Changes in Blubber Contaminant Concentrations in California Sea Lions (*Zalophus californianus*) associated with Weight Loss and Gain during Rehabilitation

A. J. HALL,*,† F. M. D. GULLAND,‡ G. M. YLITALO,§ D. J. GREIG,‡ AND L. LOWENSTINE||

Sea Mammal Research Unit, Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife, KY16 8LB, U.K., The Marine Mammal Center, 1065 Fort Cronkhite, Sausalito, California, California 94965, Environmental Conservation Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, Washington 98112, and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California at Davis, Davis, California 95616

Received October 29, 2007. Revised manuscript received March 17, 2008. Accepted March 19, 2008.

California sea lions have high levels of persistent organic pollutants (POPs) in their blubber. Animals affected by domoic acid fast and refeed during their rehabilitation. We studied the effect of decreases in total body mass (16 ± 7% of initial body mass) on blubber POP contaminant concentrations and estimated POP burdens during fasting (12 ± 5 days) in 19 California sea lions. The effect of refeeding (92 ± 8% of initial body mass) was also investigated. Significant increases in the concentration of all POPs were found over the mass loss period and decreases during mass gain. A basic mass balance model indicated that the changes did not conform to a simple concentrating and diluting pattern and a proportion of the contaminants were lost from the lipid pool. During mass loss, the lower chlorinated polychlorinated biphenyl congeners, chlordanes, and hexachlorocyclohexanes were lost at a higher rate than the other contaminant classes (particularly polybrominated diphenyl ethers). During mass gain the behavior of all contaminant classes was more consistent with the dilution model. These results indicate the importance of considering the energetic context when sampling blubber for long-term contaminant monitoring and suggest an initial approach to adjust for such differences.

Introduction

Pinnipeds store their fat as a discrete hypodermal subcutaneous layer known as blubber. This blubber is used for thermoregulation (*1*) and buoyancy (*2*) and has immune and endocrine functions (*3*). It is the major storage tissue for lipophilic contaminants, such as persistent organic pollutants (POPs) (*4*). In pinnipeds blubber is composed primarily of triglycerides, which are readily used as an energy source during periods of natural fasting, such as during the breeding season (*5*). But negative energy balance can also be due to illness, and stranded animals are commonly emaciated (*6*). Thus the dynamics of the lipid stores in the blubber layer will dictate the concentration and fluctuation of the lipophilic contaminants measured in this tissue. However, the interactions between the lipids and contaminants are not well understood. The concentration of persistent organic pollutants such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), and organic pesticides have been monitored in blubber samples from California sea lions since the 1970s (*7, 8*). The effect of these contaminants on health of sea lions is of considerable interest as studies on captive animals have shown immunotoxic and reproductive impacts (*9*). Blubber PCB levels have been associated with a high prevalence of cancer in California sea lions (*10*), but weight loss was a confounding variable in that study. To determine the role of contaminants in the etiology of diseases, knowledge of the effect of weight loss on contaminant dynamics is needed. Although nothing is known about effects of blubber mass changes on contaminant concentrations in otariids, data from phocid seals (*11*), particularly from postweaned pups (*12*) that undergo prolonged periods of fasting and mass loss, indicate that increases in contaminant concentrations occur with decreasing lipid reserves (*12, 13*).

In this study we investigate the relationship between total body mass and blubber mass dynamics, changes in blubber lipid content and the influence of these parameters on a wide range of POP compound concentrations in the blubber of California sea lions during a period of dramatic and rapid weight loss and gain. We determine the rate of change in contaminant concentration, both on a total and congener specific basis, relate this to lipolysis in the blubber and apply a simple mass balance model to investigate the dynamic behavior of the different POP contaminant classes.

Materials and Methods

This study was carried out on 20 adult female California sea lions (9 in 2003, 2 in 2004, and 10 in 2005) that stranded live following intoxication with domoic acid due to the ingestion of contaminated fish (*14*). Clinical signs and response to treatment were consistent with domoic acid toxicosis, as described in Gulland et al. (*15*) although the dose of domoic acid received by wild sea lions prior to stranding cannot be determined. Blubber samples from each female were collected three times during the rehabilitation period, which ranged from 14 to 44 days. Acute domoic acid poisoning causes seizures (*15*) and animals were treated with lorazepam at 0.2 mg/kg, phenobarbitone at 1 mg/kg to control seizures, and subcutaneous fluids to maintain hydration (*15*), for approximately one week during which time they were obtunded and unable to feed. After completion of the course of medication they remained reluctant to feed for up to a week and continued to lose mass. They then started to eat, appeared clinically normal, and were fed thawed previously frozen herring for about a week until release from the rehabilitation facility. Animals were weighed and blubber samples were obtained; first within 2 days of admission; second at between days 6 and 24 (at the nadir of weight loss as feeding recommenced); and finally at between days 17 and 44. Full thickness blubber samples were collected with

* Corresponding author phone: +44 1334 462634; fax +44 1334 462632; e-mail: ajh7@st-andrews.ac.uk.
† University of St Andrews.
‡ The Marine Mammal Center.
§ National Oceanic and Atmospheric Administration.
|| University of California at Davis.

