DECREASED BLUBBER THICKNESS IN BALTIC GREY SEALS *(HALICHOERUS GRYPUS)* DOES NOT CORRELATE WITH CONCENTRATIONS OF PERFLUOROALKYL SUBSTANCES

Roos, A. [1], Berger, U. [2], Kärsryd, A-S[2]. and Bäcklin, B-M. [1]

[1]Departement of Contaminant Research, Swedish Museum of Natural History, PO Box 50007, SE 104 05 Stockholm, Sweden, [2]ITM. Department of Applied Environmental Science, Stockholm University, SE-106 91 Stockholm, Sweden.

Introduction

The grey seal *(Halichoerus grypus)* population of the Baltic has decreased dramatically in numbers and distribution. In the beginning of 1900s some 100 000 grey seals were estimated to live in the Baltic but due to an intensive hunt and later other reasons the population decreased to only a few thousands in 1980 (1). In 1974 all hunting was banned, but not until a decade later could we observe an increase in numbers. Pathology of the Baltic grey seal has been studied intensively during the last four decades. Previously seals suffered from several lesions, such as claw deformations, bone loss, lesions in kidneys and many females had uterine stenosis or were sterile due to uterine occlusions (2-6). It has been suggested that these lesions were caused by a high pollutant load in the seals diet, most probably polychlorinated biphenyls (PCB) (2-8). Some of these lesions have decreased in frequency, as has the concentration of PCB in Baltic biota (5,9). However, we also know that there are other substances that have increased in concentrations during recent years, such as PFOS and similar compounds (9).Today grey seals in the Baltic face other problems namely increased frequencies of intestinal ulcers, parasitic infections and decreased blubber thickness (10). Decreased blubber thickness has been observed in the autumn/winter season among grey seals during recent years but the reason for that is unknown (Figure 1). In this study we elucidate if there is a correlation between elevated concentrations of PFASs in seals and a thin blubberlayer, since we know that these a compounds have increased in the Baltic biota during the same period as the number of lean seals have increased.

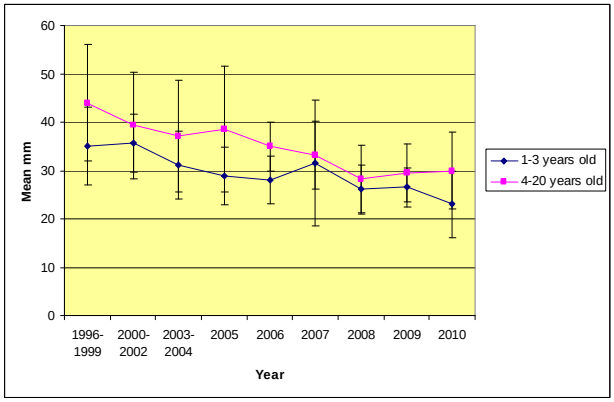

Figure 1. Significant decreasing mean blubber thickness in bycaught grey seals during the last decade.

Materials and methods

Seals

Twenty adult male grey seals, 9-12 years old, were chosen and divided into two groups: Group 1=20-30 mm sternum blubber thickness (n=9) and group 2= 40-70 mm sternum blubber thickness (n=11). Cause of death was by caught in fishing gear or shot during legal hunt. The seals were collected during 2005 and 2009, and all of them during autumn-winter season, *i.e.* the period when they should be the fattest.

Analytical method

Three gram s of liver from 20 seals were sampled from the Environmental Specimen Bank at the SMNH and analyzed at ITM (11). Fifteen fluorinated compounds, including PFOS and FOSA were analyzed. In short, 0.5 g of the homogenized sample was spiked with a suite of 8 isotope labelled internal standards. Extraction was performed with two times 5 mL acetonitrile in an ultrasonic bath. After centrifugation, the supernatant extract was removed and the combined acetonitrile phases were concentrated under nitrogen to 1 mL. The concentrated extract underwent dispersive clean-up on graphitised carbon and acetic acid. An aliquot of 0.5 mL of the clean extract was added to 0.5 mL aqueous ammonium acetate. Precipitation occurred and the extract was centrifuged before the clear supernatant was transferred to an autoinjector vial for analysis. Finally, isotopically labelled volume standards were added. Ultra performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS) was applied. The instrumental setup was: Acquity UPLC (Waters) and Xevo TQ-S MS/MS (Micromass). Separation was achieved on an Acquity UPLC BEH C18 column (Waters) with a binary gradient of buffered (ammonium acetate) methanol and water. Quantification was performed in selected reaction monitoring chromatograms using the internal standard method.

Results and discussion

Generally the concentrations of all compounds were quite low, and there were no significant differences in concentrations between lean and thicker seals for any of the 15 substances ($p>0.05$, students t-test).

The concentrations of PFOS, the by far most dominating compound, varied between 89.6 – 490 ng/g wet weight (Figure 2). It is actually somewhat lower than what was found in guillemot eggs (*Uria alge*) from the same time span, analyzed within the Swedish monitoring program, run by SMNH, and some 100 times higher than in Baltic herring (9). This shows that PFOS does bioaccumulate in seals.

