Supplementary Information

Risk assessment of organohalogenated compounds in water bird eggs from South China

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Total Pages:

Identification and quantification of organochlorines

Eggs were thawed and individually homogenized in a blender. Approximately 10 g from each egg was mixed with anhydrous Na_2SO_4 and then extracted in a Soxhlet apparatus with a mixture of dichloromethane and hexane. Before extraction, PCB 30 and tetrachloro-m-xylene were added as internal surrogate standards for calculation of recoveries. Lipid content was determined gravimetrically from an aliquot of extract. Lipids were removed from extracts by use of gel permeation chromatography column (GPC; Bio-Beads S-X3, Bio-Rad Laboratories) by elution with an equivalent mixture of dichloromethane in hexane (1:1 v/v) at a flow rate of 5 mL/min. The

concentrated extract was then transferred and further purified by elution through activated silica gel (60 Å average pore size) and deactivated alumina.

Identification and quantification of PCB congeners and chlorinated pesticides, including hexachlorobenzene (HCB), aldrin, dieldrin, endrin, mirex, total heptachlor (Σheptachlors), total chlordane (Σchlordanes) and total DDTs (ΣDDTs) and total PCBs (ΣPCBs) was accomplished by use of previously described methods (*19*). Analytes were separated by gas chromatography with a Hewlett Packard 6890 series gas chromatograph (GC) equipped with two microelectron capture detectors (μECDs) and dual columns. The limit of quantification (LOQ), defined as three times the procedural blank, ranged from 2.0×10^{-2} to 5.0×10^{-2} ng/mL. Rates of recovery were: PCB congeners: 72% - 96%; chlordane: 81% - 93%; DDTs: 83% - 98% and the other organochlorines: 80% - 89%. ΣPCBs was the sum of 41 congeners (Table S1), ΣDDT was the sum of three compounds each with two isomers (*p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDE, *o,p'*-DDD, *o,p'*-DDT), Σchlordane was the sum of *cis*- and *trans*-chlordane, *cis*- and *trans*- nonachlor, and oxychlordane, and Σheptachlor was the sum of heptachlor and heptachlor epoxide.

Quantification of toxaphene was performed by gas chromatography (Shimadzu GC-2010) equipped with MSD operated in the single ion monitoring (SIM) mode (Shimadzu GCMS-QP2010) in the negative chemical ionization (NCI) mode with methane used as the reactant gas. The GC column used for quantification was a DB-5MS fused silica capillary (J&W Scientific Inc., Folsom, CA) having 0.25 mm i.d. x 60 m x 0.25 μm film thickness for toxaphene congeners. A standard mixture of four individual congeners purchased from LGC Promochem (Middlesex, UK) (DE-USL 421) was used for the quantification of the four Parlars (Parlar No. 26 (P26), 50 (P50), 62 (P62), 32 (P32)). Total

toxaphene (Σtoxaphenes) was expressed as the sum of the hexa- through nona-chlorinated congeners in a mixture with 25 toxaphene congeners (LGC Promochem). In the NCI spectrum, the most abundant ion for toxaphene was $(M\text{-}Cl)^-$ for hepta- through nona-chlorinated congeners. For each chemical, two ions were monitored: one for quantification and one for confirmation. The detection limits were 0.2 ng/mL for hexa- to octa-homologues, and 5.0 ng/mL for nona-homologues. Recoveries of toxaphene in the spiked recovery samples ranged from 77 – 95% for the hepta-homologue, 80 – 92% for the octa-homologue, 75 – 80% for the nona-homologue, 94 – 95% for P26, 86 – 92% for P32, 77 – 89% for P50 and 85 – 95% for P62.

Identification and quantification of PBDEs

Procedures for sample preparation of PBDEs in eggs were similar to the analysis of organochlorines with some modifications. Briefly, before being subjected to GPC (Bio-Beads SX3, Bio-Rad Laboratories, Hercules, CA) for lipid removal, 5 ng of each internal standard ($^{13}C_{12}$-labeled BDE3, BDE15, BDE28, BDE47, BDE99, BDE153, BDE154, BDE183, BDE197, BDE207, and BDE209) was added to the extract. The eluent was further cleaned by use of a silica gel column. $^{13}C_{12}$-labeled BDE139 was added as the recovery spike prior to GC analysis. Quantification was performed using a GC (Agilent 6890N) equipped with a mass-selective detector (Agilent 5973) for mono- to hepta-brominated diphenyl ethers (BDEs), and by use of a GC with a mass-selective detector (JEOL GCmate II, JEOL, Tokyo, Japan) using electron impact (EI) mode for octa- to deca-BDEs (for more details, please refer to (*18*)). Fourteen major PBDE congeners (BDE3, BDE15, BDE28, BDE47, BDE99, BDE100, BDE153, BDE154, BDE183, BDE196, BDE197, BDE206, BDE207, and BDE209) were quantified using the isotope dilution method to their corresponding $^{13}C_{12}$-labeled congeners. Recovery of $^{13}C_{12}$-labeled BDE ranged between 60% and 120%. The LOQ was 0.02 ng/g of lipid for mono- to