10.1021/es702685p CCC: $40.75
Published on Web 05/03/2008

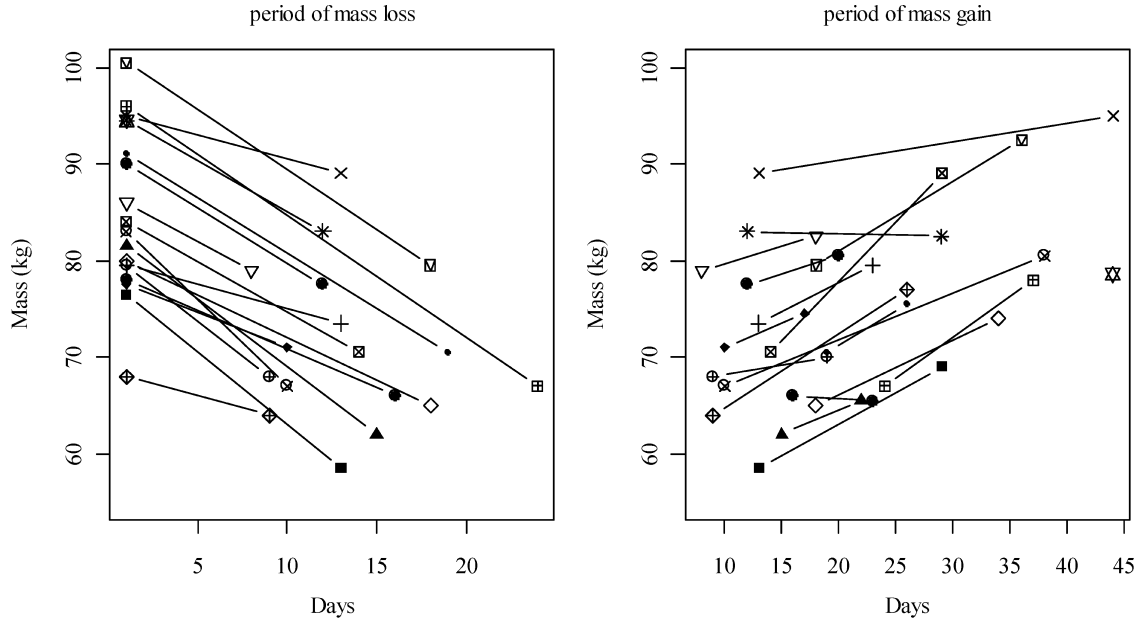

FIGURE 1. Mass loss and gain in 20 female California sea lions following domoic acid intoxication. Each symbol represents the same individual.

a sterile 8 mm biopsy punch during manual restraint following sedation with intramuscular administration of 0.2 mg/kg midazolam (Versed, Roche Laboratories, NJ) and local administration of lidocaine. Blubber was stored in solvent-rinsed Teflon sheets and frozen at −20 °C until analysis.

Persistent organic pollutants in the blubber samples were extracted using an accelerated solvent extractor (ASE) and measured by gas chromatography/mass spectrometry (GC/MS) as described in ref *16* and given in more detail in the Supporting Information (POP contaminant and blubber lipid analyses). Concentrations of lipid in blubber were measured by thin layer chromatography with flame ionization detection (TLC/FID) using an Iatroscan Mark 6, and full details including quality control results are given in the Supporting Information (POP contaminant and blubber lipid analyses).

Total PCBs (ΣPCBs) were calculated by adding the concentrations of 44 PCB congeners (PCBs 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187, 191, 194, 195, 199, 205, 206, 208, 209) and total PBDEs (ΣPBDEs) were calculated by adding the concentrations of 10 PBDE congeners (28, 47, 49, 66, 85, 99, 100, 153, 154, and 183). Total DDTs were calculated by adding the concentrations of o,p′-DDD, p,p′-DDD, o,p′-DDE, p,p′-DDE, o,p′-DDT, and p,p′-DDT; total chlordanes were calculated by adding heptachlor, heptachlor epoxide, oxychlordane, *gamma*-chlordane, nonachlor III, *alpha*- chlordane, *trans*-nonachlor, and *cis*-nonachlor concentrations and total hexachlorocyclohexanes (HCHs) is the sum of the concentrations of the *alpha*-, *beta*-, and *gamma*-HCH isomers.

In the first stage of the analysis, total body mass and blubber POP concentrations were plotted against time to visualize the relative dynamic pattern among the variables. In the second stage, linear and nonlinear least-squares mixed-effects models (with individual as the random effect) were used to investigate the relationship between blubber POP contaminant concentrations and changes in both body mass and blubber lipid content. The mass balance of the contaminants was then explored by also including an estimation of changes in the total blubber mass during the periods of mass loss and gain. This tested the hypothesis that the total amount of contaminant in the blubber did not change and conformed to a simple concentration/dilution model (i.e., burden at time t_0 = burden at t_1 = burden at t_2). Unfortunately, we were not able to directly determine body composition changes in the study animals, and there are no published data on the relationship between body mass and total body fat for California sea lions. However Kumagai et al. (*17*) have published data on the relationship between percent total body fat and total body mass in a very closely related species, the Steller sea lion (*Eumetopias jubatus*). Since pinnipeds store fat as a subcutaneous layer of blubber this equates to the total blubber mass. We fitted a linear mixed effects model to the data with percent fat as the dependent variable, mass as a fixed effect, and individual as a random effect and used the resulting equation (total body fat % = 0.20 × body mass (kg) − 11.56, $p < 0.0001$) to estimate total blubber mass in the study animals at each time point. Three animals were then excluded from the subsequent analyses because their blubber mass estimates were unrealistically low. This was because two animals had blubber lipid levels <100 mg/g and one an initial total body mass of 68 kg which was much lower than the average initial body mass. Statistical analyses were carried out using the statistical packages R (R Development Core Team, Version 2.5.0, 2007) and MedCalc for Windows, version 9.2.0.0 (MedCalc Software, Mariakerke, Belgium). All data used in the analysis (total body mass, blubber lipid, estimated blubber mass, and contaminant POP concentrations by homologue group) are given in Table S2 in the Supporting Information.

