

Cod: 8.4020

EVIDENCE OF DDTs IN THE GREENLAND SHARK SOMNIOSUS MICROCEPHALUS FROM GREENLAND SEAWATERS

S. Cotronei¹, K. Pozo², O. Audy³, J. Nielsen⁴, S. Corsolini¹

¹ *Department of Physics, Earth and Environmental Sciences, University of Siena, Via P.A. Mattioli 4, I-53100 Siena, Italy*

² *(RECETOX) Research Centre for Toxic Compounds in the Environment, Masaryk University, Kamenice 3/126, 625 00 Brno, Czech Republic; Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Alonso de Ribera 2850, P.C. 407 01 29 Concepción*

³ *(RECETOX) Research Centre for Toxic Compounds in the Environment, Masaryk University, Kamenice 3/126, 625 00 Brno, Czech Republic*

⁴ *Marine Biological Section, University of Copenhagen, Strandpromedan 5, 3000 Helsingør, Denmark; Greenland Institute of Natural Resources, P.O. Box 570, 3900 Nuuk, Greenland*

Introduction

Dichlorodiphenyltrichloroethane (DDT) is chlorinated hydrocarbon compound with peculiar insecticidal properties. It was first synthesized in 1874, but its insecticidal properties were not discovered until 1939¹ and large-scale industrial production started in 1943. DDT was banned in industrialized countries during 1970s because of the potential harmful effects on wildlife and humans but is currently being used in many developing countries for insect vector of disease²⁻³⁻⁴.

The Greenland Shark *Somniosus microcephalus* is a top predator of cold sea-waters that may reach a total length of up to six meters. For this reason, it is one of largest shark species in the world and presumably the largest fish in the Arctic⁵. Very little is known about migrations or habitat preferences of this predator. Recent studies demonstrate that *S. microcephalus* may extend from the temperate North Atlantic Ocean to the Arctic Ocean⁵⁻⁶. It hunts actively throughout the water column in the Arctic Sea, as shown by some recent tagging experiments⁷⁻⁸. Considering that the *S. microcephalus* is an opportunistic top predator, extremely long lived with slow growth⁹⁻¹⁰, the bioaccumulation process of pollutants generated by human activities might be enhanced.

The aim of this study was first to investigate the concentrations of DDTs and its metabolites in samples of liver and white muscle of *S. microcephalus* from Greenland seawaters, secondly to evaluate if the bioaccumulation is affected by sex.

Materials and methods

DDTs were analyzed in 1-5 g of white muscle and liver tissue samples from 11 Greenland Sharks (6 ♀, 5 ♂, body length (bl) 104-454 cm). The sharks were collected at different depths (114-745 m). Eight specimens were caught by long-lines in the fjords of north-west Greenland, the remaining specimens were caught from offshore areas in the south-west and south-east Greenland. Samples were kept frozen at -20°C until analysed.

DDT compounds (p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, o,p'-DDE, o,p'-DDD) were investigated following the methods described elsewhere¹¹⁻¹². Briefly, samples were extracted with dichloromethane in a Büchi System B-811 automatic extractor. After extraction, the sample volume was reduced under a gentle stream of nitrogen at ambient temperature and an aliquot of each sample was used to determine the lipid contents gravimetrically. The remaining POPs aliquot was cleaned up with a modified silica column, 25 mm i. d. (2 g silica activated + 35 g activated silica gel modified with sulphuric acid). The sample was loaded and then eluted with 200 mL of a mixture of n-Hexane: DCM (1:1). The solvent was reduced in TurboVap II and transferred into a GC conical, recovery standard were added. Samples were analyzed using a GC-MS instrument (GC 7890 / MS-MS Triple Quadrupole 7000B (Agilent)). One laboratory blank and one reference material were analyzed with each set of ten samples. Internal standards (PCB-121 for PCBs + OCPs) were spiked for each sample after evaporation to a final volume of 50 µL.

Result and discussion

The results are showed in Table 1. The o,p'- and p,p'-DDT, DDD and DDE isomers were detected in all samples. The lipid content was 46 ± 17% in white muscle and 41 ± 18% in the liver.

The concentrations of Σ DDTs were higher in the muscle (748 ± 434 ng/g lipid basis) than in the liver samples (699 ± 374 ng/g). Differences between the sex and size of sharks and the type of tissues were observed in this study. In the female sharks, the mean concentrations of Σ DDTs were 984 ± 421 ng/g l.w. and 787 ± 310 ng/g l.w. in the muscle and the liver, respectively, in males levels were lower than in females in both tissues (465 ± 256 ng/g l.w. and 592 ± 450 ng/g l.w.). In particular, the females were larger (body length (bl) 310-454 cm) than males (body length (bl) 104-326 cm) and the highest concentrations observed in female sharks were presumably correlated to their age and/or different feeding behaviours (Figure 1). However, the highest concentration of Σ DDT (1336 ng/g l.w.) was observed in the liver of the smallest shark (body length (bl) 104 cm); liver being the primary detoxification organ, the evidence of a high level of o,p'-DDT and p,p'-DDT in this immature individuals might be due to its limited biotransformation capacity in comparison with mature individuals that may metabolize DDT to DDE more easily⁹. The percentage of contribution for p,p'-DDT isomers in all samples was 25% in the liver and 24% in white muscle, regarding o,p'-DDT isomers the percentage contribution was 11% and 10% in the liver and white muscle respectively. Among DDT metabolites, the p,p'-DDE was the main contributor, accounting for 52% and 54% in the liver and white muscle, respectively (Figure 2). The high percentages of p,p'-DDE (>50%) in all samples, is probably due to the high degree of biomagnification of this metabolite in the arctic food web¹³.

