Absorption of decabromodiphenyl ether and other organohalogen chemicals by grey seals (*Halichoerus grypus*)

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Abstract

An input-output balance study was performed for polybrominated diphenyl ethers, polychlorinated biphenyls and some organochlorine pesticides on three captive, juvenile grey seals (*Halichoerus grypus*). The animals were fed a diet of herring for six months, during the last three months of which this study was performed. A supplement of decabromodiphenyl ether was included in the diet during the second month of the study. Consistently high absorption (>89%) was observed for all of the chemicals studied, whereas work on other animals has generally shown high (>80%) net absorption at log K_{OW} <~6, dropping towards higher log K_{OW}, and very low absorption of decabromodiphenyl ether. The half-life of decabromodiphenyl ether in blood was

estimated to be between 8.5 and 13 days. Measurable concentrations of decabromodiphenyl ether were detected in seal blubber at the end of the study, indicating that this chemical can be stored in adipose and may bioaccumulate. Current understanding of the mechanism of absorption of organohalogen chemicals and the potential for accumulation of decabromodiphenyl ether will need reassessing in the light of these results.

Introduction

Of the three main technical mixtures in use, the pentabromodiphenyl ether (PeBDE) and octabromodiphenyl ether (OcBDE) mixtures are currently being phased out in Europe but are still in high use in other parts of the world. However, in the UK in particular, stringent fire retardancy regulations for furniture led to previously high use of PeBDE. The decabromodiphenyl ether (DeBDE) mixture, a high usage flame retardant (approx. 54,800 tonnes annual usage estimated in 1999 (Alaee *et al.*, 2003)), is currently undergoing a comprehensive risk assessment in the European Union (EU, 2002). This chemical is seen as a very useful flame retardant for use in electrical equipment housing, rubber insulating materials and other applications. However, there is some uncertainty over the possibility of DeBDE entering the environment, and if it does so, whether it is available to biota. The DeBDE mixture almost entirely comprises BDE 209, which has a high octanol-water partition coefficient (K_{OW}), a relatively high molecular weight (959 amu) and is relatively labile to heat and light (Söderström *et al.*, 2003). PBDEs from the PeBDE mixture have been reported in a wide range of environmental media (de Wit, 2002), and concentrations in human breast milk have recently been reported to be substantially higher in North America than in Europe

(Petreas et al, 2003). DeBDE, despite it's higher usage, has only been found in a small number of environmental studies, generally linked to sediments near sites of production and use (Sellström *et al.*, 1998, Allchin *et al.*, 1999), and, recently, in the eggs of wild birds of prey in Scandinavia (Lindberg *et al.*, 2004), although laboratory studies have generally shown low or negligible absorption by animals (El Dareer *et al.*, 1987; Mörck and Klasson Wehler, 2001). However, Sandholm *et al.* have reported that about 26% BDE-209 was bioavailable in rats and suggested that the relatively high concentrations of phenolic metabolites present in the blood plasma compared to the parent BDE-209 indicated that total adsorption was higher than 26%. (Sandholm *et al.*, 2003). Jakobsson *et al.* (2003) have reported up to 36 pmol BDE-209 /g lipid in the blood of occupationally exposed workers.

Polychlorinated biphenyls (PCBs) and many organochlorine pesticides (OCPs) have been banned or restricted in many countries since the 1970s due to their bioaccumulative and persistent properties, and have been dropping in concentration in many environmental media for the last two decades or so (e.g. Bignert *et al.*, 1998). However, there are concerns about the concentrations of these chemicals in the marine environment (Tanabe, 1988; Aguilar *et al.*, 2002), and recent work has highlighted the possibility that these chemicals may increase carcinogenic effects on biota at doses much lower than previously thought (Kalantzi *et al.*, 2004).

Very high concentrations of PBDEs, PCBs and OCPs have been recorded in a range of marine mammals (Aguilar *et al.*, 2002; Law *et al.*, 2002). Organohalogen contamination has been implicated in endocrine disruption effects, leading to increased susceptibility to a range of diseases, some of which have been seen as epizootic episodes in recent years (Oberdörster and Cheek, 2000). Recently, PBDEs have been shown to affect

thyroid hormone activity in mammals (Fowles *et al.*, 1994; Meerts *et al.*, 1998), and we have recently reported a correlation between PBDE and thyroid hormone levels in North Sea grey seals (Hall *et al.*, 2003).