di-BDEs, 0.1 ng/g of lipid for tetra-BDEs, 0.05 ng/g of lipid for tri- and penta- to hepta-BDEs, 0.02 ng/g of lipid for octa- to nona-BDEs, and 0.5 ng/g of lipid for deca-BDE. Total PBDE concentrations (ΣPBDE) were reported as the sum of the masses of the 14 individual PBDE congeners quantified (Table S1).

H4IIE-*luc* cell culturing and bioassay

H4IIE-*luc* cells were cultured in Dulbecco's Modified Eagle's Medium (D2902, Sigma, St. Louis, MO) at 37 ºC and 5% CO_2. Cell bioassays were conducted as previously described (*21*). Briefly, each of the inner 60 wells of 96-well culture plates (PerkinElmer, Boston, MA, USA) was seeded with 250 µl of cell solution/well at a density of 8 x 10^5 cells/ml. Three sample extracts representing 2 g of each little egret egg from each study site (Hong Kong, Xiamen, and Quanzhou) were tested, as well as extraction blanks. A 1:3 dilution series in hexane (six concentrations) was prepared for each sample. After 24 h incubation, the cells were dosed with sample extracts at 1% v/v in triplicate. Solvent controls (hexane) and blanks were dosed on all plates. Cells were incubated for 72 h, visually checked for signs of cytotoxicity, and then used in the luciferase assay with 50 ml Luc-Lite reagent (PerkinElmer) per well. Sample responses were solvent-corrected and expressed as a percentage of the maximum response of the cells compared to the appropriate standard. The responses were quantified by comparing the activities of the extracts to that of a 2,3,7,8-TCDD standard curve (*22*).

Table S1. Concentrations (mean ± SD, ng/g lipid wt.) of trace organic pollutants in eggs (n = 5 for each species) of little egret (LE) and black-crowned night heron (NH) from Hong Kong, Xiamen and Quanzhou, South China in 2004.

	Hong Kong, LE			Xiamen, LE			Quanzhou, LE			Xiamen, NH			Quanzhou, NH		
Lipid %	10.4	±	2.42	8.12	±	3.50	4.75	±	2.40	6.39	±	3.34	4.08	±	1.54
Aldrin	0.357	±	0.775	0.227	±	0.129	0.0553	±	0.101	0.228	±	0.175	0.0384	±	0.0636
Endrin	56.4	±	35.1	10.5	±	10.9	8.28	±	3.58	8.27	±	5.00	6.75	±	1.38
Dieldrin	135	±	69.8	66.9	±	54.8	62.3	±	41.8	51.8	±	20.3	49.3	±	14.8
HCB	149	±	67.6	76.8	±	76.2	327	±	263	64.6	±	66.5	254	±	99.0
Mirex	194	±	101	89.3	±	45.9	65.3	±	40.7	317	±	318	145	±	39.9
Heptachlor	3.66	±	1.57	0.462	±	0.315	1.59	±	3.03	0.376	±	0.176	0.720	±	1.05
Heptachlor epoxide	68.0	±	56.9	19.7	±	8.80	13.2	±	2.56	31.1	±	14.5	11.1	±	2.25
Σheptachlor	71.7	±	58.0	20.2	±	9.10	14.8	±	3.26	31.5	±	14.6	11.8	±	2.51
***cis*-chlordane**	127	±	127	37.9	±	29.5	21.2	±	16.6	32.3	±	18.5	14.9	±	6.61
***trans*-chlordane**	11.2	±	6.70	3.02	±	1.90	5.03	±	4.27	2.77	±	1.68	3.79	±	1.59
***cis*-nonachlor**	441	±	243	166	±	163	91.6	±	42.9	117	±	62.4	74.5	±	21.6
***trans*-nonachlor**	656	±	594	471	±	487	106	±	62.7	317	±	194	84.1	±	17.8
oxychlordane	524	±	444	406	±	377	83.7	±	42.9	280	±	166	73.1	±	38.2
Σchlordane	1760	±	1200	1080	±	1050	308	±	155	749	±	417	250	±	60.2
***o,p'*-DDD**	1.79	±	1.25	3.64	±	4.92	58.6	±	28.0	5.89	±	5.29	6.89	±	8.93
***o,p'*-DDE**	79.2	±	33.8	326	±	287	519	±	340	270	±	157	394	±	95.7
***o,p'*-DDT**	0.828	±	0.812	11.1	±	8.38	104	±	86.4	23.7	±	28.9	13.5	±	22.8
***p,p'*-DDD**	200	±	179	627	±	456	2040	±	599	1430	±	830	2150	±	1370
***p-p'*-DDE**	7370	±	3310	27400	±	13600	37800	±	11200	30700	±	13900	31800	±	8460
***p-p'*-DDT**	220	±	137	2790	±	2990	15400	±	12100	8030	±	6970	6970	±	11700
ΣDDTs	7870	±	3550	31100	±	16100	55900	±	23400	40400	±	19000	41400	±	12500
Parlar: P32	2.55	±	1.16	1.57	±	1.18	1.11	±	0.21	1.71	±	1.08	0.68	±	0.24
Parlar: P26	3.08	±	1.88	4.21	±	3.31	2.58	±	0.91	3.82	±	1.33	1.14	±	0.96

