This item is the archived peer-reviewed author-version of:

Lipophilicity of PCBs and fatty acids determines their mobilisation from blubber of weaned northern elephant seal pups

Reference:

Louis Caroline, Covaci Adrian, Crocker Daniel E., Debier Cathy.- Lipophilicity of PCBs and fatty acids determines their mobilisation from blubber of weaned northern elephant seal pups

The science of the total environment - ISSN 0048-9697 - 541(2016), p. 599-602

Full text (Publishers DOI): <http://dx.doi.org/doi:10.1016/J.SCITOTENV.2015.09.094>

To cite this reference: <http://hdl.handle.net/10067/1301860151162165141>

Lipophilicity of PCBs and fatty acids determines their mobilisation from the blubber of weaned northern elephant seal pups

Caroline Louis¹, Adrian Covaci², Daniel E. Crocker³, Cathy Debier¹

¹ Institut des Sciences de la Vie, UCLouvain, Croix du Sud 2/L7.05.08, 1348 Louvain-la-Neuve, Belgium

² Department of Pharmaceutical Sciences, Toxicological Center, Campus Drie Eiken, Universiteit Antwerpen, Universiteitsplein 1, 2610 Wilrijk, Belgium

³ Department of Biology, Sonoma State University, 1801 East Cotati Ave, Rohnert Park, CA 94928, USA

ABSTRACT

Polychlorinated biphenyls (PCBs) exhibit lipophilic properties that lead to their bioaccumulation in adipose tissue. Being characterised by large adipose tissue stores, northern elephant seals (*Mirounga angustirostris*) are contaminated by PCBs. During lipolytic periods such as the post-weaning fast, fatty acids (FAs) from triglycerides and PCBs are mobilised from their blubber. Our results showed that the degree of lipophilicity of FAs and PCBs was a determinant factor affecting their release: the more lipophilic FAs and PCBs tended to be more conserved in blubber over the fast than the less lipophilic ones. This led to an enrichment of more lipophilic compounds within adipocytes with the progression of the fast.

KEYWORDS

Polychlorinated biphenyls; fatty acids; adipose tissue; lipolysis

Polychlorinated biphenyls (PCBs) are highly lipophilic environmental pollutants that are mainly stored within the adipose tissue (Gevao et al., 2009; Harrad, 2009). During periods of negative energy balance, such as fasting, the adipose tissue is considered as a source of PCBs, leading to both their release in blood as well as their concentration in the remaining amount of adipose tissue (Kim et al., 2011; Louis et al., 2014). The dynamics of their release appears to depend on their physico-chemical properties (Debier et al., 2006). It has been suggested that the heterogeneous release of PCBs could be influenced by the differential mobilisation of fatty acids (FAs) from the adipose tissue (Debier et al., 2003). Nevertheless, no link has been clearly established yet between the release of PCBs and lipids from the adipose tissue during a lipolytic process.

The purpose of this work was thus to study the potential link between the release of PCBs and FAs during lipolysis. In order to reach this goal, our study focused on the northern elephant seal (NES), which undergoes several natural periods of fast during its life. It can thus be considered as a good model to study the lipolysis within the adipose tissue. Furthermore, the NES is a marine mammal predator at the top of the food chain that is characterised by a significant contamination by lipophilic pollutants, such as PCBs (Debier et al., 2012).

Twenty-two and twenty free-ranging weaned NES pups from Año Nuevo State Reserve (CA, USA) were sampled at 1- and 7-week post-weaning (also called, early and late fast) in 2010 and 2012, respectively. Blubber collection procedures and details regarding PCB and FA analyses are described in Louis et al. (2014), Covaci et al. (2008) and Schneider et al. (2012), respectively. Briefly, blubber biopsies were sampled with 6-mm biopsy punches and were cut into three equal parts. Only the inner layer (i.e. close to the muscle) was exploited for this study, as it is preferentially mobilised during lipolysis. For the PCB analyses, blubber samples from 2010 were mixed with anhydrous Na₂SO₄. Lipophilic pollutants were extracted by automated Soxhlet. Samples were then cleaned up through an acid silica cartridge. Fourteen PCBs (IUPAC numbers: CB-47, -74, -99, -105, -118, -128, -138, -146, -153, -170, -177, -180, -183 and -187) were quantified by gas chromatography (GC) coupled with a mass spectrometer system. For the FA analyses, blubber samples from 2012 were crushed with ceramic beads and lipids were extracted by a mixture of chloroform/methanol/water. Neutral lipid fraction (i.e. TGs, diglycerides, monoglycerides and cholesterol esters) was isolated by SPE columns. A methylation step was then performed. FA methyl esters were extracted and

separated by GC. Thirty FA methyl esters were quantified in NES samples (C10:0; C12:0, C14:0, C14:1n-5; C16:0, C16:1n-7, C18:0, C18:1n-7, C18:1n-9, C18:2n-6, C18:3n-3, C18:3n-6, C18:4n-3, C20:0, C20:1n-9, C20:2n-6, C20:3n-3, C20:3n-6, C20:4n-3, C20:4n-6, C20:5n-3, C22:0, C22:1n-9, C22:4n-6, C22:5n-3, C22:5n-6, C22:6n-3, C24:1n-5, C24:5n-3, C24:6n-3).