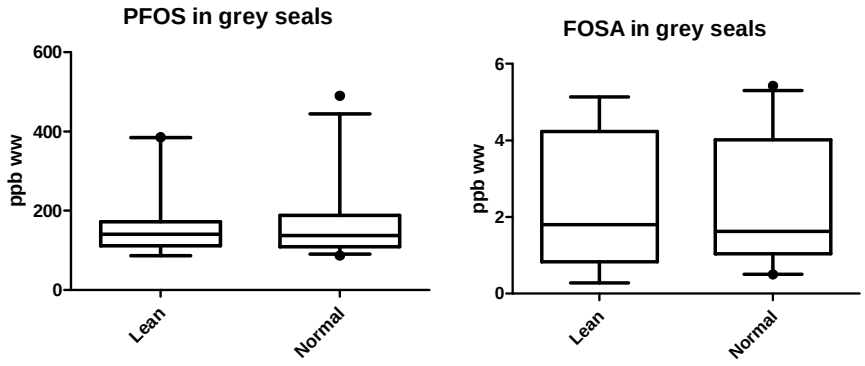

Figure 2. Concentrations of PFOS (left) and FOSA (right) in grey seal liver (ng/g wet weight). Group 1= leaner seals, group 2= seals with normal blubber thickness.

Other PFASs

All other PFASs were found in much lower concentrations compared to PFOS (see Table 1).

	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFPeDA	PFBS	PFHxS	PFOS	PFDS	FOSA
min-max	15,4-97,5	5,0-56,6	4,9-52,7	0,8-9,0	2,5-24,6	0,5-5,8	0,2-2,6	0-0	0,3-1,6	89,6-490	0,1-1,9	0,3-5,4
Mean	43,3	14,5	15,5	2,4	7,9	1,7	0,8		0,7	171	0,4	2,4
Median	37,0	10,8	14,0	2,0	6,7	1,3	0,7		0,7	138	0,3	1,7

Table 1. Results for all PFASs (all values in ng/g wet weight).

Compared to concentrations of PFASs in otters from Sweden, the concentrations in grey seals are lower, or much lower despite the fact that the seals are older than the otters (12). One possible explanation is that the seals live further away from the contaminant source.

However, the sample size is rather small (n=20) and it is not possible to draw any firm conclusions. It cannot be ruled out that these compounds do affect seals negatively, but it is not correlated to blubber thickness, at least not in the concentrations found in the present study.

Acknowledgements

This study was made possible thanks to funding from World Wide Fund for Nature, WWF:

References

1. Hårding, K. and Härkönen, K. (1999). Development in the Baltic Grey Seal (*Halichoerus grypus*) and Ringed Seal (*Phoca hispida*) Populations during the 20th Century. *Ambio* 28:619-627.
2. Helle, E., Olsson, M., and Jensen, S. (1976). PCB levels correlated with pathological changes in seal uteri. *Ambio*, 5: 261-263.
3. Bergman, A., and Olsson, M. (1985). Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: Is environmental pollution the cause of a widely distributed disease syndrome? *Finnish Game and Research*, 44 : 47-62.
4. Bergman, A., and Olsson, M., and Reiland, S. (1992). Skull-bone lesions in the Baltic grey seal (*Halichoerus grypus*). *Ambio*, 21:517-519.
5. Bergman, A. (1999). Health condition of the Baltic grey seal (*Halichoerus grypus*) during two decades: Gynaecological health improvement but increased prevalence of colonic ulcers. *APMIS*, 107:270-82.
6. Bergman, A., Bergstrand, A., and Bignert, A. (2001). Renal lesions in Baltic grey seals (*Halichoerus grypus*) and ringed seals (*Phoca hispida bothnica*). *Ambio*, 30:397-409.
7. Bredhult, C., Bäcklin, B-M., Bignert, A., and Olovsson, M. (2008). Study of the relation between the incidence of uterine leiomyomas and the concentrations of PCB and DDT in Baltic grey seals. *Reproductive Toxicology*, 25: 247-255
8. Roos, A., Bergman, A., Greyerz, E. and Olsson, M. (1998). Time trend studies on sDDT and PCB in juvenile grey seals (*Halichoerus grypus*), fish and guillemot eggs from the Baltic Sea. Proceedings from the 18th Symposium on Halogenated Environmental Organic Pollutants, Stockholm, Sweden, August 17-21, 1998. In: DIOXIN-98. Environmental Levels II. (Eds.) N. Johansson, Å. Bergman, D. Broman, H. Håkansson, B. Jansson, E. Klasson Wehler, L. Poellinger and B Wahlström. Organohalogen Compounds 39:109-112.
9. Bignert, A., Boalt, E., Danielsson, S., Hedman, J., Johansson, A.-K., Miller A., Nyberg E. Berger, U., Borg, H., Eriksson, U., Holm, K., Nylund, K. & Haglund, P.( ). Comments Concerning the National Swedish Contaminant Monitoring Programme in Marine Biota. *Report to EPA (Naturvårdsverket)* nr 7:2011, 224 pp.
10. Bäcklin, B-M., Moraeus C.,Kunnasranta, M., and Isomursu, M. 2010. Health assessment in the Baltic grey seal (*Halichoerus grypus*). *HELCOM Indicator Fact Sheets 2010*. Online., http://www.helcom.fi/environment2/ifs/en_GB/cover/.
11. Berger U., Glynn A., Holmström K., Berglund M., Halldin Ankarberg E., Törnkvist A. (2009) Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden – Analysis of edible fish from Lake Vättern and the Baltic Sea, *Chemosphere 76*, 799-804.
12. Roos, A., Järnberg, U., Berger, U. & Bignert, A. **(**2009). Perfluorinated compounds in liver from Swedish otters *(Lutra lutra)* collected between 1972 and 2008 — a new threat to Swedish otter population? *PFC Workshop, Stockholm 2009. Abstract.*