Parlar: P62	*L-LOQ			*L-LOQ			*L-LOQ			*L-LOQ			*L-LOQ		
Parlar: P50	2.78	±	2.26	2.95	±	2.34	2.36	±	1.00	2.68	±	1.23	1.12	±	0.86
***Σ toxaphene**	54.4	±	79.3	26.8	±	13.5	47.4	±	28.1	28.0	±	11.7	14.1	±	17.8
***ΣPCBs**	4150	±	1820	863	±	605	1500	±	425	784	±	484	921	±	57.3
***ΣPBDEs**	470	±	271	43.7	±	9.86	214	±	76.1	115	±	56.9	316	±	46.0
***$TEQ_{WHO\text{-}avian\text{-}TEF}$**	58	±	13.5	15.7	±	16.7	12.2	±	18.9						
*** $TEQ_{H4IIE\text{-}luc}$**	35.0	±	21.8	3.04	±	2.50	7.26	±	4.91						
*** $TEQ_{H4IIE\text{-}luc\text{-}ReP}$**	16.2	±	3.92	3.68	±	3.52	3.09	±	4.28						
$TEQ_{H4IIE\text{-}luc\text{-}ReP}$ (PCDD/F data, 2006), (pg/g wet wt.)	11.6	±	5.94												

*ΣPCBs: sum of 41 congeners (8, 18, 28, 31 49, 52, 66, 70, 74, 77, 81, 87, 99, 101, 105, 110, 114, 118, 119, 123, 126, 128, 138, 149, 151, 153, 156, 157, 158, 159, 163, 167, 168, 169, 180, 182, 183, 187, 189, 194 and 206)

*ΣPBDEs: sum of 14 congeners (BDE3, BDE15, BDE28, BDE47, BDE99, BDE100, BDE153, BDE154, BDE183, BDE196, BDE197, BDE206, BDE207 and BDE209)

*Σtoxaphene: sum of 25 different polychlorobornanes, from penta- to nona-chlorinated congeners

* $TEQ_{WHO\text{-}avians}$ (pg/g wet wt.): TEQs for bird eggs were calculated using avian TEFs proposed by (*24*)

* $TEQ_{H4IIE\text{-}luc}$ (pg/g wet wt.) : Total dioxin-like activities of the samples were obtained from a cell line-based assay (H4IIE-*luc* cell bioassay)

* $TEQ_{H4IIE\text{-}luc\text{-}ReP}$ (pg/g wet wt.): TEQs were calculated using TEFs derived from the H4IIE bioassay (*57,58*)

*L-LOQ: Less than Limit of Quantification (LOQ) (5.0 ng/mL)

Table S2. DDE residues (μg/g wet wt.) in eggs of piscivorous birds related to reproductive success

	ΣDDE	***Percentage of young fledged**	**Reference**
Osprey	12	77	(59)
(*Pandion haliaetus*)	6	162	(59)
	6	123	(60)
Brown pelican (*Pelecanus occidentalis*)	1.5	40	(61)
Black-crowned night heron (*Nycticorax nycticorax*)	0.9	95	(62)
	1	81	
	4	71	
	8	52	
	12	48	
	16	38	
	25	19	
Great blue heron (*Ardea herodias*)	3	89	(63)
Snowy egret (*Egretta thula*)	0.9	100	(64)
	1	109	
	5	45	
	10	45	

*Percentage of young fledged: percentage of young that must be fledged per pair of breeding age in order to maintain a stable population; 100% fledging success represents a stable population of a particular species based on recruitment standards and number of young produced per active nest for each species.

