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Bioaccumulation of organochlorine pesticides in the liver of birds from Boraphet wetland, Thailand

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ABSTRACT: The accumulation of toxic and persistent organochlorine pesticides (OCPs) in the liver tissues of nine species of birds collected from the wetland, Bueng Boraphet, central Thailand was studied during April 2007 to September 2010. Contamination at different trophic levels (in carnivorous, omnivorous, and insectivorous birds) was also analysed. The study indicates that birds in the Boraphet wetland are still subject to OCP contamination. Total dichlorodiphenyltrichloroethane and metabolites (ΣDDTs) and total aldrin (ΣALD) accumulated the most, followed successively by total hexachlorocyclohexanes (ΣHCH) and total chlordane compounds (ΣCHL). Carnivorous birds were at highest risk of contamination by OCPs due to their highest trophic level. Omnivorous birds were contaminated with moderate levels of ΣALD and ΣHCH. The insecticide usage seems to be well managed, as insectivorous birds were the least contaminated, with only low concentrations of ΣALD and ΣHCH. Regular monitoring of OCP contamination is recommended due to a continued usage of OCPs in agriculture around the Boraphet wetland.

KEYWORDS: contamination, DDT, ALD, HCH

INTRODUCTION

Extensive use of organochlorine pesticides (OCPs) in agriculture during past decades poses an environmental problem. Owing to their persistence and lipid solubility, OCPs have a high potential of accumulation in the adipose tissue exposed of organisms, and biomagnification in the food web. Birds are especially vulnerable to OCPs due to their widespread distribution and high rates of food consumption. Carnivorous species are at highest risk for OCP accumulation [1]. Furthermore, OCPs have been found to impact the breeding success of many bird species because they inhibit carbonic anhydrase activity and therefore lower the levels of calcium, which in turn leads to eggshell thinning and breakage [2–5]. OCPs may also affect other reproductive properties of birds, including the induction of male feminization [6], inhibiting egg-laying, decreasing clutch size [7] and hatching success [8], and inducing deformities of the embryos [6,7].

OCPs were used extensively in Thailand until 1983; when many compounds were banned by the Thai government (www.thailand.ipm-info.org/pesticides/pesticides_banned.htm). Despite restrictions and bans on the use of many OCPs, their bioaccumulation and detection in the egg yolk of birds at the Wat Tan-En Non-hunting Area, Phranakhon Si Ayutthaya province, central Thailand [9], and in the aquatic food web at Klong Rangsit, Patum Thani province, central Thailand [10] indicates either continual use or persistence of the compounds in the environment.

Boraphet wetland is located in Nakhon Sawan province, central Thailand (15°40′–45′ N and 100°10′–23′ E). Lying at a maximum elevation of 24 m above sea level, it is the largest freshwater wetland in Thailand (212 km^2), and is considered to be of international conservation importance [11,12]. The wetland encompasses a reservoir and surrounding agricultural areas. Its ecosystems include floodplains of the Chao Phraya River and its tributaries [13]. The reservoir is an important breeding ground for aquatic animals, particularly fish from the Chao Phraya River, because the river tributaries carry nutrients and sediments into the reservoir. The wetland also serves as a major habitat for wintering and nesting birds; holding more than 20 000 birds of at least 187 species [13].

Table 1 Locations of ten sampling sites in the Boraphet wetland.

Site	Location	Coordinates	Description
1	Fai Kao	15°41′58″ N 100°10′44″ E	Paddy fields
2	Ko Wat	15°42′14″ N 100°12′38″ E	Central lake area
3	Ban Tha Din Daeng	15°43′ 3″ N 100°13′17″ E	Fish farm
4	Pluak Sung	15°40′55″ N 100°13′33″ E	Communities
5	Pramong Choeng Panit	15°42′38″ N 100°16′21″ E	Paddy fields
6	Laem Na – Ko Ta Ruang	15°41′32″ N 100°16′12″ E	Central lake area
7	Khlong Huai Hin	15°40′ 2″ N 100°15′50″ E	Paddy fields and watermelon farm
8	Laem Ta Seng Research Station	15°42′ 5″ N 100°17′12″ E	Central lake area and no farming
9	Noen Rakang	15°41′ 9″ N 100°17′33″ E	Paddy fields
10	Khlong Khao Phanom Set	15°41′44″ N 100°19′53″ E	Paddy fields

Although the Boraphet wetland is a major waterbird site, there has been only scant pesticide monitoring. Pesticides used in surrounding rice paddies and farmland can accumulate in the reservoir, and in aquatic organisms to become biomagnified through the food chain[14,15]. Collecting live specimens is prohibited; so that previous studies have mostly been confined to analysis of pesticide residues in carcasses.

