

SUPPORTING INFORMATION PART A

Newly Identified DDT-Related Compounds Accumulating in Southern California Bottlenose Dolphins

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Supporting Information Summary

Glossary of acronyms, two supplemental figures and four tables are included in SI Part A. A separate supporting information, SI Part B, includes information associated with identification of the 45 DDT related compounds (mass spectra, interpretation of mass spectral fragmentation patterns, chromatographic retention times, and corresponding standards' mass spectra). In addition, two mass spectral library reports for DDT and Dicofol technical mixtures are provided in PDF format. These materials are available free of charge via the Internet at <http://pubs.acs.org/>.

Glossary of Acronyms

DDT: dichlorodiphenyltrichloroethane

p,p'- DDT: 2,2-bis(chlorophenyl)-1,1,1-trichloroethane

o,p'- DDT: 2,4-bis(chlorophenyl)-1,1,1-trichloroethane

p,p'- DDE: 2,2-bis(chlorophenyl)-1,1-dichloroethene

o,p'- DDE: 2,4-bis(chlorophenyl)-1,1-dichloroethene

p,p'- DDD: 2,2-bis(chlorophenyl)-1,1-dichloroethane

o,p'- DDD: 2,4-bis(chlorophenyl)-1,1-dichloroethane

p,p'- DDMU: 2,2-bis(chlorophenyl)-1-chloroethene

DDMU: bis(chlorophenyl)chloroethane

p,p'- DDM: 2,2-bis(chlorophenyl)methane

DDM: bis(chlorophenyl)methane

p,p'- DBP: 4,4-dichlorobenzophenone

DDNS: bis(chlorophenyl)ethane

p,p'- DDNU: 2,2-bis(chlorophenyl)ethene

DDNU: bis(chlorophenyl)ethene

DDOH: bis(chlorophenyl)ethanol

DDMS: bis(chlorophenyl)chloroethene

DDCN: bis(chlorophenyl)acetonitrile

DDPU: 1,1- bis(4-chlorophenyl)-1-propene

DDPS: 3,3- bis(4-chlorophenyl)-1-propene

4,4',4''-TCPM: 4,4',4''-tris(chlorophenyl)methane

TCPM: tris(chlorophenyl)methane

4,4',4''-TCPMOH: 4,4',4''-tris(chlorophenyl)methanol

TCPMOH: tris(chlorophenyl)methane

Dicofol: trade name Kelthane, 2,2,2,-trichloro-1,1-bis(4-chlorophenyl)ethanol

PBDEs: Polybrominated diphenyl ethers

PVS: Palos Verdes Shelf

BCF: Bioconcentration Factor

eV: electron volts

GC×GC: comprehensive two-dimensional gas chromatography

TOF-MS: time-of-flight mass spectrometry

GC/MS: gas chromatography/mass spectrometry

GPC: gel permeation chromatography

HOCs: Halogenated organic compounds

MS: mass spectrum

RT: retention time

Σ_6 DDT: sum of *p,p'*- and *o,p'*- DDT, DDD, DDE isomers

Σ_4 DDT: sum of *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, and *o,p'*-DDT

Quantitative Analysis Methods

Sample Preparation

Samples were randomly labeled 1 to 8 prior to extraction to eliminate analytical bias.

Approximately 4 g wet weight of blubber was homogenized, ground with kilned Na₂SO₄ and extracted by pressurized fluid extraction (Dionex ASE 300, Dionex, Sunnyvale, CA, USA) with dichloromethane (100 °C, 1500 psi). Approximately 1 g or 0.1 g of extracted blubber (lipid) was dissolved in 4.5 mL of 1:1 ethyl acetate/cyclohexane and spiked with a known amount of the internal standard mixture (BDE 77, ¹³C-TCPM, and 6F BDE-47). The solution was injected into a gel permeation chromatography (GPC) system (J2 Scientific, Columbia, MO) to remove lipids based on a previously validated method that maximized recovery of several classes of halogenated organic compounds (HOCs).^{S1} The GPC column, with a 2-cm i.d. and length of 22.5 cm, was packed with 24 g of BioBeads S-X3 in 1:1 ethyl acetate/cyclohexane. The flow rate was 5 mL/min, and the mobile phase was 1:1 ethyl acetate/cyclohexane. The eluent fraction between 8.5 and 20.5 min was collected and reduced to 1 mL under N₂ gas for the 1 g extracted blubber sample and 50 μL for the 0.1 g extracted blubber sample. The 1 g sample extract was brought to 4.5 mL with the mobile-phase solvent and re-injected into the GPC system to remove residual lipids. The 0.1 g sample was injected on the GPC system only once. Following the second GPC clean-up of the 1 g sample extract the sample was evaporated to 50 μL. Fifty microliters of a recovery standard mixture of known concentration (4F BDE-69) was then added to bring the extracts to a final volume of 100 μL prior to analysis by GC×GC/TOF-MS.