Materials and Methods

Study design and sampling - An input-output balance study was performed on three captive (wild) juvenile grey seals (*Halichoerus grypus*). The seals were fed a constant diet of North Sea herring for 3 months before this study started, by which time we anticipated that steady-state conditions, with respect to the chemicals of interest found in the diet, would have been approached. This study then lasted 3 months, during which fish were all taken from one frozen batch caught in the North Sea, defrosted as necessary. Each seal consumed between 1 and 2.5 kg (fresh weight) fish per day. In the second month of the study the seals were fed a supplement of 12 µg decabromodiphenyl ether per day, dissolved in cod liver oil in a capsule, and in the final month the seals were fed a control (unspiked) cod liver oil capsule each day. On a weekly basis fish samples (3 fish) were taken from the defrosted fish batch, a blood sample was taken and a 24 hour faeces sample was collected whilst each seal was kept in an individual dry enclosure. A blubber biopsy was taken from each seal at three times during the study.

Chemical analysis - Samples were analysed for a range of PCBs, OCPs and PBDEs using methods based on those previously published (Thomas *et al.*, 1998; Hovander *et al.*, 2000; Bayen *et al.*, 2003), which briefly entailed the following. Whole fish were ground separately in a steel blender and frozen until analysis. Fish, faeces or blubber samples were mixed well with anhydrous sodium sulphate before dichloromethane (DCM) extraction using an accelerated solvent extraction system. Blood samples were modified with hydrochloric acid and extracted with a mixture of hexane and MTBE,

then partitioned with aqueous KOH to remove phenolic chemicals. All samples were spiked with seven ^{13}C-labelled PCBs and ^{13}C-labelled BDE 209 before extraction. Fish, faeces and blubber samples were cleaned-up on a column containing silica gel modified with concentrated sulphuric acid, eluted with hexane. Blood samples were cleaned-up using concentrated sulphuric acid. All samples received a secondary clean-up using gel permeation chromatography before being concentrated to a small volume with internal standards added. PBDEs (except BDE 209) were analysed on a Fisons MD800 GC-MS in SIM mode, with separation on a 30 m long, 0.18 mm id, DB5 MS column. The mass spectrometer was used in ECNI (electron capture negative ion) mode with ammonia as the reagent gas. PCBs and OCPs were analysed on a Thermo Trace GC-MS in SIM mode using an EI+ source. Separation was achieved on a 50 m 0.25 mm id CP-Sil 8 column. BDE 209 was analysed on a HP6890 GC attached to a VG Autospec Ultima used in EI+ mode, with a resolution of at least 10,000. Separation was achieved on a 15 m long, 0.18 mm id, DB5 MS column.

The method detection limit was defined as the higher of three times the standard deviation of the blank values or the instrument detection limit. Recoveries averaged 67 –81 % for each of the ^{13}C labelled standards.

Results and Discussion

Calculation of apparent absorption A range of PCBs, OCPs and PBDEs were found in the herring and faeces; daily input and output fluxes were calculated for each seal, for each chemical which was found. The geometric mean concentration in fish (9 samples analysed) was used to calculate the input fluxes because the concentrations determined appeared to follow a log-normal distribution. Faeces concentrations were used to

calculate output fluxes directly for the relevant day. Chemical concentrations found in fish are shown in Table 1, and the input and output fluxes are shown in Table 2.

The apparent amount of each chemical absorbed from the diet was defined as the difference between the input and output fluxes, and is expressed as a percentage of the total input flux, for a selection of the chemicals, in Table 3. Because of the log-normal distribution of chemical concentrations normally found in biota (for which there was some evidence in this study) the data were log-transformed before calculation of the apparent absorption using the formula presented by Juan *et al.* (Juan *et al.*, 2002). Apparent absorption may be an over- or under-estimate of true absorption, because of the possibility of degradation within the gastro-intestinal tract or excretion of chemicals from previous exposure. BDE 209 was not found in the fish analysed, and apparent absorption was calculated solely for the occasions when this chemical was added to the diet.

