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Levels and Trends of Brominated Diphenyl Ethers in Blubber of Harbor Porpoises (*Phocoena phocoena*) from the U.K., 1992−2008

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Controls were placed on the production and use of the penta-mix polybrominated diphenyl ether (PBDE) formulation within the European Union in 2004. In porpoises stranded or bycaught around the U.K., BDE congeners from this product predominate. Lipid-normalized concentrations of 9 (summed) BDE congeners in the blubber of 415 porpoises sampled during the period 1992−2008 have been investigated for possible time trends resulting from the regulatory action. Our analysis suggests that, overall, median Σ9BDE concentrations peaked around 1998 and have since reduced by between 53.8% and 73.5% to 2008. Our best point estimate is that the reduction has been 67.6%. This decline was highly statistically significant ($p < 0.001$) and was not confounded by a range of other factors which were also considered (area, season, nutritional status, bycaught/stranded, and age class).

Introduction

Polybrominated diphenyl ethers (PBDEs) have been used extensively as additive flame retardants in textiles, thermoplastics, polyurethane foams, and electronic products. Over the past 25 years, since they were first identified in environmental samples remote from sources (*1*), brominated diphenyl ether (BDE) congeners have become the subject of intense research activity (*2, 3*). Within the European Union (EU), risk assessments have been undertaken for all three PBDE formulations, the penta-, octa- and, deca-mix products. Controls on the production and use of the penta- and octa-mixes were recommended, and these products were withdrawn from the EU market prior to August 2004 (*4*). Following a ruling by the European Court of Justice, the deca-mix product was also banned from use in electrical and electronic goods within the EU from July 2008 (*4*). BDE183, considered as a marker for the octa-mix PBDE product, has been detected in only a few U.K. porpoises at concentrations close to the detection limit (maximum concentration 0.077 mg kg^{-1} wet weight), and BDE209 (from the deca-mix PBDE product) has not been detected in any animals in which that compound was determined. BDE contamination in U.K. porpoises, therefore, results primarily from release of the penta-mix PBDE product to the environment.

There have been a number of previous studies of temporal trends of BDEs in organisms (*2, 5*). Recently, in peregrine falcon eggs from California during 1986−2007, for example, Park et al. (*6*) reported ΣBDE levels more than tripling each decade. Summarizing a number of other recent studies, Law et al. (*3*) indicated that, in the environment of the Northern Hemisphere generally, ΣBDE levels have been stabilizing since the mid-1990s, other than for BDEs undergoing long-range atmospheric transport to the Arctic and for BDE209 in sediments. Further details of time-trend studies for BDEs in marine mammals are given below.

The ban on the penta-mix PBDE products within the EU was widely foreseen, and it is likely that some switch to alternatives took place before that date, though industry sales figures are not available. In this study, we wished to see whether the cessation of use had, as yet, led to declining levels of contamination in the blubber of porpoises from the U.K.

Materials and Methods

The porpoises sampled were collected within the Cetacean Strandings Investigation Programme funded by the U.K. government. Selected animals which have been found stranded, or were bycaught, were taken for postmortem study in order to establish cause of death. Only freshly dead to moderately decomposed animals were selected. The distribution of sampling locations around the U.K. is shown in Figure S1 of the Supporting Information. Contaminant analyses were conducted in blubber tissue with the aim of investigating possible links between contaminant burden and death due to infectious disease. The detailed protocols applied in the postmortem studies and the classification criteria are given elsewhere (*7*). Tissue samples were stored frozen at −20 °C prior to analysis and were then thawed and homogenized prior to extraction.

Analysis of a suite of BDE congeners was conducted using a method based upon determination using gas chromatography with detection by electron capture negative ion mass spectrometry (GC-ECNIMS), monitoring the bromine ions at 79 and 81 Da (*8*). Briefly, the method involved Soxhlet extraction of blubber samples with *n*-hexane/acetone (1:1) and cleanup using alumina and silica columns prior to GC-MS analysis. Limits of quantification (LOQs) ranged from 0.001 to 0.01 mg kg^{-1} wet weight depending on the lipid content of the tissue sample. The method was previously validated within an international interlaboratory study (*8*) and has subsequently been used within laboratory intercomparisons (NIST and IAEA) and in reference material certification studies for the EU Joint Research Centre. The Centre for Environment, Fisheries and Aquaculture Science (Cefas) has also participated successfully in the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) Laboratory Proficiency Scheme, submitting data twice annually for BDEs in biota. Analyses were conducted under full analytical quality control procedures. These involved the analysis within each sample batch of a procedural blank and the certified reference material (CRM) BCR-349 (cod liver oil) which has been characterized in-house for BDE28, BDE47, BDE99, and BDE100. Blank values were all below LOQs and were not

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deducted from sample concentrations. The results obtained for the BCR-349 replicates were used to plot individual Shewhart control charts and to decide upon acceptance or rejection of the data produced for each sample batch. Batches in which the CRM data were beyond the upper and lower control limits (set at ±3 standard deviations of the mean) were rejected and reanalyzed.