Instrumental Analysis

Sample extracts were analyzed on a Pegasus 4D GC×GC/TOF-MS (LECO, St. Joseph, MI, USA) connected to an Agilent 6890 gas chromatograph (Palo Alto, CA, USA) with a secondary oven, a split/splitless injector, and a non-moving quad-jet dual stage modulator. A splitless sample injection was conducted using an Agilent autosampler (Santa Clara, CA). All instrument parameters are listed in Table S1.

Quantitative Analysis

Quantitative analysis was performed using multipoint internal standard calibration curves. Standard concentrations ranged from 0.01 ng/mL to 2 ng/mL. When an exact authentic standard could not be obtained, semi-quantitation was performed using the calibration curve of an isomer or structurally related compound. The internal standard used for quantitation was ^{13}C -TCPM. The 0.1 g lipid extract was used to prevent chromatographic overloading and to allow quantitation of higher abundance compounds. Quantification of *p,p'*-DDE (the most abundant analyte) required sample dilution; therefore, an external calibration curve was used. PBDEs were quantified using a single-point calibration response factor, based on a previously established linear instrument response.

Table S1. Summary of the GC×GC/TOF-MS instrumental parameters.

GC×GC/TOF-MS	
Instrument	GC×GC/TOF-MS 6890 Gas Chromatogram with secondary oven (Agilent Technologies, USA)
MS Ionization Mode	Pegasus 4D GC×GC/TOF-MS (LECO, USA) EI (Electron Ionization)
Electron Energy	-70 eV
Detector Voltage	1600 V
Data Acquisition Rate	150 spectra/s
Capillary Column	(1D) Restek (Bellefonte, PA) Rtx5-MS (30-m length, 0.25-mm i.d., 0.25- μ m film thickness) integrated with a 5 m guard column (2D) Restek Rxi-17 (1-m length, 0.10-mm i.d., 0.10- μ m film thickness)
Carrier Gas	Helium at 1 mL/min
Sample Injection	Splitless injection
Sample Injection Volume	2 μ L
Modulator	Non-moving quad-jet dual stage, 3.5 second modulation period, 0.9 s hot pulse duration, and a 35 °C modulator temperature offset versus the primary oven temperature
Temperatures	
Injector	300°C
Ion Source	250°C
Primary Oven	60 °C (hold for 1 min), 10 °C/min to 300 °C (held for 3 min), 20 °C/min to 320 °C (held for 20 min)
Secondary Oven	20 °C higher than the primary oven temperature
Transfer Line	285 °C
Software	ChromaTOF (LECO, USA)

Table S2. The concentration ($\mu\text{g/g}$ lipid) of DDT-related compounds in dolphin blubber ($n=8$). Average concentrations were determined for compounds with more than one isomer by summing the isomers in each dolphin sample (labeled D1-D8) and calculating the mean. Results listed as semi-quantitative show the structurally-related compound used to generate the calibration curve.

Compound	Concentration ($\mu\text{g/g}$ lipid)								Average \pm SE* ($n=8$)	Semi-Quantitative Standard
	D1	D2	D3	D4	D5	D6	D7	D8		
Lipid Content in Blubber (g lipid/g blubber)	0.410	0.736	0.773	0.648	0.837	0.832	0.651	0.658		
<i>DDT and major transformation products that are typically monitored</i>										
DDT										
p,p'-DDT	1.3	1.0	0.37	2.7	1.3	0.86	0.64	0.73	1.1 \pm 0.3	
o,p'-DDT	0.12	0.061	0.032	0.25	0.42	0.097	0.11	0.056	0.14 \pm 0.05	
<i>Average (ug/g lipid)</i>	1.2 \pm 0.3									
DDE										
p,p'-DDE	277	19	20	496	262	53	101	85	164 \pm 60	
o,p'-DDE	3.1	4.6	2.5	11	6.6	17	3.1	2.7	6.3 \pm 1.8	
<i>Average (ug/g lipid)</i>	171 \pm 60									
DDD										
p,p'-DDD	12	5.2	3.7	27	32	7.8	4.8	5.5	12 \pm 4	
o,p'-DDD	0.36	0.35	0.14	1.5	1.0	0.45	0.62	0.23	0.58 \pm 0.16	
<i>Average (ug/g lipid)</i>	13 \pm 4									
DDMU										
p,p'-DDMU	0.30	0.29	0.14	0.82	1.8	0.58	0.26	0.11	0.53 \pm 0.192	
DDMU 1	0.045								0.05	p,p'-DDMU
DDMU 2	0.050			0.080			0.023		0.05 \pm 0.02	p,p'-DDMU
<i>Average (ug/g lipid)</i>	0.6 \pm 0.2									
<i>DDT transformation products that are not typically monitored</i>										
DDM										

