

Supplementary Material

Ecotoxicological characterization of Type C killer whales from Terra Nova Bay (Ross Sea, Antarctica): Molecular biomarkers and legacy and emerging persistent organic contaminants

Cristina Panti^{a*}, Juan Muñoz-Arnanz^b, Letizia Marsili^{a,c}, Simone Panigada^d, Matteo Baini^a, Begoña Jiménez^d, Maria Cristina Fossi^a, Giancarlo Lauriano^e

(a) Department of Environmental, Earth and Physical Sciences, University of Siena, Siena, Italy

(b) Institute of Organic Chemistry (IQOG-CSIC), Madrid, Spain

(c) CIRCE – Centro Interuniversitario di Ricerca sui Cetacei, c/o Department of Environmental, Earth and Physical Sciences, University of Siena, Italy

(d) Tethys Research Institute, Milan, Italy

(e) Italian Institute for Environmental Protection and Research (ISPRA), Rome, Italy

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1. Reagents and standards for POP analysis

Solvents of high purity (pesticide residue grade): n-hexane, dichloromethane and nonane were purchased from Merck (Darmstadt, Germany). Silica gel (70-230 mesh) and sulfuric acid (analytical reagent grade, 95-97%) were supplied by Merck while anhydrous sodium sulfate by J.T. Baker (Deventer, Netherlands). The standards used in this study are summarized in the following table:

Table S1. Labeled and native chemical standards used in this study.

Group	Congeners	Acquired from
native HCHs and HCB	α -HCH, β -HCH, γ -HCH, HCB	Dr.Ehrenstorfer (Augsburg, Germany)
labeled HCHs and HCB	$^{13}\text{C}_6$ - α -HCH, $^{13}\text{C}_6$ - β -HCH, $^{13}\text{C}_6$ -HCB	
native PCBs	PCB-28, -52, -95, -101, -105, -114, -118, -123, -132, -138, -149, -153, -156, -157, -167, -170, -180, -183, -189, -194	
labeled PCBs	$^{13}\text{C}_{12}$ -PCB-28, -52, -101, -138, -153, -180, -209 (PCB mix EC-4058)	Cambridge Isotope Lab. Inc. (Andover, USA)
	$^{13}\text{C}_{12}$ -PCB-77, -81, -105, -114, -118, -123, -126, -156, -157, -167, -169, -189 (PCB mix WP-CVS); $^{13}\text{C}_{12}$ -PCB-70, -111, -138, -170 (PCB mix WP-ISS)	Wellington Laboratories (Guelph, Ontario, Canada)
native DDTs	<i>p,p'</i> -DDT, <i>o,p'</i> -DDT, <i>p,p'</i> -DDE, <i>o,p'</i> -DDE, <i>p,p'</i> -DDD, <i>o,p'</i> -DDD (Pesticide-Mix 164)	Dr.Ehrenstorfer (Augsburg, Germany)
labeled DDTs	D_8 - <i>p,p'</i> -DDE, D_8 - <i>o,p'</i> -DDT, D_8 - <i>p,p'</i> -DDT	Dr.Ehrenstorfer (Augsburg, Germany)
	$^{13}\text{C}_{10}$ - <i>p,p'</i> -DDT	Cambridge Isotope Lab. Inc. (Andover, USA)
native PBDEs	BDE-28, -47, -66, -85, -99, -100, -153, -154, -183, -184, -191, -196, -197, -209	Wellington Laboratories (Guelph, Ontario, Canada)
labeled PBDEs	$^{13}\text{C}_{12}$ -BDE-138, -139	Wellington Laboratories (Guelph, Ontario, Canada)
native DP	syn-DP, anti-DP	Wellington Laboratories (Guelph, Ontario, Canada)
labeled DP	$^{13}\text{C}_{10}$ -syn-DP, $^{13}\text{C}_{10}$ -anti-DP	Cambridge Isotope Lab. Inc. (Andover, USA)

Table S2. Quantity of each surrogate spiked in blubber samples for the analysis of DDTs, HCB, HCHs, PCBs, PBDEs and DP.