The present study was carried out as a subproject of the Surveillance and Protection of All Viruses in Wild Birds Programme, authorized by the Department of National Parks, Wildlife and Plant Conservation of Thailand (DNP). The programme carried out surveys of viral infections among wild birds following outbreaks of the high pathenogenicity avian influenza during 2005−2008. The present study investigated the accumulation of OCPs in the liver tissues of birds collected under the above programme to assess environmental contamination by pesticides. Furthermore, the concentrations of OCPs in carnivorous, omnivorous, and insectivorous birds were analysed to evaluate OCP contamination at different trophic levels.

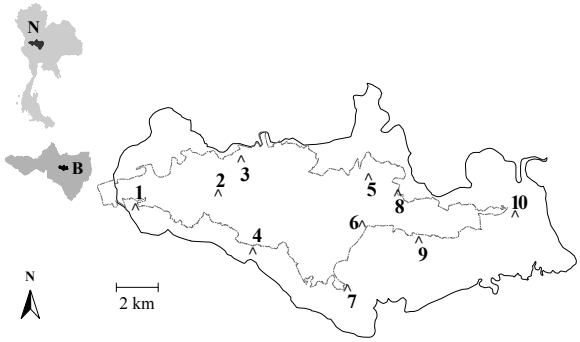

Fig. 1 Locations of sampling sites in Boraphet wetland non-hunting area (B), Nakhon Sawan province (N), Thailand: Fai Kao (1), Ko Wat (2), Ban Tha Din Daeng (3), Pluak Sung (4), Pramong Choeng Panit (5), Laem Na-Ko Ta Rueang (6), Khlong Huai Hin (7), Laem Ta Seng Research Station (8), Noen Rakang (9), Khlong Khao Phanom set (10).

MATERIALS AND METHODS

A total of 49 birds comprising nine species were captured during April 2007 − March 2010 in 10 different sites in the Boraphet wetland (Table 1 and Fig. 1) using mist-nets. The samples of a particular species from different locations were analysed and reported as one group. Captured birds were categorized according to their feeding habits into three groups: (1) carnivorous birds that fed on animals such as fish, prawns, and frogs: Little Cormorant (*Phalacrocorax niger*), Yellow Bittern (*Ixobrychus sinensis*), and Long-tailed Shrike (*Lanius schach*); (2) omnivorous birds that fed on young leaves, flowers, grains, insects, shrimp, and fish: Pheasant-tailed Jacana (*Hydrophasianus chirurgus*), Asian Pied Starling (*Sturnus contra*), and Purple Swamphen (*Porphyrio porphyrio*); and (3) insectivorous birds: Striated Grassbird (*Megalurus palustris*), Streak-eared Bulbul (*Pycnonotus blanfordi*), and Dusky Warbler (*Phylloscopus fuscatus*).

After collection, samples were prepared by a veterinary physician from DNP, following animal welfare regulations. The abdominal cavity of each bird was cut open. The liver was resected, weighed, and individually stored at −20 °C for further analysis.

The protocol for analysis was carried out with a modification of the method suggested by Cid et al[16]. Liver samples were defrosted, thawed, and washed in deionized water. Each bird was prepared separately. Each sample was homogenized in a blender with anhydrous Na_2SO_4 in a ratio of 1:4. The samples were then extracted with 100 ml n-hexane and acetone (4:1 v/v) using soxhlet extractors for 2 h. Each extract was dried under a nitrogen stream and then redissolved in 2 ml of n-hexane. Precisely 1 ml of extract from the chloroform layer was drawn to determine the lipid content by gravimetry[17]. The

Table 2 The liver mass and the concentration of each OCP isomer in birds of the Bueng Boraphet wetland.