On the other hand, the evidence of the parent compounds, p,p'-DDT and o,p'-DDT in all studied specimens may be due to their low metabolic capacity and/or to a recent use of this pesticide in countries, where the malaria is still endemic, allowed to use DDT by the Stockholm Convention to control the mosquito vectors of malaria²⁻³⁻⁴. However, the ratio of DDT to DDE in this study was below 1.0 in all samples studied, suggesting an old DDT contamination event¹³ together with new exposure.

In general, our study confirms the presence of high DDT levels in this species from such pristine and remote areas and its bioaccumulation in this species. Moreover, the highest DDT concentrations were observed in the liver of the smallest shark, and female sharks showed higher concentrations respect to males. Further studies are needed to confirm these results in order improve our knowledge on the relationship between bioaccumulation and sex/size of the Greenland shark.

Acknowledgements

The Italian National Antarctic Research Programme (PNRA) funded this research (PdR2013 C1_04). The laboratory analyses were supported by the National Sustainability Program of the Czech Ministry of Education, Youth and Sports (LO1214) and the RECETOX research infrastructure (LM2015051). The samples were collected during expeditions led by the Greenland Institute of Natural Resources. We are very grateful to Dr Rasmus Hedeholm for his kind support during the expedition on board the R/V Sanna in August 2014. We thank the crew of the R/V Sanna and Pamiut. S. Cotronei would like to thank the Erasmus Program for the five month placement at Masaryk University.

References

1. UNEP, 2009. Marine litter: A global challenge. UNEP publications, Nairobi. 232-233.
2. van den Berg H, (2009) *Env. Health Persp.* 117: 1656-63
3. Jaga K, Dharmani C, (2003) *Rev. Panam. Salud Publica.* 14: 171-85.
4. SC-POPs, 2013. Stockholm convention on persistent organic pollutants (POPs). <http://chm.pops.int/default.aspx>.
5. MacNeil MA, McMeans BC, Hussey NE, Vecsei P, Svavarsson J, et al. (2012) *J. Fish. Biol.* 80: 991-1018
6. Nielsen J, Hedeholm RB, Simon M, Steffensen JF, (2014) *Polar Biol.* 37: 37-46
7. Fisk AT, Lydersen C, Kovacs KM, (2012) *Mar. Ecol. Prog. Ser.* 468: 255-265
8. Skomal GB, Benz GW, (2004) *Mar. bio.* 145: 489-498.
9. Corsolini S, Ancora S, Bianchi N, Mariotti G, Leonzio C, Christiansen JS, (2014) *Mar. Pollut. Bull.* 87: 381-387.
10. Strid A, Jörundsdóttir H, Päpke O, Svavarsson J, Bergman Å (2007) *Mar. Pollut. Bull.* 54: 514-1522
11. Pozo et al, (2015) *Mar. Pollut. Bull.* 95: 480-483
12. Chropenova M, Grekusková Klemmova E, Karasková P, Pribylová P, Kukucka P, Baráková D, Cupr P, (2016) *Ecol. Indic.* 66: 132-142
13. Fisk AT, Tittlemier S, Pranschke J, Norstrom R.J, (2002) *Ecology.* 83: 2162-2172.

Table 1: Concentrations of DDT compounds (ng/g l.w.) in the Greenland shark tissues depending on the sex (SD = standard deviation).

	<i>Liver</i>			<i>White muscle</i>		
	<i>Range</i>	<i>Mean</i>	<i>SD</i>	<i>Range</i>	<i>Mean</i>	<i>SD</i>
♂ (n=5)						
<i>o,p'</i> -DDE	2-12	5	4	1-6	4	2
<i>p,p'</i> -DDE	8-751	327	254	106-426	267	142
<i>o,p'</i> -DDD	4-26	9	9	3-14	7	4
<i>p,p'</i> -DDD	24-163	57	60	21-71	44	21
<i>o,p'</i> -DDT	12-104	55	35	13-92	46	35
<i>p,p'</i> -DDT	26-280	140	96	25-185	97	76
Σ 6DDTs	174-1.336	592	450	194-735	465	256
Σ <i>p,p'</i> -DDT + <i>p,p'</i> -DDE	131-1030	467	348	149-600	364	210
♀ (n=6)						
<i>o,p'</i> -DDE	3-8	5	2	3-8	6	2
<i>p,p'</i> -DDE	179-566	398	159	308-980	520	255
<i>o,p'</i> -DDD	4-56	9	4	4-30	15	10
<i>p,p'</i> -DDD	36-114	74	29	37-163	88	47
<i>o,p'</i> -DDT	50-160	94	45	69-134	101	26
<i>p,p'</i> -DDT	99-304	207	84	137-444	254	103
Σ 6DDTs	371-1.121	787	310	591-1750	984	103
Σ <i>p,p'</i> -DDT + <i>p,p'</i> -DDE	279-870	604	240	446-1424	774	352