Table S3. Geometric mean NOAELs for embryo mortality and developmental impairment in birds exposed to TEQs (*54*).

Species	**NOAEL (pg/g ww)**
Laboratory Studies	
White leghorn chicken (*Gallus gallus*)	66
American kestrel (*Falco sparverius*)	230
Ring-necked pheasant (*Phasianus colchicus*)	710
Double-crested cormorant (*Phalacrocorax auritus*)	3670
Turkey (*Meleagris gallopavo*)	10000
Mallard duck (*Anas platyrhynchos*)	35360
Greylag goose (*Anser anser*)	50000
Common golden eye (*Bucephala clangula*)	50000
Black-headed gull (*Larus ridibundus*)	50000
Herring gull (*Larus argentatus*)	50000
Field Studies	
Wood duck (*Aix sponsa*)	5
Great blue heron (*Ardea herodias*)	13
Osprey (*Pandion haliaetus*)	140
Forster's tern (*Sterna forsteri*)	350
Caspian tern (*Sterna caspia*)	1440

Figure S1. Map showing sampling locations of Ardeid eggs in South China.

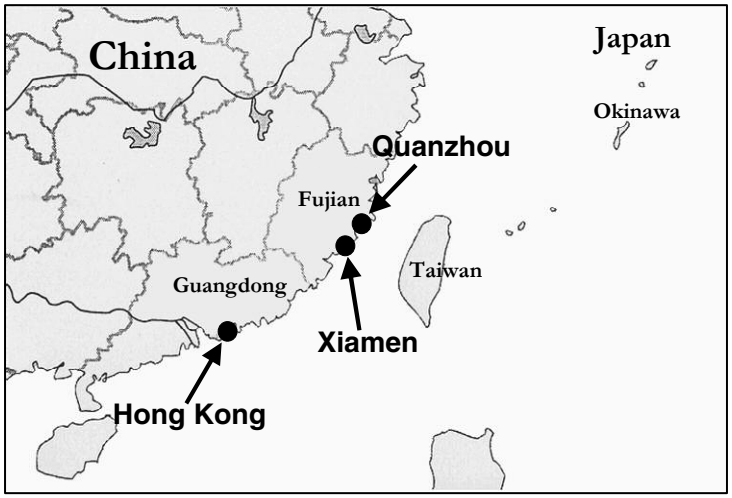
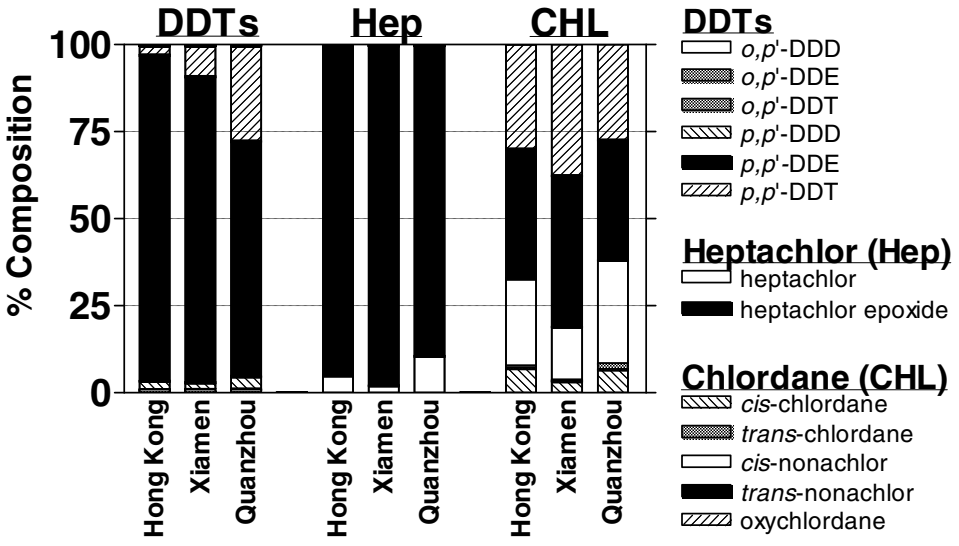


Figure S2. Relative proportions of total organochlorine insecticides attributable to ΣDDTs, Σheptachlor and Σchlordane in Ardeid eggs.