	Phalacrocorax niger	*Ixobrychus sinensis*	*Lanius schach*	Carnivores[b]	*Hydrochirurgus phasianus*	*Sturnus contra*	*Porphyrio porphyrio*	Omnivores[b]	*Megalurus palustris*	*Pycnonotus blanfordi*	*Phylloscopus fuscatus*	Insectivores[b]
Common name	Little Cormorant	Yellow Bittern	Long-tailed Shrike		Pheasant-tailed Jacana	Asian Pied Starling	Purple Swamphen		Striated Grassbird	Streak-eared Bulbul	Dusky Warbler	
Number of samples	6	8	6	20	10	4	3	17	5	3	4	12
Liver mass (g)	$1.3_{\pm 0.7}$	$1.4_{\pm 1.0}$	$0.3_{\pm 0.1}$	$1.2_{\pm 0.8}$	$1.4_{\pm 1.0}$	$1.9_{\pm 0.0}$	$1.5_{\pm 0.7}$	$1.5_{\pm 0.7}$	$0.7_{\pm 0.4}$	$1.1_{\pm 0.4}$	$0.2_{\pm 0.1}$	$0.7_{\pm 0.5}$
Lipid determination (% w/w)	70	80.5	33.5	33.5–80.5	70.5	79.5	73.5	70.5–79.5	36	53.5	9	9–53.5
4,4′-DDD	74.2[a]	$59^{[116.5]}_{\pm 81}$	ND	27.2 ± 6.7	ND	ND	8.2[a]	$2.7^{[8.2]}_{\pm 0.4}$	ND	ND	ND	ND
4,4′-DDE	$9.8^{[10.6]}_{\pm 1.1}$	$152^{[300.7]}_{\pm 209}$	0.6[a]	$42_{\pm 15}$	ND	0.2[a]	ND	$< 0.1_{\pm 0.0}$	ND	ND	0.4[a]	$< 0.1_{\pm 0.0}$
4,4′-DDT	159.6[a]	ND	ND	$26.6_{\pm 7.9}$	ND	ND	ND	ND	ND	ND	ND	ND
Aldrin	$9.1^{[9.2]}_{\pm 0.1}$	$11^{[21.3]}_{\pm 15}$	$1.3^{[3.5]}_{\pm 1.9}$	$6.5_{\pm 1.0}$	$3.0^{[7.3]}_{\pm 3.7}$	ND	$11.0^{[11.5]}_{\pm 0.81}$	$8.2_{\pm 0.9}$	$9^{[17]}_{\pm 11}$	4.3[a]	$0.8^{[2.4]}_{\pm 1.4}$	$5.7_{\pm 1.4}$
Dieldrin	ND	6.7[a]	ND	$0.8_{\pm 0.3}$	ND	ND	$60^{[112.3]}_{\pm 75}$	$39.7_{\pm 6.5}$	ND	ND	ND	ND
α-endosulphan	ND	ND	ND	ND	4.7[a]	0.2[a]	ND	$0.5_{\pm 0.2}$	ND	ND	ND	ND
β-endosulphan	119.4[a]	$178^{[346.5]}_{\pm 238}$	ND	$64_{\pm 18}$	ND	ND	ND	ND	ND	ND	ND	ND
endosulphan sulphate	ND	$24^{[48.6]}_{\pm 19}$	ND	$12.1_{\pm 2.8}$	ND	ND	ND	ND	27[a]	ND	ND	$5.4_{\pm 2.2}$
endrin	ND	2.7[a]	0.3[a]	$0.4_{\pm 0.1}$	ND	ND	ND	ND	24.7[a]	ND	ND	$4.9_{\pm 2.0}$
endrin aldehyde	ND	ND	ND	ND	ND	8.6[a]	11.6[a]	$3.9_{\pm 0.8}$	ND	ND	ND	ND
α-HCH	66.4[a]	$3.7^{[9.6]}_{\pm 5.1}$	0.3[a]	$12.5_{\pm 3.3}$	$0.9^{[4.4]}_{\pm 1.7}$	$0.4^{[0.8]}_{\pm 0.3}$	ND	$0.9_{\pm 0.2}$	6[a]	ND	ND	$1.2_{\pm 0.5}$
β-HCH	ND	$1.3^{[1.5]}_{\pm 0.3}$	$3.3^{[11.8]}_{\pm 5.7}$	$2.5_{\pm 0.5}$	$11^{[31.5]}_{\pm 18}$	21.1[a]	ND	$0.9_{\pm 2.1}$	$30.4^{[30.4]}_{\pm 0.0}$	ND	ND	$6.1_{\pm 2.5}$
γ-HCH	$18^{[41.8]}_{\pm 21}$	$6.5^{[10]}_{\pm 5.0}$	$0.3^{[0.4]}_{\pm 0.2}$	$11.2_{\pm 2.1}$	ND	$5.7^{[16.3]}_{\pm 9.2}$	$19^{[35.3]}_{\pm 23}$	$16.9_{\pm 2.2}$	$1.5^{[2.3]}_{\pm 1.1}$	$1.5^{[1.9]}_{\pm 0.6}$	$0.1^{[0.2]}_{\pm 0.1}$	$1.6_{\pm 0.2}$
δ-HCH	ND	$19^{[29.2]}_{\pm 15}$	ND	$4.7_{\pm 1.4}$	ND	ND	ND	ND	ND	2.7[a]	ND	$0.9_{\pm 0.2}$
heptachlor	$34.4^{[34.4]}_{\pm 0.0}$	0.4[a]	ND	$5.8_{\pm 1.7}$	$2.0^{[3.6]}_{\pm 2.3}$	$0.5^{[0.5]}_{\pm 0.0}$	ND	$0.5_{\pm 0.2}$	4.8[a]	$1.3^{[1.3]}_{\pm 0.0}$	ND	$1.4_{\pm 0.4}$
heptachlor-exo-poxide	ND	0.6[a]	ND	$0.1_{\pm 0.0}$	0.7[a]	$0.7^{[1.2]}_{\pm 0.8}$	ND	$0.4_{\pm 0.1}$	ND	2.3[a]	ND	$0.8_{\pm 0.1}$

$\text{Mean}^{[\text{max}]}_{\pm \text{SD}}$ ng/g. ND = Not determined.