p,p'-DDM	0.053			0.084		0.065		0.032	0.059 ± 0.011	
DBP										
p,p'-DBP	0.21			0.43	0.12		0.084	0.035	0.18 ± 0.07	
DDNS										
DDNS	0.22	0.086	0.14	0.87	0.33	0.11	0.085	0.20	0.25 ± 0.09	p,p'-DDNU
DDNU										
p,p'-DDNU	0.057							0.031	0.044 ± 0.013	
<i>TCPM or TCPMOH related compounds</i>										
TCPM										
4,4',4''-TCPM	1.2	0.92	0.63	12	27	2.7	2.7	1.4	6 ± 3	
TCPM 3	0.62	0.35	0.20	4.2	10	0.85	0.89	0.58	2.3 ± 1.3	4,4',4''-TCPM
TCPM 4	0.16	0.074	0.039	1.2	15	0.19	0.19	0.21	2.1 ± 1.82	4,4',4''-TCPM
TCPM 5	1.1	1.1	0.35	8.6	30	2.4	2.1	1.1	6 ± 4	4,4',4''-TCPM
TCPM 6	0.22	0.19	0.039	1.9	15	0.45	0.46	0.29	2.3 ± 1.8	4,4',4''-TCPM
<i>Average (ug/g lipid)</i>	19 ± 10									
TCPMOH										
4,4',4''-TCPMOH	0.92	0.27	0.29	7.0	2.8	0.68	1.3	1.0	1.8 ± 0.8	
TCPMOH 2	0.063	0.030	0.011	1.7	0.66	0.078	0.13	0.10	0.4 ± 0.2	4,4',4''-TCPMOH
TCPMOH 3	0.027	0.012	0.011	0.49	1.9	0.029	0.068	0.041	0.3 ± 0.2	4,4',4''-TCPMOH
<i>Average (ug/g lipid)</i>	2 ± 1									

* SE stands for standard error.

Table S3. PBDE concentrations ($\mu\text{g/g}$ lipid) in bottlenose dolphin blubber from the Southern California Bight (n=8). Individual samples are labeled D1-D8.

Compound	Concentration ($\mu\text{g/g}$ lipid)								Average \pm SE* (n=8)	
	D1	D2	D3	D4	D5	D6	D7	D8		
<i>PBDE</i>										
BDE-28	0.33	0.07	0.53			0.10				0.3 \pm 0.1
BDE-47	16	16	35	106	14	42	6.2	6.0		30 \pm 10
BDE-99	3.2	1.5	1.7	2.4	4.5	5.4	1.4	0.90		2.6 \pm 0.6
BDE-100	4.1	2.2	5.0	2.1	4.2	7.5	2.0	1.8		3.6 \pm 0.7
BDE-153	0.21	0.08	0.23	0.49	0.25	0.32				0.26 \pm 0.05
BDE-154	0.58	0.27	0.68	1.0	0.52	0.93	0.28	0.22		0.6 \pm 0.1

* SE = standard error

Table S4. Average concentrations of \sum_6 DDT (*p,p'*- and *o,p'*- DDT, DDD, and DDE isomers) and \sum_4 DDT (*p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, and *o,p'*-DDT) reported within the United States and globally.

Location	Year	Species	n	\sum DDT Concentration ($\mu\text{g/g}$ lipid)	Reference
Southern California Bight (off the coast of California)	1995-2010	<i>Tursiops truncatus</i> -Adult Male	8	\sum_6 DDT- 184 \sum_4 DDT- 178	<i>This Study</i>
Southeastern US (off the coast of Florida)	2003-2005	<i>Tursiops truncatus</i> -Adult Male	33	\sum_6 DDT- 19	S2
Southeastern US(off the coast of Georgia)	2003-2005	<i>Tursiops truncatus</i> -Adult Male	36	\sum_6 DDT- 29	S2
Northern Gulf of Mexico	2010-2012	<i>Tursiops truncatus</i> -Male	108	\sum_6 DDT- 20	S3
Gulf of Mexico (off the coast of Louisiana and Florida)	2011	<i>Tursiops truncatus</i> -Male and Female mixed ages	22	\sum_6 DDT- 11	S4
East Coast of the US, the Gulf of Mexico, and Bermuda*	2000-2007	<i>Tursiops truncatus</i> -Male	261	\sum_6 DDT- 28	S5
Georgia*	2006-2008	<i>Tursiops truncatus</i> -Male	74	\sum_6 DDT- 28	S6
North Carolina, South Carolina, and Florida*	1995-2002	<i>Tursiops truncatus</i> -Male	18	\sum_6 DDT- 33	S7
Canary Islands Spain*	2003-2011	<i>Tursiops truncatus</i> -Male mixed ages	64	\sum_6 DDT- 105	S8
Brazil*	2000-2005	<i>Tursiops truncatus</i> -Male	2	\sum_4 DDT- 5	S9
Tanzania*	2000-2002	<i>Tursiops aduncus</i> -Adult Male	4	\sum_4 DDT- 43	S10