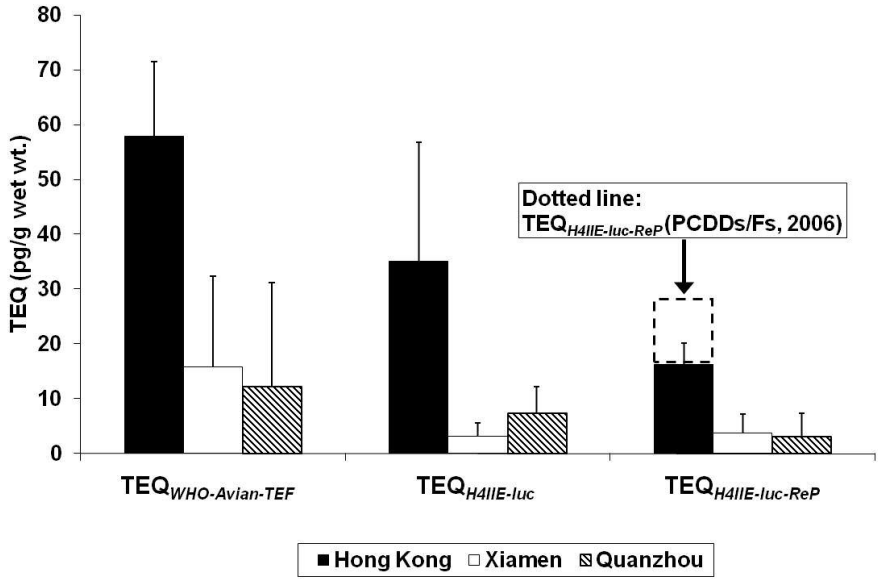


Figure S3. Concentrations of $TEQ_{WHO\text{-}Avian\text{-}TEF}$, $TEQ_{HII4E\text{-}luc}$, $TEQ_{HII4E\text{-}luc\text{-}ReP}$ in little egret eggs from Hong Kong, Xiamen, and Quanzhou.

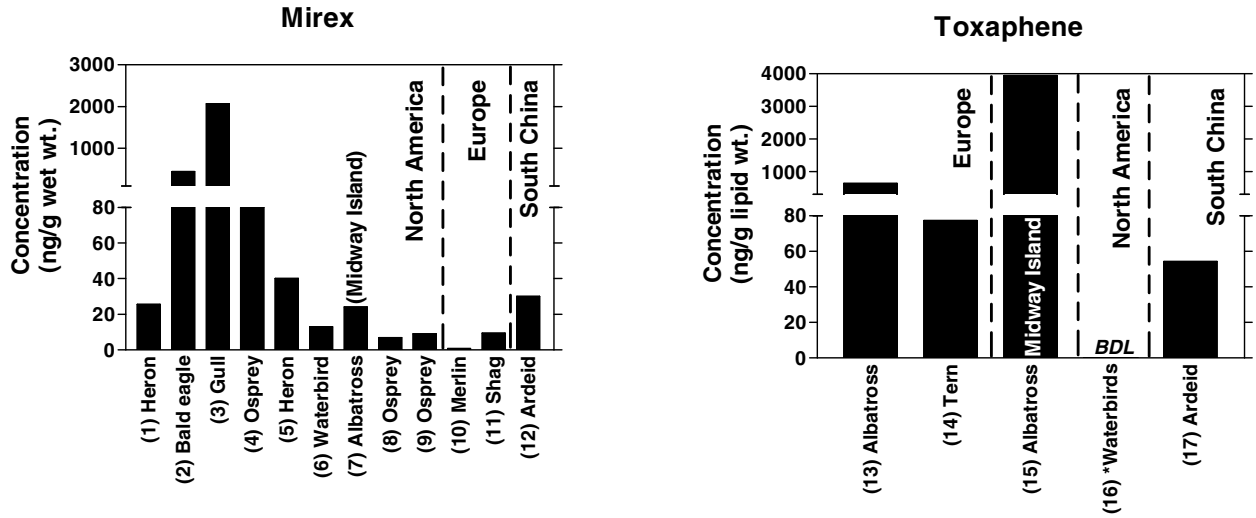


Figure S4. Comparisons of (a) mirex and (b) Σtoxaphene levels in Ardeid eggs from South China with those in other avian eggs reported elsewhere (References for mirex: (1) *12*; (2) *33*; (3) *36*; (4) *65*; (5) *16*; (6) *9*; (7) *10*; (8) *34*; (9) *66*; (10) *35*; (11) *67*; (12) present study. References for toxaphene: (13) *48*; (14) *68*; (15) *10*; (16) *Water birds consists of six studies: *69*, *70*, *71*, *72*, *73*, *67*; (17) present study). BDL: Below detection limits.

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