Surrogate	Quantity (pg)	Surrogate	Quantity (pg)
DDTs		PCBs	
D ₈ - <i>p,p'</i> -DDE	5,000	¹³ C ₁₂ -PCB-28	2,500
D ₈ - <i>o,p'</i> -DDT	5,000	¹³ C ₁₂ -PCB-52	2,500
D ₈ - <i>p,p'</i> -DDT	5,000	¹³ C ₁₂ -PCB-101	2,500
		¹³ C ₁₂ -PCB-138	2,500
HCB		¹³ C ₁₂ -PCB-153	2,500
¹³ C ₆ -HCB	5,000	¹³ C ₁₂ -PCB-180	2,500
		¹³ C ₁₂ -PCB-209	2,500
HCHs		PBDEs	
¹³ C ₆ - α -HCH	5,000	¹³ C ₁₂ -BDE-138	3,750
¹³ C ₆ - γ -HCH	5,000		
DP			
¹³ C ₁₀ -syn-DP	2,000		
¹³ C ₁₀ -anti-DP	2,000		

Table S3. Injection standards used to reconstitute samples prior to instrumental analysis.

Volume and concentration of the internal standards used
20 μ L of ¹³ C ₁₂ -PCB-111,170,178 [50 pg/ μ L], ¹³ C ₁₂ -BDE-139 [50 pg/ μ L] and ¹³ C ₁₀ - <i>p,p'</i> -DDT [100 pg/ μ L] in nonane

2. QA/QC

For POP analysis, metal and glassware material was cleaned (3x) with three solvents of decreasing polarity: acetone, dichloromethane and n-hexane. A procedural blank was analyzed within each five-sample-batch covering each analytical step. Special care was taken to minimize exposure to UV light throughout the entire analytical procedure. Quantitation was carried out according to: (i) ratio between the two monitored ions within $\pm 15\%$ of the theoretical value, and (ii) limits of quantification (LOQ) corresponding to relationship signal-to-noise (S/N) of 10. Quantifications based on the isotopic dilution technique were inherently recovery corrected and when quantifiable levels of a given analyte were found in a procedural blank, these were subtracted from the corresponding sample batch. Calibration curves (ten points from 1 to 1000 pg/ μ L for PCBs, DDTs and HCB and seven points from 1 to 250 pg/ μ L for DP and PBDEs, save BDE-209 (5.0 to 600 pg/ μ L for BDE-209)) were daily checked. The precision for the quantification methods was assessed by reanalyzing one blubber sample in four different days obtaining RSDs

lower than 9% for all target analytes. Average recoveries for the used surrogates and average LOQ are summarized in Table S4.

Table S4. Recovery values of labeled surrogates and average LODs for target compounds.

Compound	Recovery (%) \pm SD	Average LOQ (ng/g l.w.)	Compound	Recovery (%) \pm SD	Average LOQ (ng/g l.w.)
DDTs			PCBs		
o,p'-DDE		0.673	PCB-28		0.19
p,p'-DDE		1.31	¹³C₁₂-PCB-28	93.6 \pm 8.5	
D₈-p,p'-DDE	119 \pm 13		PCB-52		0.467
o,p'-DDD		1.79	¹³C₁₂-PCB-52	101 \pm 12	
p,p'-DDD		0.627	PCB-95		1.52
D₈-o,p'-DDT	94.5 \pm 12.2		PCB-101		1.02
o,p'-DDT		0.987	PCB-105		0.383
p,p'-DDT		1.03	¹³C₁₂-PCB-101	118 \pm 11	
D₈-p,p'-DDT	99.2 \pm 16.5		PCB-114		1.62
			PCB-118		0.39
HCB			PCB-123		0.833
HCB		0.847	PCB-132		0.723
¹³C₆-HCB	72.9 \pm 9.0		PCB-138		0.690
			PCB-149		1.20
HCHs			¹³C₁₂-PCB-138	127 \pm 12	
α -HCH		0.480	PCB-153		0.340
¹³C₆-α-HCH	63.5 \pm 10.7		¹³C₁₂-PCB-153	132 \pm 10	
β -HCH		0.063	PCB-156		0.372
γ -HCH		0.685	PCB-157		0.710
¹³C₆-γ-HCH	72.9 \pm 11.2		PCB-167		0.303
			PCB-170		0.717
			PCB-180		0.293
			¹³C₁₂-PCB-180	110 \pm 11	
PBDEs			PCB-183		0.540
BDE-17		0.370	PCB-189		0.380
BDE-28		0.463	PCB-194		0.457
BDE-47		0.32	¹³C₁₂-PCB-209	89.7 \pm 7.8	
BDE-66		0.613			
BDE-85		0.33	DP		
BDE-99		0.317	syn-DP		0.005
BDE-100		0.567	¹³C₁₀-syn-DP	97.3 \pm 8.2	
¹³C₁₂-BDE-138	104 \pm 15		anti-DP		0.007
BDE-153		0.243	¹³C₁₀-anti-DP	98.1 \pm 9.1	
BDE-154		0.293			
BDE-183		0.343			
BDE-184		0.323			
BDE-191		0.357			
BDE-196		1.403			
BDE-197		1.06			
BDE-209		13.1			