* Off the coast of the location

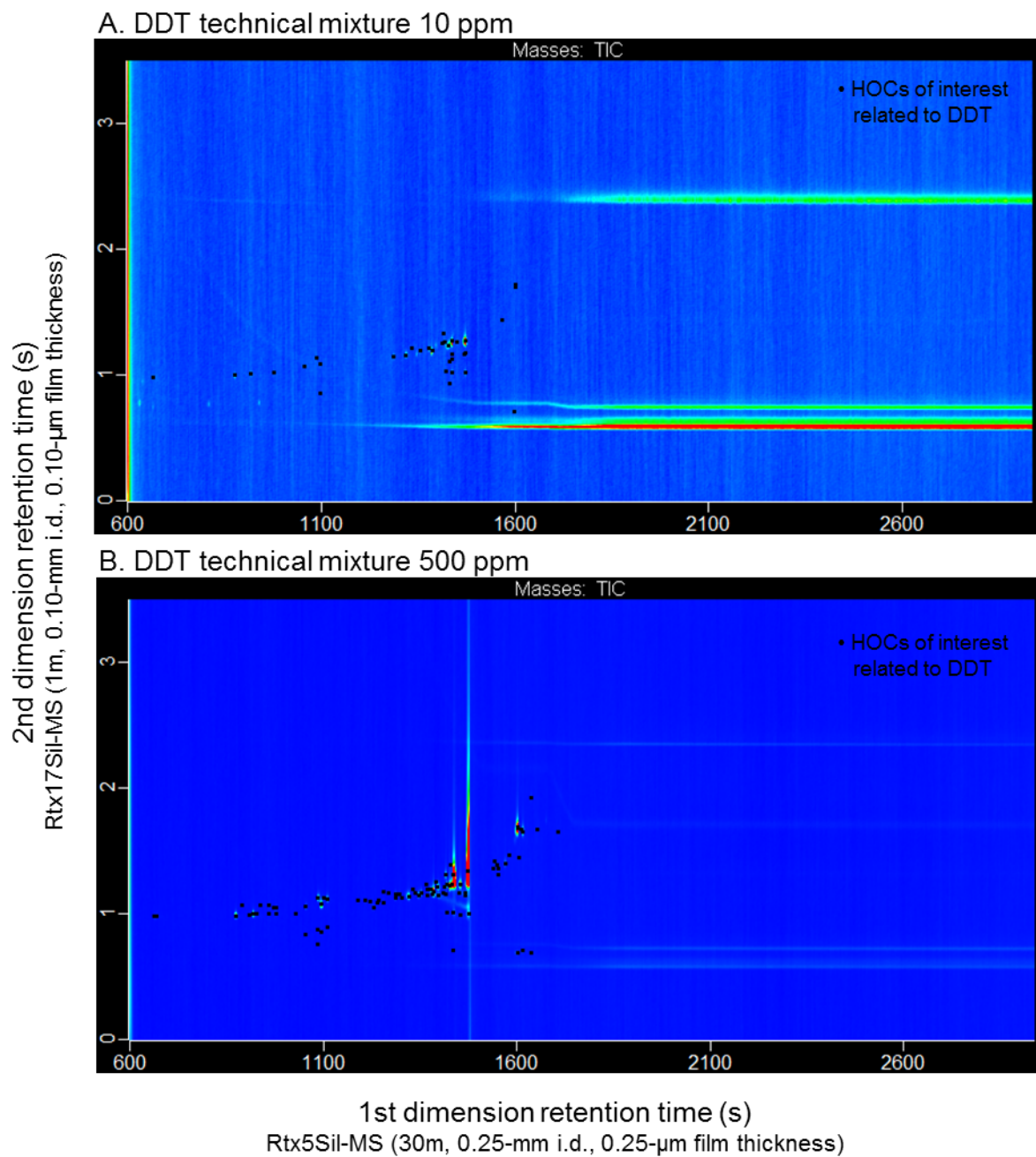
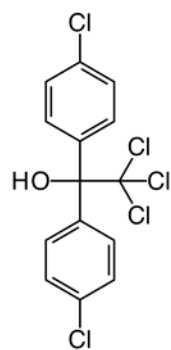


Figure S1. GC \times GC chromatogram of the DDT technical mixture at A. 10 ppm (μ g/mL) and B. 500 (μ g/mL). Black dots represent halogenated organic compounds (HOCs) related to DDT.



Dicofol

Figure S2. Chemical structure of 2,2,2-Trichloro-1,1-bis(4-chlorophenyl)-ethanol, aka Dicofol (trade name *p,p'*-Kelthane).

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