Results

Mass and Lipid Changes. At the start of the study the mean total body mass of the sea lions was 86 kg ($n = 20$, SD ± 8.1). The change in body mass over the 44 days of the study (taking the first sample time-point following admission for each animal as its reference, t_0) by individual sea lion are shown in Figure 1a and b for the periods of fasting and refeeding respectively. One animal was only weighed at the start and end of the study and was excluded from subsequent analyses. In all the remaining animals the initial decline in mass was followed by a corresponding increase, so that animals were regaining lost weight and condition. The mean (±SD) mass loss was 14.3 ± 6.8 kg, corresponding to a mean (±SD) loss of 16.5 ± 7.1% of their mass on admission, and the mean length of fasting was 12 ± 5 days. The mean (±SD) mass

TABLE 1. Contaminant Concentrations in Female California Sea Lion Blubber at Each Sampling Point (ng/g Lipid Weight) (n = 19)

	first sample		second sample		third sample	
	geometric mean	95% CI	geometric mean	95% CI	geometric mean	95% CI
estimated blubber mass (kg)[1]	5.40	4.46–6.34	2.34	1.64–3.04	3.73	2.92–4.58
blubber lipid content (mg/g)[1]	318	265–371	265	213–317	295	252–338
total PCBs	2780	1860–4140	6580	4040–10730	3890	2800–5390
tri- CBs	30	20–40	60	40–110	30	30–40
tetra- CBs	130	90–190	300	190–500	170	130–220
penta- CBs	500	340–740	1190	740–1920	660	480–900
hexa- CBs	790	520–1200	1870	1140–3070	1100	780–1550
hepta- CBs	400	270–600	930	570–1540	570	400–810
octa- CBs	110	80–160	260	160–420	170	120–240
nona- CBs	40	30–50	80	50–130	60	40–80
deca- CBs	20	10–20	40	20–60	30	20–40
total DDT	5700	3760–8630	13200	8110–21470	6870	4710–10020
4,4′, DDE	260	170–420	630	390–1020	350	230–520
total PBDEs	830	500–1380	2100	1210–3650	1180	810–1710
tri- BDEs	30	20–40	70	40–100	30	20–40
tetra- BDEs	480	290–790	1190	700–2030	630	430–930
penta- BDEs	280	170–450	680	380–1190	400	270–570
hexa- BDEs	70	50–120	170	100–310	110	70–170
hepta- BDEs	10	10–20	20	10–20	10	10–20
total chlordanes	260	170–420	630	390–1020	350	230–520
total HCHs	90	60–140	200	130–300	120	90–170

[1] Arithmetic means.

regained was 7.1 ± 5.2 kg in a period with a mean (±SD) of 14 ± 7 days. The mean (±SD) blubber lipid loss during fasting was −21 ± 115 mg/g and the mean (±SD) gain during feeding was 49 ± 112 mg/g. There was a high degree of individual variability during both periods. During the period of mass loss 11/19 (58%) of animals lost lipid and during the period of mass gain 11/19 (58%) gained lipid (although these were not the same 11 individuals). The lipid classes in the blubber samples were totally dominated by the triglycerides with only 6/60 (10%) of the samples also containing a small amount of polar lipids, at between 0.8 and 3.7% of the total.

Changes in Blubber Contaminant Concentrations. The concentrations of POP contaminants in the blubber of the study animals, on a lipid weight basis at each time point (geometric means and 95% confidence intervals around the mean), the estimated blubber mass and the lipid content of the blubber are given in Table 1. As in other studies of California sea lions, total DDTs dominated the contaminant profiles, followed by total PCBs and total PBDEs (*7*). In all cases the second sample, when the animals were at their lowest mass, had the highest concentrations. There was a highly significant correlation between blubber contaminant concentrations among all POP contaminant groups (p < 0.0001 in all cases, see Supporting Information, Table S1). Therefore investigations of the changes in concentrations during mass loss and gain focused on the total PCB group. Given the high degree of correlation among all the groups, the temporal patterns observed were the same for all the contaminants measured (see Supporting Information Figure S2).

Concentrations of total PCBs in the blubber on a lipid weight basis by day of collection (taking the first sample as day 1) are shown in Figure 2. The lines joining the points for each individual animal illustrate the matching pairs of samples and it should be noted that these changes may not necessarily be linear. All animals showed an increase in total PCB concentrations during mass loss, (Figure 2a) and the majority (17/19, 89%) a decline again during mass gain (Figure 2b).