Observation on apparent absorption Consistently high absorption (>89%) of all chemicals analysed was observed. This extended over a log K_{OW} range from approximately 3.8 (alpha-HCH) to 10.3 (BDE 209). This contrasts with work on fish (Gobas *et al.*, 1993) and dairy cows (McLachlan, 1994; Thomas *et al.*, 1999), showing a reduction in absorption efficiency with increasing K_{OW} once log K_{OW} exceeds approximately 6. There are several possible explanations for the consistently high absorption seen in these seals:

1) The high total body fat content of seals, compared to most land animals, will provide a large capacity for storage of these hydrophobic chemicals, keeping the chemicals in the blood (the supplying medium) at a constant relatively low concentration, and creating a relatively large fugacity gradient between the gastro-

intestinal (GI) tract contents and the blood (the receiving medium). Assuming that absorption is a purely passive process, this will cause the equilibrium between the GI tract content and blood to be consistently in favour of movement to the blood, i.e. that consistently high absorption is maintained.

2) Seals, being carnivores, generally have a high assimilation efficiency (AE) from their diet. A high AE implies that the organic matter content (and hence the fugacity capacity) of the GI tract contents is greatly reduced compared to the food, and passive transport across the GI tract wall would be in favour of movement to the blood. Herbivores and omnivores, which most previous mass balance and absorption studies have used, have a lower AE, because their diet is less digestible. They are likely to have a higher GI tract content fugacity capacity, and a lower POPs absorption efficiency. In this study the AE (dry matter) was found to be 96.9 ± 2.3 %.

3) Incomplete collection of faeces, or reduced faeces output (due to increased stress in the faeces collection period) may have caused the total output flux to be underestimated. However, in any 24 hour faeces collection period it is estimated that not more than 10% of the faeces produced was lost due to dispersion by rainwater or by the seals behaviour. Comparison of the AE found in this study to AE values for herring eaten by ringed and harp seals (Lawson *et al.*, 1997a; 1997b) suggests that the seals produced at least 50% of the faeces which might have been expected in each collection period.

4) It has been suggested that POPs can be degraded in the GI tract, which would result in an overestimation of net absorption. It is possible that this happened in this study, but we found no evidence in the concentrations of lower brominated BDE congeners in faeces, which remained constant, to suggest that it may have occurred.

Transfer of BDE 209 to blood and body-fat – Blood BDE 209 concentrations are shown in Figure 1. BDE 209 was not detected in blood before the seals were fed the spiked capsules, nor was it found in blood after three days of BDE 209 consumption. Measurable concentrations of BDE 209 had been reached in all three seals by day 45, and the concentrations remained measurable for the remainder of the study. Maximum concentrations of BDE 209 were found in the blood between 5 and 11 days after cessation of spiked capsule consumption, and these maxima were followed by a rapid drop in BDE 209 concentrations in each seal. On day 83 BDE 209 concentrations had dropped to between 14 and 15% of the maximum concentration for each seal. Fitting first order reaction curves to the BDE 209 concentration data for each seal, from day 59 to the end of the study, gave BDE 209 half-lives in blood between 8.5 and 13 days.

BDE 209 was found in the blubber of two of the seals within 3 days of them starting to ingest DeBDE-spike capsules, and was found in all three seals at the end of the study, 29 days after the seals had ingested the last DeBDE-spiked capsule. No BDE 209 was found in blubber at the beginning of the study. The total body-fat content was estimated roughly mid-way through the study by an isotope dilution method, using deuterated water, that has been described elsewhere (Reilly and Fedak, 1990). The total body-burdens for each seal, estimated by multiplying the blubber concentration by the estimated total body-fat content, are shown in Table 4. It can be seen that at the end of the study between 11 and 15% of the cumulative amount of BDE 209 ingested by each seal (~320 μg per seal) was stored in the blubber after 29 days on a DeBDE-free diet, but that after 3 days ingesting DeBDE a larger proportion of the cumulative amount of BDE 209 ingested was found in the blubber of two of the seals. BDE 209 was not detected in the blubber of seal 2 on day 30, although it was detected in the other two

animals. Seal 2 had 10.7 kg of body-fat, compared to 6.3-6.4 kg for the other seals. The probable explanation for the difference between the seals is that the BDE 209 absorbed in the first few days will have been diluted into this bigger fat store, giving lower concentrations which were below the method detection limit.