Results and Discussion

BDE Concentrations in Porpoise Blubber. During the extended period during which these samples were analyzed, the congener suite determined changed, primarily because some congeners were never detected and so were removed from the list of determinands and as new BDE congeners became available. Throughout the period, nine BDE congeners were determined in all samples. These were BDE28, BDE47, BDE66, BDE85, BDE99, BDE100, BDE138, BDE153, and BDE154. In all, summed BDE congener concentrations (Σ9BDE) were available for 415 individuals (see Table S1 of the Supporting Information). These ranged from not detected in a 15 year old male porpoise from Eastern England sampled in 1998 (reference number SW1998/115) to 15.7 mg kg^{-1} lipid weight (lw) in a juvenile female porpoise from the Shetland Islands sampled in 1993, off the north coast of the Scottish mainland (SW1993/10b). Of these 415 animals, 181 came from Scotland; 95 were from eastern England (East: Northumberland to Kent); 123 were from western England and Wales (West: Cumbria to Cornwall); and 16 were from southern England (South: East Sussex to South Devon). By area, the concentration ranges were the following: Scotland 0.1−15.7 mg kg^{-1} lw; East not detected to 8.2 mg kg^{-1} lw; West 0.1−6.0 mg kg^{-1} lw; South 0.2−2.0 mg kg^{-1} lw. The highest concentration was found in one of the remotest and least populated areas of the U.K. This may be explainable by changes and spatial variability in prey availability. Christensen and Richardson (*9*) reported a temporal change in stable isotope ($\delta^{15}N$) distributions in porpoises from the North Sea in the middle of the 20th century, which implied fundamental changes in food web structure. In a separate study investigating stable isotopes and trace elements, it was concluded that there had been a shift in porpoises' feeding habits from pelagic prey species in deep northern waters to more coastal and/or demersal prey in the relatively shallow North Sea waters (*10*). Finally, from a study of stomach contents of porpoises stranded between 1992 and 2003, Santos et al. (*11*) demonstrated a greater reliance on gadoid fish around Shetland than on the Scottish east coast (North Sea), where sandeels predominated.

Four of the congeners (BDE28, BDE66, BDE85, and BDE138) were present only at low concentrations relative to the other five congeners and remained at similar levels throughout the study period. For the other five, there were changes in the BDE profile over time. Looking at the mean values in 1992 and 2008, the relative proportions of BDE47 and BDE99 declined (67−47% and 16−12%, respectively) and those of BDE100, BDE153, and BDE154 increased (13−19%, 0.6−5.7%, and 3−13%, respectively) over the 16 year period. The trends were linear for BDE153 and BDE154 and more variable for BDE100. This could represent changes in sources but is more likely to reflect debromination of more highly brominated congeners, such as BDE209, in the environment. As no BDE209 was detected in porpoises, this probably occurred in either their fish prey (as reported for other species (*12, 13*), in which BDE100, BDE153, and BDE154 have all been produced) or in bottom-living organisms exposed to BDE209 in sediments and subsequently entering the food chain. McKinney et al. (*14*) investigated in vitro metabolism of individual BDE congeners using hepatic microsomes from beluga whale and found significant metabolism of BDE47, though not of BDE99. BDE99 was metabolized in experiments with control rat microsomes, however, in the same study.