Concentration, Dilution, and Mass Balance of Contaminants. Of particular interest is the concentration and dilution of contaminants in the blubber depending on whether animals are in positive or negative energy balance and how this is related to the chemical structure of the individual contaminants. The simplest explanatory relationship might be an inverse function, whereby during mass loss halving the lipid content in the blubber would double the concentration of contaminants, assuming no further removal of toxicant into the circulation, to other organs for storage or loss from the body through metabolism and excretion. Figure 3 shows the total PCB ratios on a lipid weight basis as a function of the ratio of the proportion of blubber lipid, at the first sample to the second sample (t_1/t_0) or the third sample to the second (t_1/t_2). The solid line shows the fitted nonlinear least-squares model ($y = a/x$) and the dotted line shows the expected functional relationship $y=1/x$. The estimate for the numerator from this model for the total PCBs was 1.25 (SE = 0.10, t = 12.7, p < 0.0001), indicating that overall the concentrating effect due to changes in blubber lipid was higher than might have been expected and the diluting effect less than expected, using a simple concentrating model. However, this approach does not account for any change in total blubber mass between the sampling points. In order to test the hypothesis that there was no overall change in POP contaminant burden, we incorporated an estimate of the total blubber mass (Table 1) and determined whether the blubber contaminant concentration ratios were inversely proportional to the animals' total lipid. We then investigated how this relationship varied among the different contaminant classes. Thus, if the animals blubber POP burden remained the same then eq 1

$$C_1/C_0 = 1/(L_1/L_0) \times (M_0/M_1) \text{ and } C_2/C_1 = 1/(L_2/L_1) \times (M_1/M_2) \quad (1)$$

Where C_0, C_1, and C_2 are the concentration of contaminants at each of the three time points respectively (ng/g lipid), L_0, L_1, and L_2 are the lipid content of the blubber (ng/g wet weight), and M_0, M_1, and M_2 are the blubber masses (g wet weight). Figure 4 shows the concentration ratio for total PCBs plotted against the inverse of the total body lipid changes for the periods of mass loss and gain. If the ratios were equivalent the slope of the line would equal one. During mass loss the concentration ratio in the blubber was lower than predicted

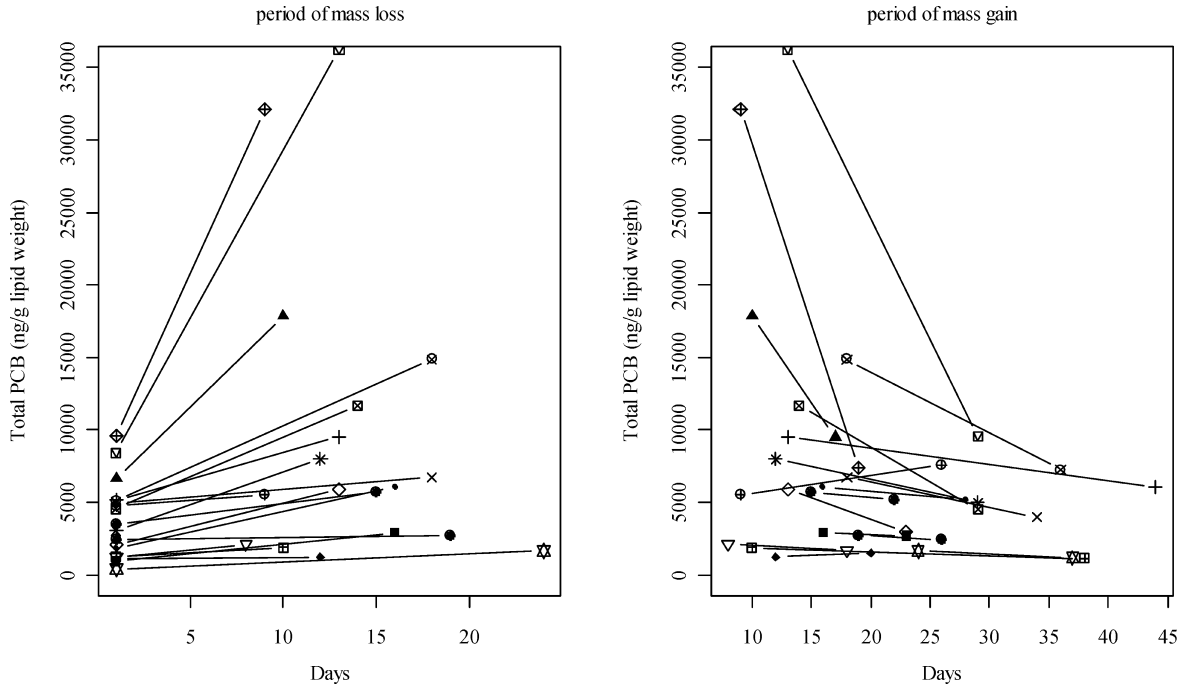

FIGURE 2. Changes in total PCB concentrations on a lipid weight basis, during periods of mass loss and gain.