Implications for risk assessments There are some important implications for the environmental and human risk assessment of BDE 209 and other non-ionised chemicals from this study.

1) It has been assumed that non-ionised non-nutrient molecules with a molecular mass of more than 700 amu cannot cross biological membranes (Mörck and Klasson Wehler, 2001). However, we cannot find convincing reports of this being true in the literature, and suggest that this assumption requires reassessment in the light of the high absorption of BDE 209 seen in this study.
2) This study shows that BDE 209 can be stored in the blubber of grey seals if they are exposed to it, although it generally appears not to be present in marine animals in the natural environment. BDE 209 has, however, been found to be present in the eggs of birds of prey (Lindberg *et al.*, 2004) and has been found at low concentrations in salmon (Burreau, 2001). BDE 209 has also been found in sediments from some locations (Allchin *et al.*, 999). Exposure to and absorption of BDE 209 is likely to be dependent on the species, food web and a combination of the ingested matrix with which the chemical is associated and the specific characteristics of the animal involved. For example, BDE 209 attached to organic rich sediment ingested by bottom dwelling fish is likely to be absorbed less than BDE 209 present in the bodies of the prey species of the fish. We believe that the cod liver oil used in this study

should be a comparable carrier to whole fish, since if BDE 209 were present in fish it would most likely be contained within the fish lipids.

3) BDE 209 has been shown to be readily metabolised or excreted in other animals (e.g. Mörck and Klasson Wehler, 2001; Sandholm *et al.*, 2003; Sjödin *et al.*, 1999; Kierkegaard *et al.*, 1999; Stapleton *et al.*, 2003; Hagmar *et al.*, 2000). Blood concentrations of BDE 209 in the seals dropped rapidly after the maximum concentration was reached, which may be due to continued transfer to the blubber or metabolism, or a combination of both. BDE 209 would probably not be metabolised appreciably whilst stored in the blubber. Thus, animals may continue to carry a body-burden of BDE 209 after exposure, even though concentrations in the blood may rapidly become undetectable. In the wild, compounds stored in the blubber are likely to be re-mobilised during periods of fasting.

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Table 1 – Concentrations of chemicals in fish

	Median concentration (min-max) ng/g lipid		Median concentration (min-max) ng/g lipid
PCB18	1.3 (0.46-2.4)	alpha-chlordane	5.2 (2.7-8.7)
PCB28	2.7 (1.1-4.7)	alpha-HCH	3.5 (1.8-6.7)
PCB31	1.9 (0.74-4)	HCB	11 (5.1-19)
PCB44	2.4 (0.86-4.3)	p,p'-DDD	9.2 (4.6-18.1)
PCB49	1.5 (0.58-3.3)	p,p'-DDE	35 (8.8-88)
PCB52	5.4 (1.7-8.8)	p,p'-DDT	3.5 (1.4-11)
PCB70	3.7 (1.2-7.5)		
PCB74	2.2 (0.85-4.3)	BDE28	1.8 (1.1-2.8)
PCB90 + 101	9.2 (3.6-25)	BDE32	1.5 (1.2-2.7)
PCB95	5.3 (2-12)	BDE35	1.3 (1.1-1.4)
PCB99	4.2 (1.2-11)	BDE47	4.9 (1.1-10.9)
PCB105	2.6 (0.71-6.2)	BDE49	2.3 (1.7-6.3)
PCB110	6.3 (1.8-17)	BDE99	2.7 (0.83-5.4)
PCB118	7.7 (2.2-20)	BDE100	3.1 (1.8-7.2)
PCB132	2.8 (1.1-9)	BDE153	1.1 (1-1.1)
PCB138	21 (5.8-60)	BDE154	0.93 (0.92-1.3)
PCB149	11 (4.7-32)	Total BDE	17 (3.3-33)
PCB151	3.3 (1.9-9.6)		
PCB153	20 (6.5-57)		
PCB170	2.2 (0.77-5.9)		
PCB174	1.7 (0.77-3.5)		
PCB180	3.1 (1.5-8.9)		
PCB183	1.1 (0.69-3)		
PCB187	5.9 (2.1-18)		
PCB194	0.67 (0.36-1)		
PCB203	0.76 (0.38-1.3)		
Total PCB	130 (49-360)		