In order to study the mobilisation of PCBs and FAs from blubber, we calculated the fractional mobilisation (FM) values as following (adapted from Mustonen et al. (2007) and Noren et al. (2013)):

$$\frac{\text{Concentrations at week 1} - \text{Concentrations at week 7}}{\text{Concentrations at week 1}}$$

The PCB and FA concentrations were expressed per wet weight. A compound with a small FM value tends to be conserved in the blubber layer over the fast, whereas a compound with a greater FM value tends to be mobilised. The log K_{ow} values for PCBs were compiled from Hansen et al. (1999). The log K_{ow} values for FAs were estimated thanks to Advanced Chemistry Development, Inc. Software (version 12.01).

The FM values of studied PCBs were all negative (Figure 1), meaning that the PCB concentrations in blubber were higher at late fast compared to early fast. This phenomenon was also visible when PCBs were expressed per unit of lipid weight. The observed increase of PCB concentrations in the inner blubber layer, despite their mobilisation into the circulation (Louis et al., 2014), appears to result from a combination of two phenomena: (i) the less efficient mobilisation of PCBs than cell TGs and, (ii) the sampling of a higher number of cells, which have a smaller size, at late fast (Figure 2). Indeed, the sampling at early and late fast was performed with 6-mm biopsy punches, which allows taking off blubber biopsies of same diameter. However, since weaned NES pups mobilised TGs from blubber (which is reflected by a loss of animal weight), the size of adipocytes decreased, as confirmed by the cell profile area of blubber adipocytes (Louis et al., 2014). The proportion of connective tissue being small in NES blubber (< 5%; D.E. Crocker, personal communication) and adipocytes being the main cell type of this tissue, we most likely sampled a higher number of adipocytes at late fast than at early fast. As illustrated in Figure 2, even if the quantity of PCBs per cell most probably decreased throughout the fast, as a result of their release into the circulation, the fact that we sampled a higher number of cells at late fast contributed to obtain higher PCB concentrations. In order to monitor the changes of PCB quantities at cell levels, expressing the

levels of PCBs per unit of cells (through cell counts or DNA quantification after adipocyte isolation) rather than per unit of lipid or wet weight would allow to better understand the dynamics of cell emptying. The FM values of studied FAs were more heterogeneous compared to PCBs, with negative and positive values, and were discussed elsewhere (article FAs NES).

A selective mobilisation from adipocytes was noticed for PCB congeners for which the degree of lipophilicity increased with the number of chlorine atoms. The more lipophilic PCBs (i.e. with the higher $\log K_{ow}$ values) were preferentially retained within the blubber, while the less lipophilic PCBs were more easily released (Figure 1A). Indeed, a linear decrease of FM values occurred with the increase of the $\log K_{ow}$ values of the molecules ($y=5.86-1.07x$, $R^2 = 0.863$). A similar relationship was also noticed for the mobilisation of PCBs and polybrominated diphenyl ethers from blubber to serum of grey seal females between early and late lactation (Vanden Berghe et al., 2012). The comparison between the FM values of each FA and their $\log K_{ow}$ values highlighted that the FAs with a higher degree of lipophilicity also tended to be conserved within the blubber, whereas the FAs with a lower degree of lipophilicity seemed to be more readily mobilised from adipocytes (Figure 1B). Similar to PCBs, there is a linear decrease of FM with $\log K_{ow}$ values ($y=0.71-0.09x$, $R^2 = 0.636$). Selective mobilisation of FAs was already observed in marine mammal species, such as lactating NES and lactating Weddell seals (Fowler et al., 2014; Wheatley et al., 2008). Raclot (2003) suggested that the discrimination in the released FAs observed in rat and human adipocytes could be linked to their molecular structure. Indeed, Raclot's study showed that the relative mobilisations of FAs increase with the number of unsaturation for a given chain length and decrease with the chain length for a given number of unsaturation.