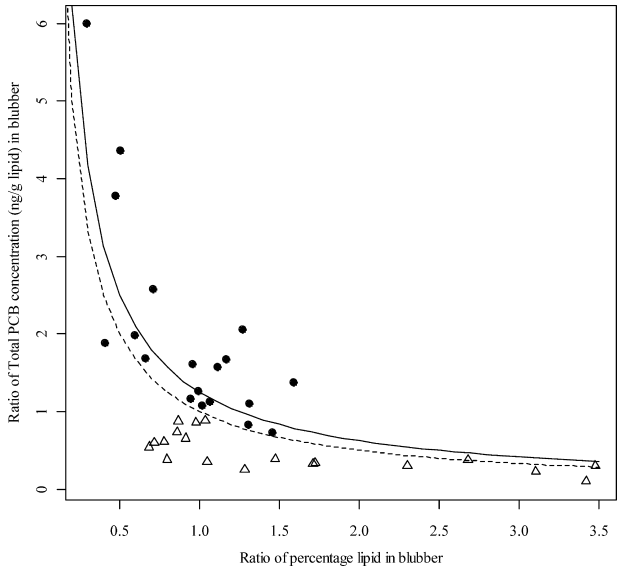

FIGURE 3. Ratio of total PCB concentration in blubber as a function of the ratio of lipid in blubber: Period of mass loss; lipid in 2nd sample/ lipid in the first sample (t_0/t_1) against total PCB in the second sample/total PCB in the first sample. Period of mass gain; lipid in the third sample/lipid in the second sample (t_2/t_1) against total PCB in the third sample/total PCB in the second sample. Solid circles = mass loss, open triangles = mass gain, solid line = nonlinear least-squares fit for inverse function $y = a/x$, dotted line = $y = 1/x$).

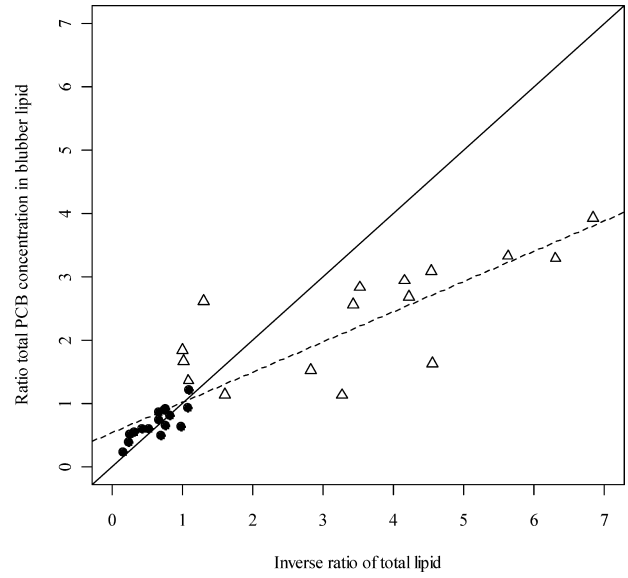

FIGURE 4. Ratio of total PCB concentration in blubber lipid (C_1/C_0 and C_2/C_1) against inverse ratio of total lipid ($1/(L_1/L_0) \times (M_0/M_1)$ and $1/(L_2/L_1) \times (M_1/M_2)$). Solid circles = mass loss, open triangles = mass gain, solid line = line of equivalence, dotted line = linear least-squares relationship.

(slope = 0.34, SE = 0.08, $p = 0.0009$, Table 2) which suggests that although the blubber total PCB concentrations significantly increased, some PCBs were being lost from the total lipid pool. During the period of mass gain, the change in concentration conformed more to the predicted model (Figure 4, slope = 0.67, SE = 0.013, $p = 0.0001$, Table 2) although the slope was still less than one.

This hypothesis was then tested for each of the contaminant classes, including the PCBs by homologue group, the total DDTs and its main metabolite p,p'DDE, the total PBDEs and the PBDE homologue groups, the total chlordanes and the total HCHs. The slopes (±SE) of the relationships, the R^2 and p values are given in Table 2, both overall and for the periods of mass loss and gain separately. During mass loss the slope coefficients were all less than one. They were lowest for the lower chlorinated compounds (and for the triCBs, tetraCBs, and total HCHs the linear relationship was not significant) and the total chlordanes (0.25), highest for the heptabrominated diphenyl ether congeners (0.81) and varied between 0.33 and 0.65 for all the other contaminant groups, being generally higher for the PBDE congeners than the PCBs and DDTs. This suggests varying degrees of contaminant loss through redistribution or excretion related to the chemical structure and lipophilicity of the compounds. During mass gain, the slopes were all higher (>0.5) than

TABLE 2. Coefficient Estimates for the Slope of the Relationship between Change in Contaminant Concentration against Inverse of Change in Total Lipid Content of Animal