TABLE 1. Mean and Median Σ9BDE Concentrations (mg kg^{-1} lw) in Porpoise Blubber by Year, 1992−2008

year	no. of animals	mean BDE	median BDE
1992	3	0.94	0.29
1993	9	2.85	1.04
1994	5	1.90	2.04
1995	10	1.42	0.61
1996	20	1.94	1.27
1997	39	2.39	2.23
1998	33	1.98	1.30
1999	30	1.26	0.71
2000	20	2.53	1.54
2001	56	1.39	0.86
2002	29	1.16	0.81
2003	32	1.27	0.75
2004	35	1.10	0.83
2005	23	1.01	0.87
2006	23	0.64	0.47
2007	39	0.46	0.32
2008	9	0.67	0.51

Investigation of Time Trends. For statistical treatment, congener concentrations below the LOQ were set to one-half of the LOQ. The overall Σ9BDE mean and median lipid-normalized concentrations are given in Table 1, summary statistics by area and age class are given in Table 2. We investigated the effects of days since January 1, 1992 and the potentially confounding factors (area, season, nutritional status (dorsal blubber thickness), bycaught/stranded, and age class) on lipid-normalized Σ9BDE concentrations. We did this by fitting a generalized additive model to the data using the function *gam* in the R package mgcv (*15*). We used thin plate regression splines to smooth the data, and the degree of smoothing was determined by generalized cross validation (GCV) (*16*). To avoid overfitting the model, we took the advice of Kim and Gu (*17*) and modified the GCV criterion by setting the gamma parameter in *gam* to 1.4. We thus modeled ln(Σ9BDE) as a function of the following: smoothed days, smoothed nutritional status, and factors representing area, season, bycaught/stranded, and age class. The natural log transformation of BDE concentrations was undertaken so as to stabilize the variance. Errors in the model were assumed to be Gaussian with mean zero and constant variance. The results of this model fitting are summarized by the analysis of variance table given in Table 3. The table shows no evidence of any effect of whether a porpoise was bycaught or stranded or of the season in which it died. However, the other factors and variables are all strongly statistically significant ($p < 0.001$ in all cases).

Thus, in terms of confounding factors when considering the trend in Σ9BDE concentrations over time, we will consider only area, age class, and nutritional status. The sample size, minimum and maximum, median, mean, and quartiles of Σ9BDE concentrations in each area and age class are shown in Table 2. Σ9BDE concentrations were highest in the East and lowest in the South, although in the latter case the sample size is very small. We can also see that, as expected, concentrations are lowest in adult females due to them offloading a large proportion of their burden to their offspring. For all categories, median values are lower than mean values, reflecting the positively skewed nature of the data. Dorsal blubber thickness stayed fairly constant over the period of the study (Figure S2 of the Supporting Information). Its overall distribution can be summarized as: min = 4.0, Q1 = 11.0, med = 16.0, mean = 16.5, Q3 = 21.0, max = 50.0. The blubber lipid level varied from 50 to 99%, with over 90% of animals having greater than 80% blubber lipid content, and no

TABLE 2. Summary Statistics by Area and Age Class of Levels of Σ9BDE Concentrations (mg kg^{-1} lw) in Porpoise Blubber between 1992 and 1998

area/age class	n	minimum	lower quartile	median	mean	upper quartile	maximum
east	95	0.03	0.79	1.65	2.28	3.19	8.19
west	123	0.10	0.34	0.61	0.86	0.98	5.98
scotland	181	0.10	0.52	0.89	1.43	1.65	15.73
south	16	0.15	0.27	0.45	0.59	0.72	2.04
adult males	95	0.03	0.53	0.87	1.34	1.62	7.32
adult females	93	0.09	0.41	0.64	1.03	1.07	8.19
juveniles	227	0.04	0.49	0.91	1.62	2.04	15.73

TABLE 3. Analysis of Variance Table from a Generalized Additive Model to Explain Variation in ln(Σ9BDE) Concentrations (mg kg^{-1} lw) in Porpoise Blubber between 1992 and 2008 to the Following: Smoothed Functions of Days and Nutritional Status and the Factors Area, Season, Bycaught, and Age Class[a]

source of variation	F	p-value
S(days)	21.507	<0.001
S(nutrition)	9.938	<0.001
area	20.520	<0.001
season	0.067	0.80
bycaught	1.063	0.30
age class	13.465	<0.001

[a] Note that S(days) and S(nutrition) represent smoothed functions of these variables.