3. Pollutant profiles

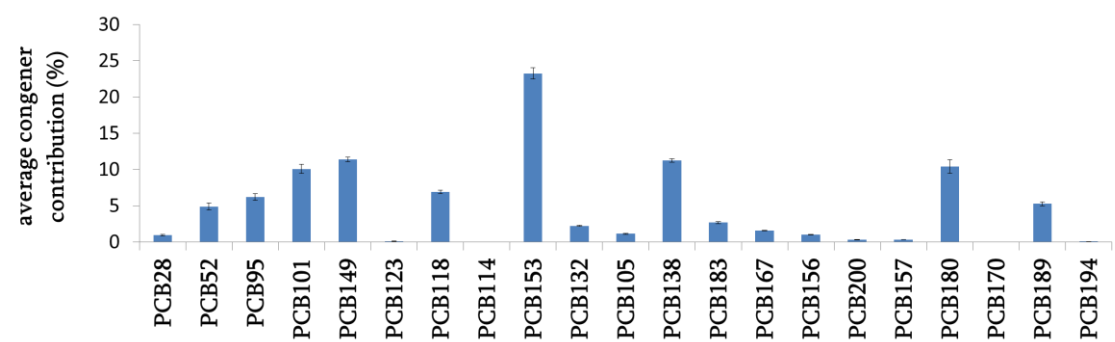


Figure S1. Average PCB congener profile in biopsies of killer whales. Error bars represent standard errors (SE).

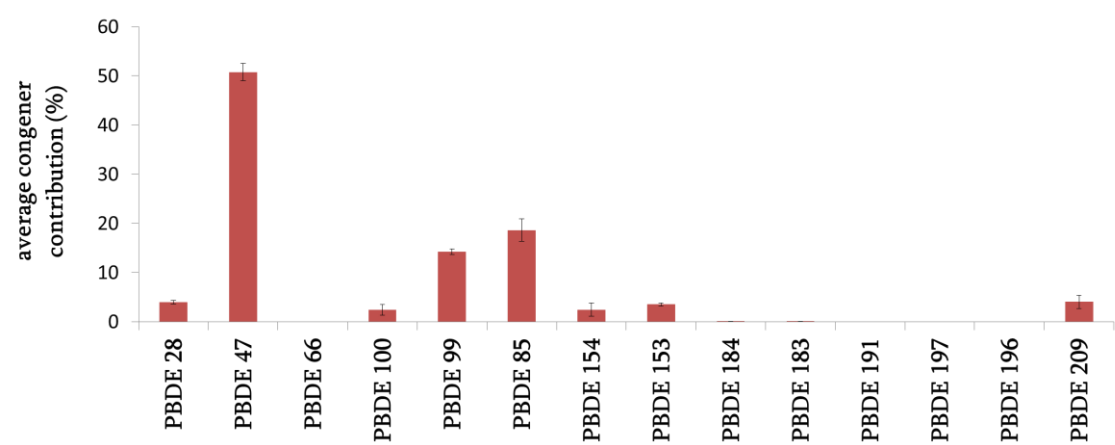


Figure S2. Average PBDE congener profile in biopsies of killer whales. Error bars represent standard errors (SE).