					mass loss period				mass gain period			
contaminant	coefficient estimate	standard error	R^2	p value	coefficient estimate	standard error	R^2	p value	coefficient estimate	standard error	R^2	p value
total PCBs	0.48	0.04	0.79	<0.0001	0.34	0.08	0.55	0.0009	0.67	0.13	0.66	0.0001
tri- CBs	0.39	0.12	0.27	0.002	0.16	0.24	0.03	N.S.	0.82	0.25	0.43	0.006
tetra- CBs	0.17	0.08	0.12	0.05	−0.13	0.13	0.06	N.S.	0.17	0.28	0.03	N.S.
penta- CBs	0.48	0.05	0.76	<0.0001	0.33	0.09	0.50	0.002	0.71	0.14	0.65	0.0002
hexa- CBs	0.51	0.05	0.79	<0.0001	0.38	0.09	0.56	0.0008	0.66	0.13	0.65	0.0002
hepta- CBs	0.50	0.05	0.78	<0.0001	0.39	0.09	0.55	0.0009	0.64	0.14	0.60	0.0005
octa- CBs	0.48	0.04	0.78	<0.0001	0.39	0.09	0.57	0.0007	0.68	0.16	0.56	0.0009
nona- CBs	0.46	0.05	0.73	<0.0001	0.36	0.09	0.49	0.003	0.72	0.24	0.38	0.01
deca- CBs	0.45	0.06	0.66	<0.0001	0.35	0.11	0.42	0.007	0.71	0.32	0.26	0.04
total DDT	0.51	0.05	0.81	<0.0001	0.39	0.09	0.60	0.0004	0.76	0.16	0.63	0.0003
4,4′, DDE	0.52	0.05	0.81	<0.0001	0.40	0.09	0.60	0.0004	0.74	0.16	0.61	0.0003
total PBDEs	0.70	0.09	0.65	<0.0001	0.65	0.20	0.43	0.006	0.65	0.17	0.51	0.002
tri- BDEs	0.57	0.08	0.65	<0.0001	0.51	0.16	0.41	0.007	0.73	0.20	0.50	0.002
tetra- BDEs	0.67	0.08	0.68	<0.0001	0.61	0.18	0.46	0.004	0.71	0.16	0.59	0.0005
penta- BDEs	0.66	0.08	0.70	<0.0001	0.63	0.17	0.50	0.002	0.67	0.20	0.45	0.005
hexa- BDEs	0.62	0.08	0.68	<0.0001	0.59	0.16	0.48	0.003	0.59	0.31	0.21	N.S.
hepta- BDEs	0.67	0.10	0.61	<0.0001	0.81	0.19	0.55	0.001	0.53	0.42	0.10	N.S.
total Chlordanes	0.45	0.06	0.66	<0.0001	0.25	0.10	0.30	0.03	0.73	0.17	0.57	0.0007
total HCHs	0.29	0.05	0.57	<0.0001	0.14	0.08	0.19	N.S.	0.67	0.19	0.47	0.004

during the mass loss period (although the tetraCBs, hexaCBs, and heptaBDEs relationships were not significant) and were closer to the predicted relationship of 1.0 (at between 0.53 and 0.82) suggesting no gain and perhaps some further loss of POP contaminants. During this phase there was less variability among the different contaminant classes.

The concentration of total PCBs in a sample of the herring fed to the sea lions during the refeeding period was 41 ng/g wet weight. The total DDTs were 12 ng/g wet weight and the total PBDEs were 5.2 ng/g wet weight. Although some of this additional intake will also have been stored in the blubber by the end of the mass gain period, without detailed information on the quantity of food eaten and the amount of POPs excreted for each individual, it is not possible to determine the overall mass balance. The results of contaminant input-output studies in other pinniped species suggest that it is likely to be low over a 30−40 day period (*18*), but this additional input may account for some of the relationship seen during the mass gain period.

Discussion

This study is, to our knowledge, the first to examine the effects of blubber composition changes on contaminant concentrations in an otariid species. The dramatic decreases in mass during the period of aphagia in adult female California sea lions affected by domoic acid resulted in a significant increase in the concentration of POPs in the remaining blubber. This mass decline was often accompanied by decreases in the proportion of lipid in the blubber and by a decline in the animals' estimated total blubber mass. Conversely, when the animals gained mass their blubber POP concentrations decreased. Although this study was carried out on adult females, we have no reason to suspect that there would be any difference in this relationship between the sexes.

A simple mass balance calculation was then carried out for the periods of fasting and refeeding. Although the POP concentrations increased significantly during mass loss and decreased again during mass gain, there was evidence that POP contaminants were being lost from the total lipid pool and that the dynamic relationship between changes in body lipid and contaminant burdens did not conform to a simple concentrating/diluting model. The loss could either be through metabolism and restorage as metabolites (*18*) or through metabolism and excretion (*19*). However, it should be noted that the total blubber masses were estimated using a model derived from data published for Steller sea lions (*17*) and it is not known how applicable this model is to the California sea lions in this study. Indeed, three animals were excluded because their total blubber estimates were unrealistically low. Nonetheless, the results from this analysis indicated that the lower chlorinated PCB compounds, chlordanes and HCHs appeared to be more readily lost during the mass loss period than the hexa- to octachlorinated biphenyls, the DDTs, and particularly the PBDEs. The preferential mobilization of the lesser chlorinated PCBs has also been reported in gray seals (*Halichoerus grypus*) during lactation fasting (*20*) and in gray and Northern elephant seals (*Mirounga angustirostris*) during the postweaning fast (*13*). However, we did not observe any difference in the loss of PBDE congeners in relation to their degree of bromination, despite the fact that lower brominated compounds accumulate more readily in marine mammals than higher brominated compounds (*12*). This finding warrants further exploration. During the mass gain period the blubber POP concentrations were diluted and although there appeared again to be some loss from the system, the amount in the lipid pool was closer to that expected by the simple lipid dilution model. The blubber POP concentrations, estimated POP burdens in the lipid pool and the contaminant profiles seen at a given time point were a function of the lipid dynamics and energy balance of the animal.