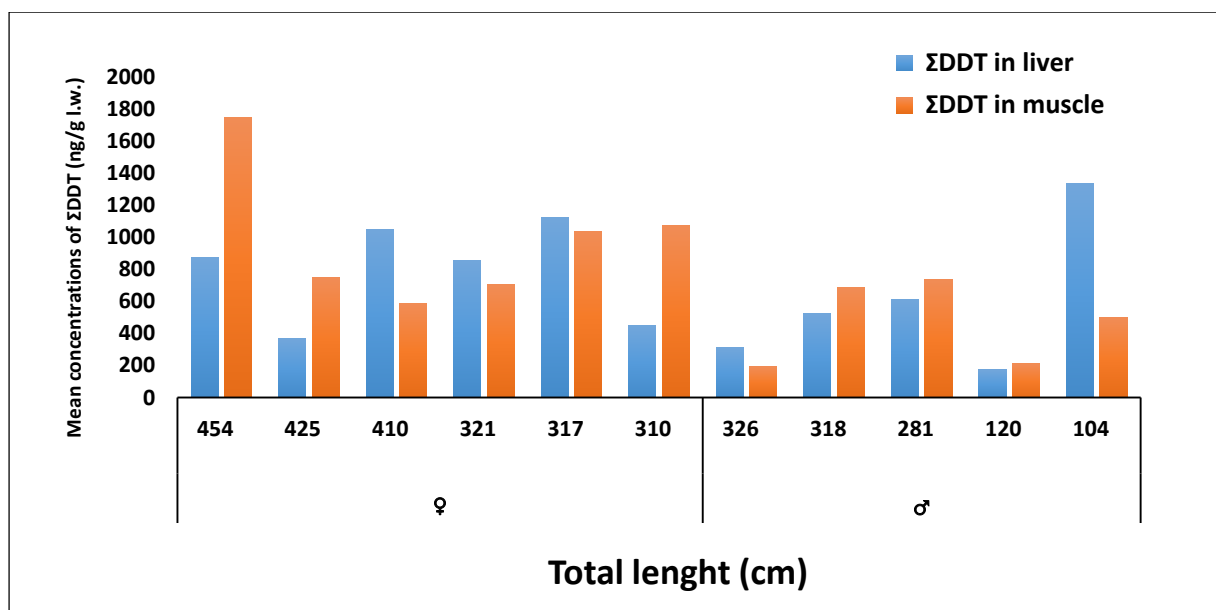


Figure 1: Concentrations of ΣDDT (ng/g l.w.) in male and female individuals (size is x axis is expressed in centimeters = cm).

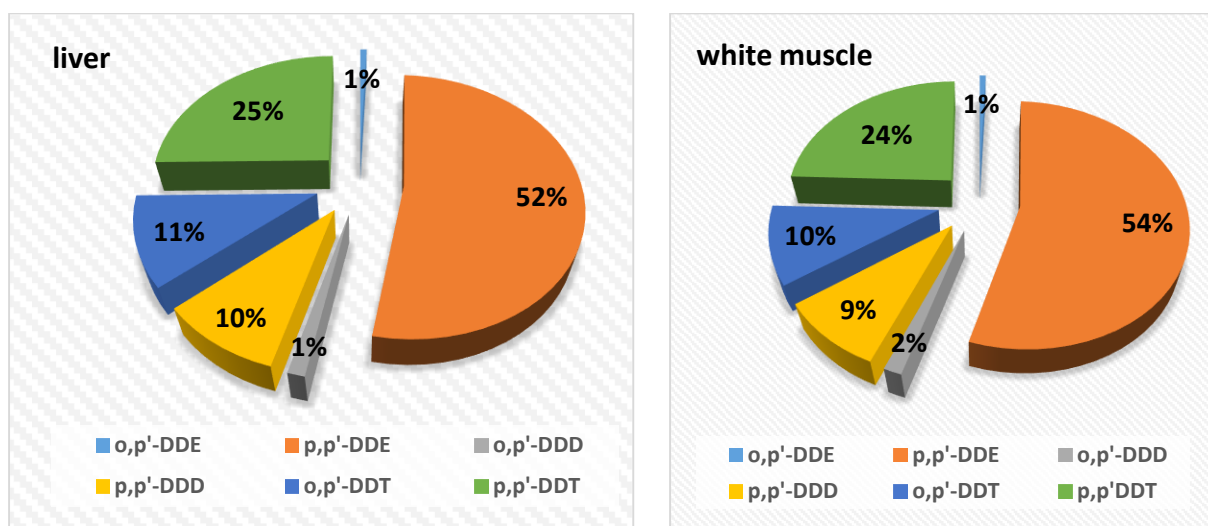


Figure 2: Percentage (%) of DDT isomers contribution and its metabolites in the Greenland shark liver and white muscle.