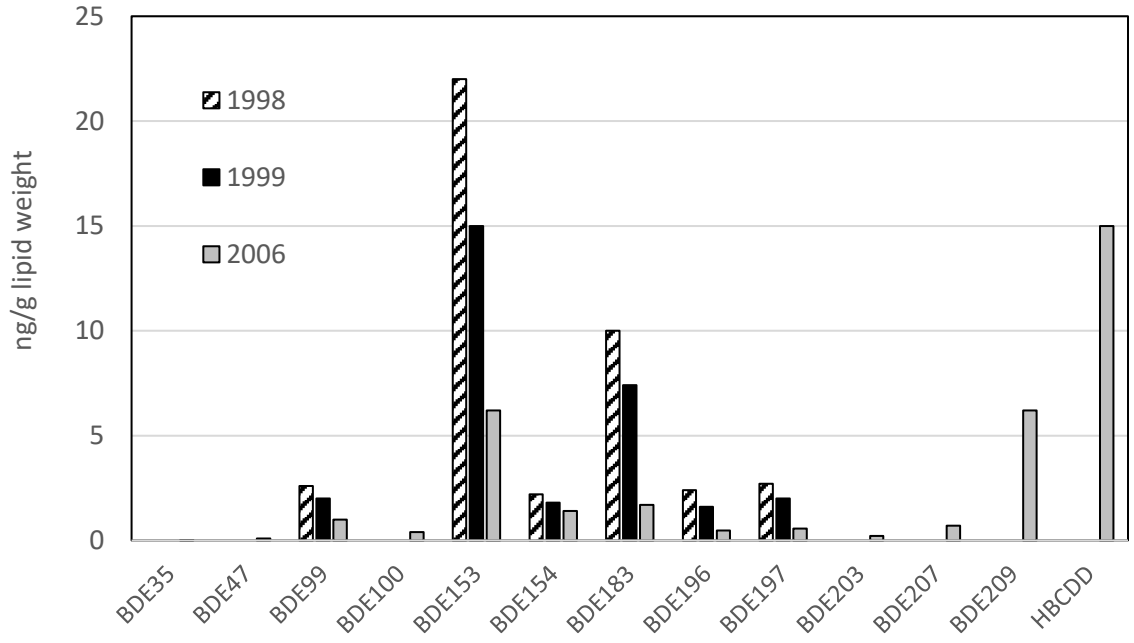

Figure S5. Time trends of PBDE and HBCDD concentrations in eggs collected in three different years from peregrine falcon 223.

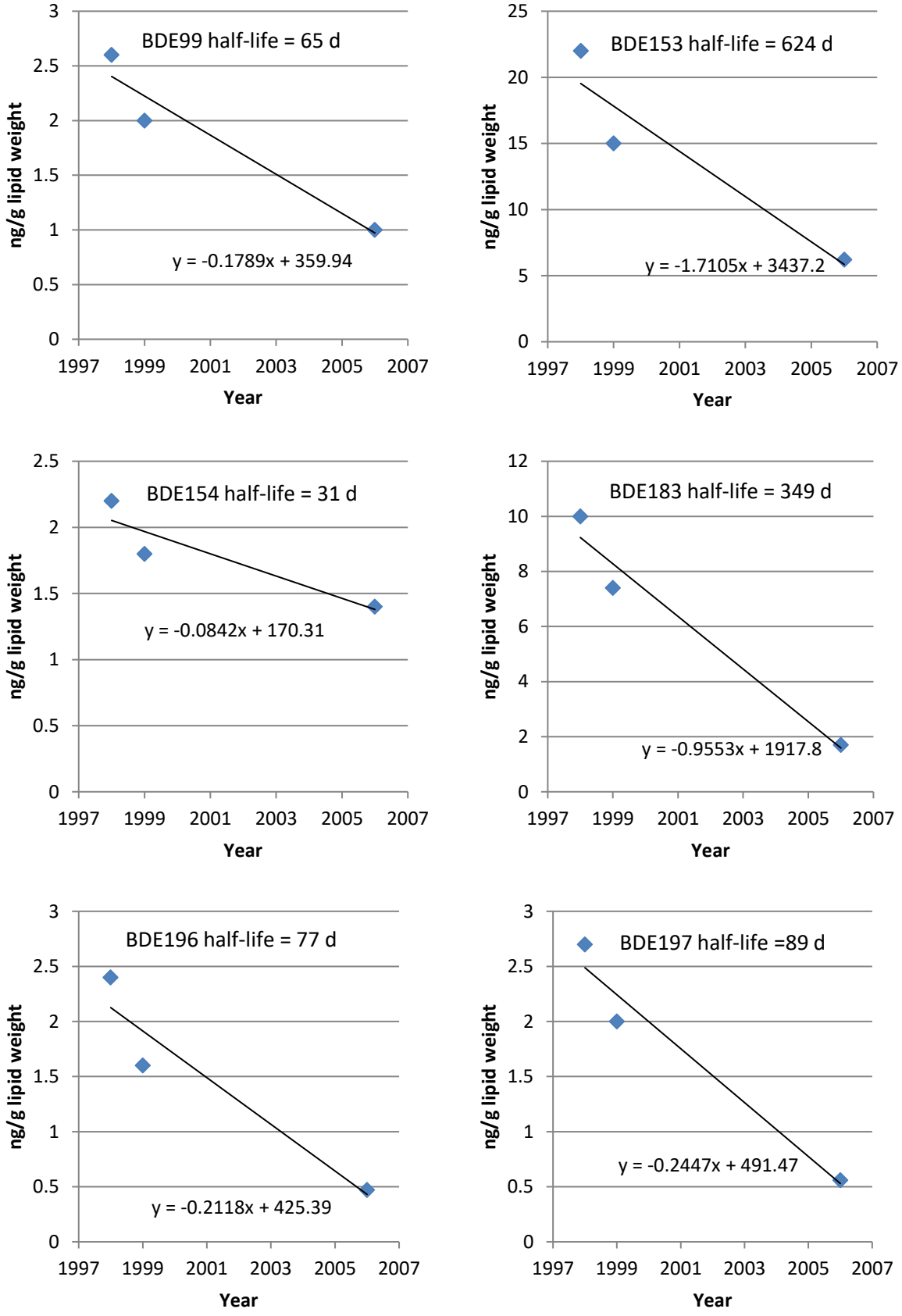

Figure S6. Linear regressions and half-lives of individual BDEs in eggs from peregrine falcon 223.