We were not, however, able to carry out a more complete mass balance and investigate simultaneous changes in blood or liver POP concentrations, tissues which are also involved in contaminant dynamics during perturbations in energy balance (*21*). Changes in blood lipids are particularly important as this may affect the steady state equilibrium between the blubber and blood (*22*). During long periods of natural fasting in phocid seals, blood lipid levels remain fairly constant (*13*) but during starvation triglyceride levels may decline (as was seen in the study animals, see Supporting Information, Figure S3) lowering the release of POPs from the blubber. Debier et al. (*20*) found that PCBs appear to be preferentially retained in

the blubber of phocid seals until a certain point when higher amounts are released into the circulation at the end of the fasting period. The authors suggest this may be due to the differential mobilization of the various fatty acids classes as the fast progresses. These may then have different PCB congener affinities so that higher chlorinated congeners are retained until the end of the fasting period. Although we did not measure different fatty acid profiles, all the blubber samples were dominated by triglycerides (neutral lipids), which alone appear to dictate the kinetics of the highly lipophilic compounds (*22*).

During extreme fasting and starvation in mammals protein catabolism also occurs (*23*) so some mass loss could have been due to changes in protein as well as lipid stores, especially in animals with low lipid stores on admission. Even fast-adapted phocid seal species have been shown to utilize approximately 10% of their protein stores for energy during the postweaning fast, although the proportion was mass dependent (*24*). Noren et al. (*25*) found that a model predicting allocation of lipid and protein during fasting in Northern elephant seals indicated protein metabolism accounted for between 12 and 32% of the energy utilization. Otariids rely less on stored blubber for energy than phocid seals do as they do not fast for such prolonged periods. Indeed 42% of the study animals showed no substantial change in the amount of lipid in their blubber during either the period of mass loss or gain, perhaps indicating the importance of protein for energy during times of fasting and starvation.

In conclusion, these results clearly demonstrate the importance of knowing the relative condition of animals when comparing blubber contaminant concentrations, either among individuals or populations. The energetic context and life history stage must be considered when sampling blubber for contaminants in comparative monitoring studies. If studies are designed to compare levels among populations (*26*) health status and regions (*27, 28*) then care must be taken to either ensure animals are in similar body condition or that differences can be accounted for. This study is the first stage toward a more complete mass balance or pharmacokinetic model for sea lions that could be used to adjust for condition differences and allow more robust temporal and spatial comparisons to be made.

Acknowledgments

We thank the staff and volunteers of The Marine Mammal Center for caring for the animals and collecting samples. We appreciate the technical analyses provided by Daryle Boyd, Ron Pearce, Catherine Sloan, and Karen Tilbury of the NOAA Fisheries's Northwest Fisheries Science Center. We also acknowledge the helpful suggestions of Mike Lonergan and Bernie McConnell. Samples were collected under National Marine Fisheries Scientific Research Permit No. 932-1489-00, and funding was provided by the National Marine Fisheries Program through the John H. Prescott Marine Mammal Rescue Assistance Program and the Marine Mammal Unusual Mortality Event Emergency Fund.