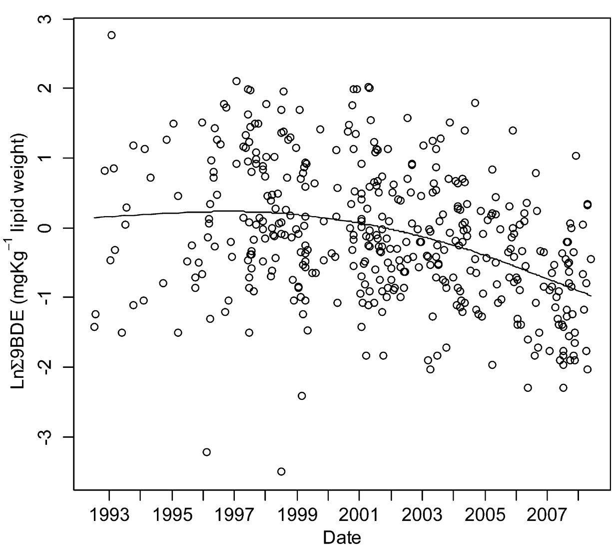

FIGURE 1. ln(Σ9BDE) (mg kg^{-1} lw) against date for all 415 porpoises. The continuous line represents the smoothed values from a generalized additive model fitted to the data.

apparent trend over time (Figure S3 of the Supporting Information).

The smoothed complete data set (Figure 1) shows a clear downward trend from about 1998. The overall model has an approximate significance level against a model of no trend of $p < 0.001$. We confirmed the trend in the series from 1998 using the standard nonparametric Mann−Kendall test, with the p-value calculated using a randomization procedure with 1000 repeat samplings (*18*). This gave a p-value of $p < 0.001$ against the null hypothesis of no change. Although there appears to be some low outliers in Figure 1, these seem to have little influence on the fitted model and so they have not been removed. The trend line shown in Figure 1 is fairly smooth and reflects the fact that BDE concentrations are unlikely to change in an erratic fashion over time. From observation of the plot, it seems that the possible peak in concentrations around 1998 has not been picked up by the trend, and also that the high outlier in 1993 has caused the trend line to be a little high in the early part of the series. However, the general conclusion of declining levels since about 1998 seems clear.

To assess the change in Σ9BDE concentrations between January 1, 1998 and January 1, 2008, we used our fitted model. By taking exponentials of the fitted values on the ln scale at these two dates, we obtained the median concentrations on the original scale. This gives a reduction in median Σ9BDE levels of 67.6% over the period. To obtain a 95% confidence interval for this prediction, we bootstrapped the residuals using 1000 replicates. Bootstrapped data sets were obtained by adding one of the residuals from the original model at random to the fitted values from the original model (*19*). The GAM model was fitted as before and the reduction in median Σ9BDE concentrations estimated as before. The 25th and 975th largest predictions from the bootstrapping were used to provide the lower and upper 95% confidence limits for the median percentage reduction. These were 53.8% and 73.5%, respectively.

Potential Confounding Factors. Potential confounding factors (area, season, nutritional status (dorsal blubber thickness), bycaught/stranded, and age class) for 1998−2008 were assessed as previously for HBCD in U.K. porpoises (*18, 20*). We do not believe that our overall conclusion of a reduction in BDE levels can be explained by any unequal sampling of the levels of the components of these confounding factors over the period of the study (e.g., if porpoises from Scotland had higher Σ9BDE concentrations than porpoises from East and if more of the latter were sampled later in the study, this could account for any overall change in BDE levels). Generally, the proportion of each component stayed fairly constant over the sampling period. In the few instances where this was not the case, the BDE levels for each of the components were similar. We illustrate our analyses by looking at concentrations of BDEs against the factors area and age class.

Figure S4 (of the Supporting Information) shows the BDE profile for East, West, and Scotland. All three plots show a general downward trend ($p < 0.05$ in all cases using the Mann−Kendall test)—although this is less clear-cut for the porpoises in the West, which show a rise around 2005. We think that this is partly due to an imbalance in the sampling design. In particular, we have looked carefully at the proportion of adult males and juveniles in the West over time. As shown in Table 2, these generally have higher BDE levels than adult females. The proportion of female porpoises was higher in 2003, and this has contributed to the apparent rise in 2004/05 when the proportion of males and juveniles was higher. Other factors may be involved but these cannot be determined from the current data. However, we are satisfied that this slight anomaly has not affected the overall downward trend because the proportion of porpoises that were sampled from the West each year stays constant at around 30% over the study period. From our models, the