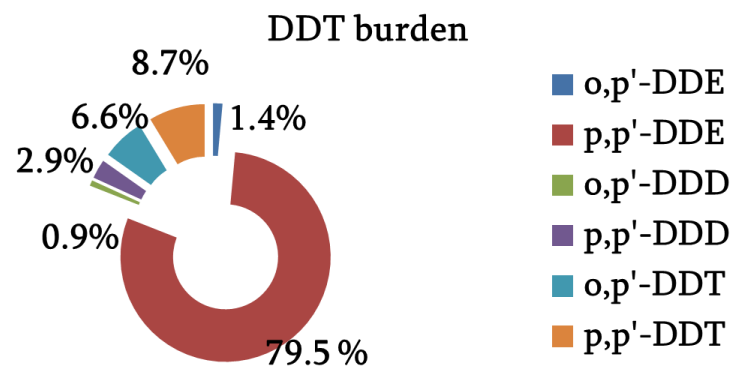


Figure S3. Average contribution of each DDT isomer to the total DDT burden in biopsies of killer whales

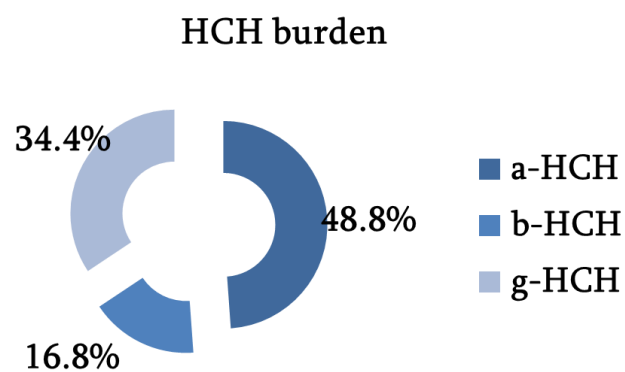


Figure S4. Average contribution of each HCH isomer to the total HCH burden in biopsies of killer whales.

4. Gene expression analysis

Table S5. qRT-PCR primer sequence and information.

Gene	Sequence (5'-3')	Amplicon Length (bp)	Efficiency	Reference
PPAR alpha	Fw-GAAGTGGTCCAGGATCAGATG Rv-GTGAACCTCCGTAATGGTAGCC	178	101.2%	This study
PPAR gamma	Fw-TCCGTCCACACTATGAAG Rv-CAGGCTCCACTTTGATTGC	113	98.2%	This study
ER alpha	Fw-GGGAGACTCGCTACTGTGC Rv-CTCCTCTGCGGTCTTTCC	234	96.4%	Panti et al 2011
AhR	Fw-ACTTGTGGCACCACCGTAGC Rv-GTCCACCATACGTACAGACCG	204	98.6%	Panti et al 2011
CYP1A	Fw-AAACGTTTGAGAAGGGCACATTC Rv -TCAAACCCAGCTCCAAAGAGGT	148	97.9	Panti et al 2011
GAPDH	Fw-CAAGGCTGTGGGCAAGGTCATC Rv-TTCTCCAGGCGGCAGGTCAG	111	97.4%	Spinsanti et al. 2006
YWHAZ	Fw-AAATGAAAGGAGACCTACTACCGC Rv-AGACCCAATCTGATAGGATGTGTTG	151	95.9%	Spinsanti et al. 2006

5. Correlation matrices

Table S6. Correlation matrix of the Spearman correlation for the p value (in red p<0.05) and rho value (in yellow the statistically significant values according to p value<0.05) and considering all the variables.