Supporting Information Available

Details of POP contaminant and blubber lipid analyses. Correlation coefficients among blubber POP contaminant concentrations, total body mass, blubber lipid, estimated total blubber mass, and contaminant POP concentrations in study animals. A representative chromatogram. Addditional figures: Changes in blubber POP contaminant concentrations by the different contaminant classes. Changes in blood triglycerides concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Kvadsheim, P. H.; Folkow, L. P. Blubber and flipper heat transfer in harp seals. *Acta. Physiol. Scand.* **1997**, *161*, 385–395.
(2) Biuw, M.; McConnell, B.; Bradshaw, C. J. A.; Burton, H.; Fedak, M. Blubber and buoyancy: monitoring the body condition of free-ranging seals using simple dive characteristics. *J. Exp. Biol.* **2003**, *206*, 3405–3423.
(3) Fruhbeck, G.; Salvador, J. Role of adipocytokines in metabolism and disease. *Nutr. Res. (N.Y.)* **2004**, *24*, 803–826.
(4) Holden, A. V. Pollutants and seals—A review. *Mamm. Rev.* **1978**, *8*, 53–66.
(5) Kovacs, K. M.; Lavigne, D. M. Maternal investment in otariid seals and walruses. *Can. J. Zool.- Rev. Can. Zool.* **1992**, *70*, 1953–1964.
(6) Gulland, F. M. Stranded seals: important sentinels. *J. Am.Vet. Med. Ass.* **1999**, *214*, 1191–1192.
(7) Greig, D. J.; Ylitalo, G. M.; Hall, A. J.; Fauquier, D. A.; Gulland, F. M. D. Transplacental transfer of organochlorines in California sea lions (*Zalophus californianus*). *Environ. Toxicol. Chem.* **2007**, *26*, 37–44.
(8) DeLong, R. L.; Gilmartin, W. G.; Simpson, J. G. Premature births in Californian sea lions: association with organochlorine pollutant residue levels. *Science* **1973**, *181*, 1168–1170.
(9) Ross, P. S.; Vos, J. G.; Birnbaum, L. S.; Osterhaus, A. D. M. E. PCBs are a health risk for humans and wildlife. *Science* **2000**, *289*, 1878–1879.
(10) Ylitalo, G. M.; Stein, J. E.; Hom, T.; Johnson, L. L.; Tilbury, K. L.; Hall, A. J.; Rowles, T.; Greig, D.; Lowenstine, L. J.; Gulland, F. M. D. The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*). *Mar. Pollut. Bull.* **2005**, *50*, 30–39.
(11) Pomeroy, P. P.; Green, N.; Hall, A. J.; Walton, M.; Jones, K.; Harwood, J. Congener-specific exposure of grey seal (*Halichoerus grypus*) pups to chlorinated biphenyls during lactation. *Can. J. Fish Aq. Sci.* **1996**, *53*, 1526–1534.
(12) Hall, A.; Kalantzi, O.; Thomas, G. Polybrominated diphenyl ethers (PBDEs) in grey seal pups during their first year of life—Are they thyroid hormone endocrine disrupters? *Environ. Pollut.* **2003**, *126*, 29–37.
(13) Debier, C.; Chalon, C.; Le Boeuf, B. J.; de Tillesse, T.; Larondelle, Y.; Thome, J. P. Mobilization of PCBs from blubber to blood in northern elephant seals (*Mirounga angustirostris*) during the post-weaning fast. *Aquat. Toxicol.* **2006**, *80*, 149–157.
(14) Brodie, E. C.; Gulland, F. M. D.; Greig, D. J.; Hunter, M.; Jaakola, J.; St Leger, J.; Leighfield, T. A.; Van Dolah, F. M. Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*). *Mar. Mamm. Sci.* **2006**, *22*, 700–707.
(15) Gulland, F. M.; Haulena, M.; Fauquier, D.; Langlois, G.; Lander, M. E.; Zabka, T.; Duerr, R. Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): clinical signs, treatment and survival. *Vet. Rec.* **2002**, *150*, 475–480.
(16) Sloan, C. A.; Brown, D. W.; Pearce, R. W.; Boyer, R. H.; Bolton, J. L.; Burrows, D. G.; Herman, D. P.; Krahn, M. M. Determining aromatic hydrocarbons and chlorinated hydrocarbons in sediments and tissues using accelerated solvent extraction and gas chromatography/mass spectrometry. In *Techniques in Aquatic Toxicology*; Ostrander, G. K., Ed.; CRC Press: Boca Raton, FL, 2005; pp 631–651.
(17) Kumagai, S.; Rosen, D. A. S.; Trites, A. W. Body mass and composition responses to short-term low energy intake are seasonally dependent in Steller sea lions (*Eumetopias jubatus*). *J. Comp. Physiol., B* **2006**, *176*, 589–598.
(18) Brandt, I.; Bergman, A. PCB methyl sulphones and related compounds: Identification of target cells and tissues in different species. *Chemosphere* **1987**, *16*, 1671–1676.
(19) Thomas, G. O.; Moss, S. E. W.; Asplund, L.; Hall, A. J. Absorption of decabromodiphenyl ether and other organohalogen chemicals by grey seals (*Halichoerus grypus*). *Environ. Pollut.* **2005**, *133*, 581–586.
(20) Debier, C.; Pomeroy, P. P.; Dupont, C.; Joiris, C.; Comblin, V.; Le Boulenge, E.; Larondelle, Y.; Thome, J. P. Dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*: differences in PCB profile between compartments of transfer and changes during the lactation period. *Mar. Ecol.-Prog. Ser.* **2003**, *247*, 249–256.
(21) Tanabe, S.; Nakagawa, Y.; Tatsukawa, R. Absorption efficiency and biological half-life of individual chlorobiphenyls in rats treated with Kanechlor products. *Agr. Biol. Chem. Tokyo* **1981**, *45*, 717–726.
(22) Emond, C.; Charbonneau, M.; Krishnan, K. Physiologically based modeling of the accumulation in plasma and tissue lipids of a

mixture of PCB congeners in female Sprague-Dawley rats. *J. Toxicol. Env. Health, Part A* **2005**, *68*, 1393–1412.
(23) MacDonald, I. A.; Webber, J. Feeding, fasting and starvation: factors affecting fuel utilization. *Proc. Nutr. Soc.* **1995**, *54*, 267–274.
(24) Biuw, M. Variations in body composition and energy utilisaiton by southern elephant seals pups over the first year. PhD Thesis, University of St Andrews: St Andrews, 2003.
(25) Noren, D. P.; Mangel, M. Energy reserve allocation in fasting Northern Elephant Seal pups: inter-relationships between body condition and fasting duration. *Funct. Ecol.* **2004**, *18*, 233–242.
(26) Barron, M. G.; Heintz, R.; Krahn, M. M. Contaminant exposure and effects in pinnipeds: implications for Steller sea lion declines in Alaska. *Sci. Total Environ.* **2003**, *311*, 111–133.
(27) Miyazaki, N. Contaminant monitoring studies using marine mammals and the need for establishment of an International environmental specimen bank. *Sci. Total Environ.* **1994**, *154*, 249–256.
(28) Aguilar, A.; Borrell, A.; Reijnders, P. J. H. Geographical and temporal variation in levels of organochlorine contaminants in marine mammals. *Mar. Environ. Res.* **2002**, *53*, 425–452.

ES702685P