Table 2 – Input and output fluxes of selected chemicals for all seals throughout the study

	Input flux ng/day median (min-max)	Output flux ng/day median (min-max)
alpha-HCH	530 (350-880)	2.2 (nd-22)
p,p'-DDD	1420 (940-2390)	10 (1-76)
PCB28	400 (270-670)	1.5 (nd-17)
p,p'-DDE	5530 (3670-9220)	270 (13-650)
HCB	1570 (1050-2620)	63 (3.2-170)
PCB52	730 (490-1220)	22 (0.8-69)
BDE28	270 (180-450)	nd (nd-5.7)
alpha-chlordane	760 (510-1270)	1.4 (nd-36)
p,p'-DDT	550 (360-920)	16 (0.6-69)
PCB90 + 101	1580 (1050-2630)	40 (1.7-130)
BDE49	340 (220-560)	3.5 (nd-41)
PCB118	1250 (830-2080)	11 (0.4-69)
BDE47	620 (410-1040)	37 (1.5-190)
PCB138	3240 (2150-5410)	260 (13-620)
PCB153	3200 (2120-5340)	280 (17-780)
BDE100	440 (290-720)	4.5 (nd-35)
BDE99	380 (250-640)	5.6 (nd-38)
PCB180	560 (360-920)	59 (5.1-210)
BDE153	150 (99-250)	2.0 (nd-10)
BDE154	170 (110-280)	1.7 (nd-7.5)

Table 3 – Average net absorption measured throughout the study.

	Log K_{OW}	% average absorption (lower – upper standard error)
alpha-HCH	3.81 [a]	99 (99 – 100)
p,p'-DDD	5.50 [a]	99 (99 – 100)
PCB 28	5.67 [b]	99 (99 – 100)
p,p'-DDE	5.70 [a]	97 (94 – 98)
HCB	5.77 [a]	97 (95 – 98)
PCB 52	5.84 [b]	98 (96 – 99)
BDE28	5.94 [c]	99 (98 – 100)
alpha-chlordane	6.00 [a]	99 (99 – 100)
p,p'-DDT	6.20 [a]	98 (96 – 99)
PCB 90 + 101	6.38 [b,d]	98 (97 – 99)
BDE49	6.60 [c,e]	98 (96 – 99)
PCB 118	6.74 [b]	99 (99 – 100)
BDE47	6.81 [c]	95 (90 – 97)
PCB 138	6.83 [b]	94 (91 – 96)
PCB 153	6.92 [b]	93 (89 – 96)
BDE100	7.24 [c]	99 (98 – 99)
BDE99	7.32 [c]	98 (97 – 99)
PCB 180	7.36 [b]	91 (85 – 95)
BDE154	7.82 [c]	98 (97 – 99)
BDE153	7.90 [c]	97 (95 – 98)
BDE 209	10.33 [c,e]	89 (57 – 97)

Note that BDE 209 was fed dissolved in cod liver oil, other chemicals were naturally present in the fish diet; a = from Mackay *et al.*, 1992; b = measured by Hawker and Connell (1988); c = from Braekevelt *et al.*, 2003; d = log Kow value for PCB 101 used; e = calculated from Hawker and Connell, 1988