p-value	Sex	CYP1A1	CYP2B	PPARG	PPARA	ER 1	Cyp1A	AhR	SPBDEs	SDPs	HCB	SHCHs	SPCBs	SDDTs
Sex	0,000	0,559	0,182	0,282	0,282	0,282	0,282	0,282	0,127	0,282	0,127	0,282	0,735	0,490
CYP1A1	0,559	0,000	0,517	0,083	0,350	0,350	0,083	0,783	0,683	0,950	0,517	0,133	0,350	0,683
CYP2B	0,182	0,517	0,000	0,683	0,450	0,783	1,000	0,450	0,083	0,233	0,017	0,350	0,133	0,083
PPARG	0,282	0,083	0,683	0,000	0,236	0,267	0,167	0,498	0,840	0,662	0,713	0,354	0,302	0,498
PPARA	0,282	0,350	0,450	0,236	0,000	0,444	0,595	0,444	0,713	0,840	1,000	0,840	0,840	0,906
ER 1	0,282	0,350	0,783	0,267	0,444	0,000	0,007	0,110	0,963	0,963	0,906	0,906	0,498	0,662
Cyp1A	0,282	0,083	1,000	0,167	0,595	0,007	0,000	0,302	0,713	0,963	0,662	0,906	0,200	0,354
AhR	0,282	0,783	0,450	0,498	0,444	0,110	0,302	0,000	0,267	0,354	0,498	0,662	0,498	0,302
SPBDEs	0,127	0,683	0,083	0,840	0,713	0,963	0,713	0,267	0,000	0,267	0,003	0,267	0,024	0,012
SDPs	0,282	0,950	0,233	0,662	0,840	0,963	0,963	0,354	0,267	0,000	0,236	0,444	0,354	0,167
HCB	0,127	0,517	0,017	0,713	1,000	0,906	0,662	0,498	0,003	0,236	0,000	0,139	0,034	0,024
SHCHs	0,282	0,133	0,350	0,354	0,840	0,906	0,906	0,662	0,267	0,444	0,139	0,000	0,498	0,556
SPCBs	0,735	0,350	0,133	0,302	0,840	0,498	0,200	0,498	0,024	0,354	0,034	0,498	0,000	0,003
SDDTs	0,490	0,683	0,083	0,498	0,906	0,662	0,354	0,302	0,012	0,167	0,024	0,556	0,003	0,000

rho-value	sex	CYP1A1	CYP2B	PPARG	PPARA	ER 1	Cyp1A	AhR	SPBDEs	SDPs	HCB	SHCHs	SPCBs	SDDTs
sex	1,000	0,354	-0,707	-0,474	-0,474	-0,474	-0,474	-0,474	0,632	0,474	0,632	0,474	0,158	0,316
CYP1A1	0,354	1,000	0,400	-0,900	-0,600	-0,600	-0,900	-0,200	-0,300	-0,100	-0,400	-0,800	-0,600	-0,300
CYP2B	-0,707	0,400	1,000	-0,300	-0,500	0,200	0,000	0,500	-0,900	-0,700	-1,000	-0,600	-0,800	-0,900
PPARG	-0,474	-0,900	-0,300	1,000	0,536	0,500	0,607	0,321	0,107	0,214	0,179	0,429	0,464	0,321
PPARA	-0,474	-0,600	-0,500	0,536	1,000	0,357	0,250	0,357	-0,179	0,107	0,000	0,107	0,107	0,071
ER 1	-0,474	-0,600	0,200	0,500	0,357	1,000	0,929	0,679	-0,036	-0,036	0,071	0,071	0,321	0,214
Cyp1A	-0,474	-0,900	0,000	0,607	0,250	0,929	1,000	0,464	0,179	-0,036	0,214	0,071	0,571	0,429
AhR	-0,474	-0,200	0,500	0,321	0,357	0,679	0,464	1,000	-0,500	-0,429	-0,321	0,214	-0,321	-0,464
SPBDEs	0,632	-0,300	-0,900	0,107	-0,179	-0,036	0,179	-0,500	1,000	0,500	0,964	0,500	0,857	0,893
SDPs	0,474	-0,100	-0,700	0,214	0,107	-0,036	-0,036	-0,429	0,500	1,000	0,536	0,357	0,429	0,607
HCB	0,632	-0,400	-1,000	0,179	0,000	0,071	0,214	-0,321	0,964	0,536	1,000	0,643	0,821	0,857
SHCHs	0,474	-0,800	-0,600	0,429	0,107	0,071	0,071	0,214	0,500	0,357	0,643	1,000	0,321	0,286
SPCBs	0,158	-0,600	-0,800	0,464	0,107	0,321	0,571	-0,321	0,857	0,429	0,821	0,321	1,000	0,964
SDDTs	0,316	-0,300	-0,900	0,321	0,071	0,214	0,429	-0,464	0,893	0,607	0,857	0,286	0,964	1,000