TABLE 4. Reported Temporal Trends in BDE Concentrations in Marine Mammals (Σ9BDE range, mg kg^{-1} lw)

species	location	observed trend	Σ9BDE range	ref
ringed seal	Arctic Canada	rose by 10× or more between 1981 and 2000	not given	*23*
beluga whales	St. Lawrence estuary	increased exponentially 1988−1999; doubling time ca. 3 years	0.2−1.1	*24*
bottlenose dolphin	Florida, USA	increased exponentially 1993−2004; doubling time ca. 3−4 years	0.03−4.5	*25*
guiana dolphins	SE Brazil	positively correlated with year of stranding 1994−2006, so increasing	0.01−1.6	*26*
harbor seals	NW Atlantic Ocean	no trend, 1991−2005	0.08−26	*27*
Atlantic white-sided dolphin	Massachsetts, USA	no trend, 1993−2000	not given	*28*
California sea lion	California, USA	no trend 1993−2003	0.6−24	*29*
California sea lion	California, USA	no trend 1994−2006	0.06−236	*30*
harbor seal			0.3−7.2	
northern elephant seal			0.04−2.0	
Indo-Pacific dolphin	Hong Kong, China	no trend, 1997−2008	0.3−51	*31*
finless porpoise			0.1−6.8	
northern fur seal	Japan	rose by 150× 1972−1994; levels decreased to 50% by 1998	0.0003−0.1	*32*
striped dolphin	Japan	rose by 100× between early 1970s and 1992, steep rise slackened by 1993	0.08−0.9	*33*
finless porpoise	South China Sea	rose by 6× between 1990−2001	0.09−0.2	*34*

estimated median percentage reductions between 1998 and 2008 are 66.5, 65.8, and 65.2 for Eastern, Western, and Scottish porpoises, respectively. The bootstrapped 95% confidence limits are (40.7, 82.2), (46.5, 79.0), and (29.9, 79.5), respectively.

Figure S5 (of the Supporting Information) shows plots for age class (the animals were classified as adult females, adult males, and juveniles). Sexual maturity was established using gonadal appearance and histological evidence of spermatogenesis in testes (*21*). BDE levels in juveniles seem to be going down the fastest, followed by those in adult females. The third plot suggests a slight rise and then fall in the levels of BDE in adult males. Our findings are reflected in the estimated median percentage reductions in Σ9BDE concentrations between 1998 and 2008. These are (95% bootstrapped confidence limits in brackets): adult females = 54.3 (16.3, 72.3); adult males = 39.1 (−21.8, 67.2); juveniles = 83.9 (69.2, 90.0) ($p < 0.05$ in all cases). Although our best estimate (39.1%) is that BDE levels are declining in adult males, the negative lower confidence interval means that it is possible that median levels are actually increasing. Looking at the sampling proportions by year, the proportion of adult males sampled stays fairly constant over the sampling period (around 23%). Only 16 animals came from the South—too few for statistical treatment.

Σ9BDE concentrations were found to be declining in both bycaught and stranded animals ($p < 0.01$). The median concentrations in stranded animals (mainly those dying due to infectious disease) were higher than those in bycaught animals, as is also the case for polychlorinated biphenyls (PCBs) (*21*).

We concluded, therefore, that our findings of declining BDE concentrations were not confounded by any other factors.

Comparison with Other Studies. Observed temporal trends in BDE concentrations in marine mammals from North and South America have been reviewed recently by Shaw and Kannan (*22*). In general, where trends could be seen, levels were rising. All available information worldwide is summarized in Table 4.

Trends in BDE concentrations in marine mammals from Europe have not been studied previously; however, Johansson et al. (*35*) determined ΣBDE concentrations in archived, freeze-dried blue mussel samples from the Seine Estuary, France, covering the period 1981−2003. They observed an exponential increase in levels from 1981−1991/1995, with doubling times of ca. 5−6 years, followed by a decline in concentrations.

It is gratifying to note that, following a formal risk assessment of the continued production and use of the penta-mix PBDE product within the European Union and the consequent ban, BDE concentrations in the blubber of porpoises around the U.K. have begun to fall significantly. As was shown in earlier work, this is also now the case for HBCD following an earlier increase, although this latter compound is still in use (*10*).

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Supporting Information Available

Table S1: concentrations of BDE congeners in the blubber of harbor porpoises from the U.K. (mg kg^{-1} wet weight; Σ9BDE also presented on a lipid weight basis) used in this temporal trend analysis. Figure S1: distribution of sampling locations around the coast of the U.K. Figure S2: trend in blubber thickness over time. Figure S3: percentage lipid content in blubber samples over time. Figure S4: Σ9BDE trend data by area. Figure S5: Σ9BDE trend data by age class. This material is available free of charge via the Internet at http://pubs.acs.org

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