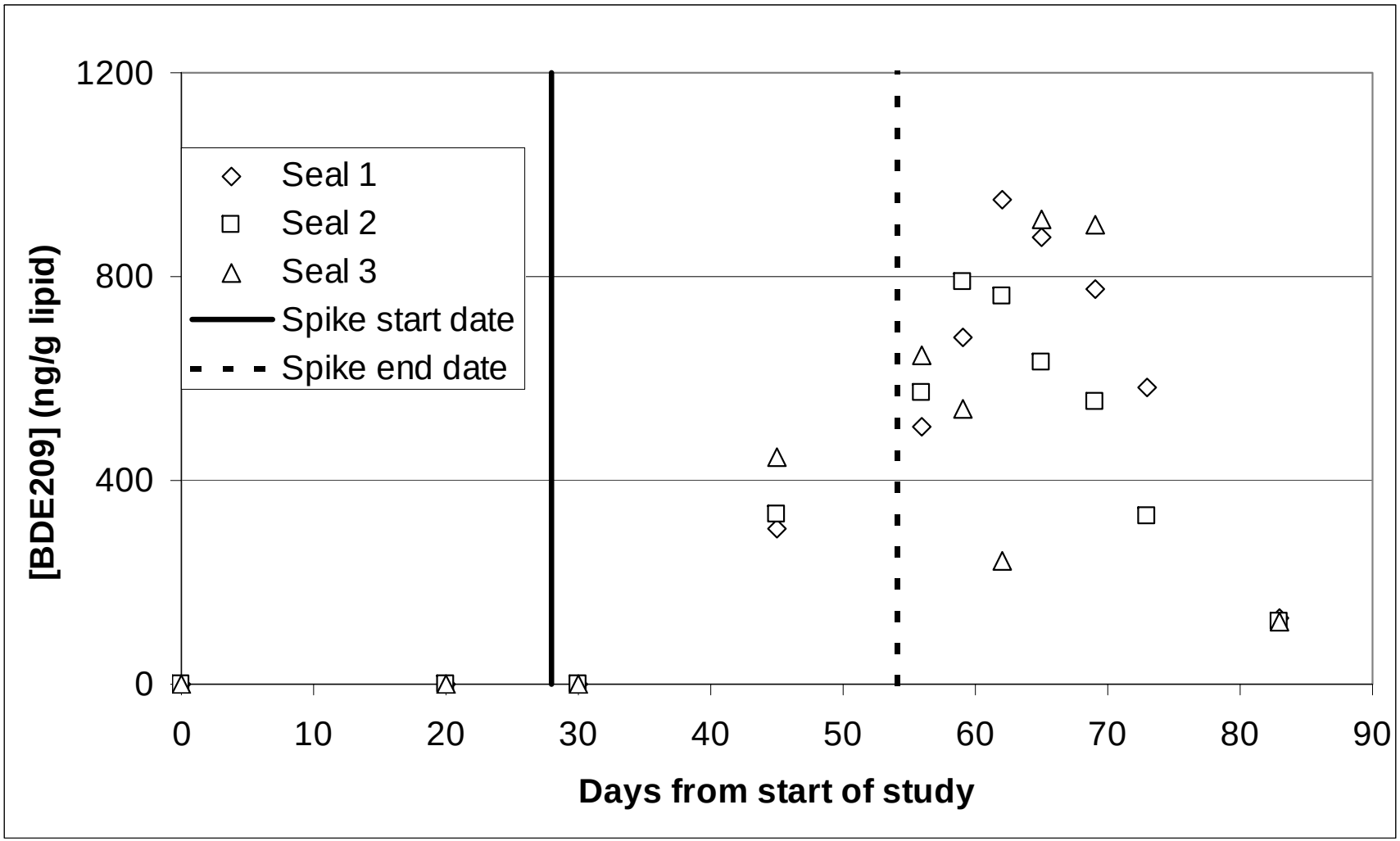
Table 4 – Estimated total body-burden of BDE 209 at the middle and end of the study

Seal	1		2		3	
Sex	Female		Female		Male	
Day of study	30	83	30	83	30	83
Total body weight (kg)	30.4	30.4	33.0	33.0	31.8	31.8
Total body-fat (kg)	6.3	6.3	10.7	10.7	6.4	6.4
BDE 209 concentration (ng/g lipid)	2.1	7.4	ND	3.4	3.9	5.3
Estimated BDE 209 body-burden (µg)	13	47	ND	36	25	34
% of total ingested BDE 209 found in blubber	36	15		11	68	11

ND = BDE 209 not detected in blubber; DeBDE was fed from day 27 until day 54

List of figures

Figure 1


1200
800
400
0
[BDE209] (ng/g lipid)
Seal 1
Seal 2
Seal 3
Spike start date
Spike end date
0
10
20
30
40
50
60
70
80
90
Days from start of study