The lipophilicity of PCBs and FAs appears to be a key factor determining their fate within adipocytes. More lipophilic FAs and more lipophilic PCBs are probably situated in the lipid droplet core, whereas less lipophilic FAs and less lipophilic PCBs are most likely situated closer to the interface (Raclot, 2003). This agrees with the lipolytic process that involves water-soluble lipases acting at the interphase formed by cytosol and lipid droplet. This selective mobilisation of PCBs and FAs leads to a reshuffle of profiles within adipocytes of the inner blubber layer. These adipocytes enriched themselves with more lipophilic PCBs and FAs and thus, acquired a higher lipophilic character over the fast. Nevertheless, we have to keep in mind that, in addition to the lipophilicity, other physico-chemical parameters could also influence the mobilisation of lipophilic pollutants from adipocytes, such as the size and

the configuration of the molecules. A higher mobilisation of those more lipophilic compounds may however happen later in the fast (Debier et al., 2003; Debier et al., 2006; Raclot, 2003).

ACKNOWLEDGEMENTS

The authors are very grateful to 2010 and 2012 field teams from Sonoma State University and University of California, Santa Cruz for the field work support. We also thank Laurent Perdaens, Eric Mignolet and Yvan Larondelle from UCLouvain for their support regarding FA analyses as well as Marie Stas, Alin C. Dirtu and Govindan Malarvannan for their support regarding PCB analyses. Research was conducted under the National Marine Fisheries Service Marine Mammal Permits #87-1743-05 and #14636-02.

LEGENDS

Figure 1 – Relationship between the fractional mobilisation of PCBs (A) and fatty acids (B) and the log K_{ow} values in the inner blubber layer of weaned northern elephant seal pups.

Figure 2 – Schematic representation of the impact of the fast on adipocyte size and PCB content (red dots). At early and late fast, blubber biopsies were sampled with 6-mm biopsy punches. Through the fast, TGs were mobilised, leading to a decrease of the size of adipocytes. Consequently, a higher amount of cells were sampled at late fast compared to early fast. The close-up represents adipocytes from the inner blubber layer.

REFERENCES

- Covaci, A., et al., 2008. Anthropogenic and naturally occurring organobrominated compounds in two deep-sea fish species from the Mediterranean Sea. *Environmental science & technology*. 42, 8654-8660.
- Debier, C., et al., 2006. Mobilization of PCBs from blubber to blood in northern elephant seals (*Mirounga angustirostris*) during the post-weaning fast. *Aquatic Toxicology*. 80, 149-157.
- Debier, C., et al., 2012. Differential changes of fat-soluble vitamins and pollutants during lactation in northern elephant seal mother-pup pairs. *Comparative Biochemistry and Physiology Part A*. 162, 323-330.

- Debier, C., et al., 2003. Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*. Marine Ecology Progress Series. 247, 237-248.
- Fowler, M. A., et al., 2014. Fatty acid mobilization and comparison to milk fatty acid content in northern elephant seals. Journal of Comparative Physiology B. 184, 1-11.
- Gevao, B., et al., 2009. Persistent organic pollutants in the developing world. Persistent Organic Pollutants. 137-169.
- Hansen, B. G., et al., 1999. QSARs for K_{OW} and K_{OC} of PCB congeners: A critical examination of data, assumptions and statistical approaches. Chemosphere. 39, 2209-2228.
- Harrad, S., 2009. Beyond the Stockholm Convention: An introduction to current issues and future challenges in POPs research. Persistent Organic Pollutants. 1-4.
- Kim, M. J., et al., 2011. Fate and complex pathogenic effects of dioxins and polychlorinated biphenyls in obese subjects before and after drastic weight loss. Environmental Health Perspectives. 119, 377-383.
- Louis, C., et al., 2014. Mobilisation of lipophilic pollutants from blubber in northern elephant seal pups (*Mirounga angustirostris*) during the post-weaning fast. Environmental Research. 132, 438-448.
- Mustonen, A.-M., et al., 2007. Lipid metabolism in the adipose tissues of a carnivore, the raccoon dog, during prolonged fasting. Experimental Biology and Medicine. 232, 58-69.
- Noren, D. P., et al., 2013. Characterization of blubber fatty acid signatures in northern elephant seals (*Mirounga angustirostris*) over the postweaning fast. Journal of Comparative Physiology B. 138, 1065-1074.
- Raclot, T., 2003. Selective mobilization of fatty acids from adipose tissue triacylglycerols. Progress in Lipid Research. 42, 257-288.
- Schneider, A.-C., et al., 2012. Conversion of t11t13 CLA into c9t11 CLA in caco-2 cells and inhibition by sterculic oil. PLoS ONE. 7, e32824.
- Vanden Berghe, M., et al., 2012. Selective transfer of persistent organic pollutants and their metabolites in grey seals during lactation. Environment International. 46, 6-15.
- Wheatley, K. E., et al., 2008. Differential mobilization of blubber fatty acids in lactating Weddell seals: Evidence for selective use. Physiological and Biochemical Zoology. 81, 651-662.

Figure 1

Figure 2