[a] Analysis from only one sample.

[b] The concentrations of OCP were not significantly different ($p > 0.05$) between three bird groups.

lipid contents are shown in Table 2. The extracts were cleaned up with concentrated H_2SO_4 until the H_2SO_4 fraction remained clear, and then packed on an 8 mm i.d. alumina/silica column from bottom to top with: neutral alumina (6 cm, 3% deactivated), neutral silica gel (10 cm, 3% deactivated), 50% (on a weight basis) H_2SO_4 silica (10 cm), and anhydrous Na_2SO_4. Alumina, silica gel, and anhydrous Na_2SO_4 were soxhlet extracted 48 h with dichloromethane and then baked for 12 h at 250, 180, and 450 °C, respectively, before use. The volume was adjusted to 2 ml, and the content was subject to GC analysis. Extracts were injected in the splitless mode on a Thermo Finnigan GC equipped with a ^{63}Ni μ-electron capture detector. Data were collected with the aid of CHEMSTATION. The GC analysis employed a DB-5 MS capillary column (30 m × 0.25 mm; with a film thickness of 0.25 μm). The GC column temperature was programmed from 150 °C to 300 °C at 8 °C/min and held at 300 °C for 10 min. Injector and detector temperatures were 250 °C and 300 °C, respectively. The helium carrier and nitrogen make-up gas flow were set at 150 °C and at 2 ml/min and 3 ml/min, respectively. All samples were analysed for the following OCPs: 4,4′-dichlorodiphenyldichloroethylene (4,4′-DDE), 4,4′-dichlorodiphenyldichloroethane (4,4′4-DDD), 4,4′4-dichlorodiphenyltrichloroethane (4,4′-DDT), aldrin, dieldrin, α-endosulphan, β-endosulphan, endosulphan sulphate, endrin, endrin aldehyde, α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane (β-HCH), δ-hexachlorocyclohexane (δ-HCH), γ-hexachlorocyclohexane (γ-HCH), and heptachlor and heptachlor-exo-epoxide.

Quality assurance and quality control

All samples were subjected to strict quality control procedures. For OCP quantification, standard solutions were used to calibrate the instrument daily. Calibration curves based on a set of concentration (5, 10, 20, 50, 100, and 200 μg/l) were drawn. Procedural blanks, solvent blanks, and field blanks were analysed using the same procedure as the used for real samples. Standards (donated by Dr Ehrenstorfer GmbH, Germany) were prepared by dissolution in n-hexane at 10 ng/ml. The analyte mixture was spiked into a matrix to determine the method detection

Table 3 The detection limit, purity, and retention time of each OCP in this study.

Organochlorine pesticide	Detection limit (ng/g)	Purity (%)	Retention time (min)
ΣDDT			
4,4′-DDD	0.008	99.0	30.45
4,4′-DDE	0.0006	98.5	29.32
4,4′-DDT	0.15	98.5	32.12
ΣALD			
aldrin	0.0001	99.0	25.50
dieldrin	0.000003	98.5	23.12
α-endosulphan	0.00001	97.0	28.38
β-endosulphan	0.10	98.0	30.82
endosulphan-sulphate	0.02	97.0	31.98
endrin	0.0003	99.5	30.10
endrin aldehyde	0.08	99.0	31.14
ΣHCH			
α-HCH	0.000004	98.0	20.04
β-HCH	0.00002	98.4	21.17
γ-HCH	0.000009	98.5	21.42
δ-HCH	0.002	98.5	22.37
ΣCHL			
heptachlor	0.00001	99.0	24.22
heptachlor-exo-epoxide*	0.000002	98.5	27.06

* (cis-, isomerB)

limit. The detection limit, purity, and retention time of each reference standard are shown in Table 3. Average surrogate recoveries ranged between 60% and 120%. The retention time and peaks of each OCP were compared with those of the reference standards; concentration of each OCP was expressed as ng/g liver (wet weight basis). For OCPs which were not detected in procedural blanks, detection limits were calculated as the amount of analyte per sample corresponding to the lowest calibration standard. In all samples, detection limits ranged between 0.01 and 0.5 ng. All the chemicals used in the study were of analytical grade and purchased from Merck, Germany. All solvents used in the study were redistilled to purify them and to reduce any of their interference effect. The glassware used was baked at 450 °C for 6 h before use.

Data analysis

One-way ANOVA was employed to evaluate differences in the concentrations of OCPs between three bird groups using the SPSS 17.0 statistical software. When significant F-values were obtained, differences between individual means were tested using the least significant different test (LSD) at $p < 0.05$.

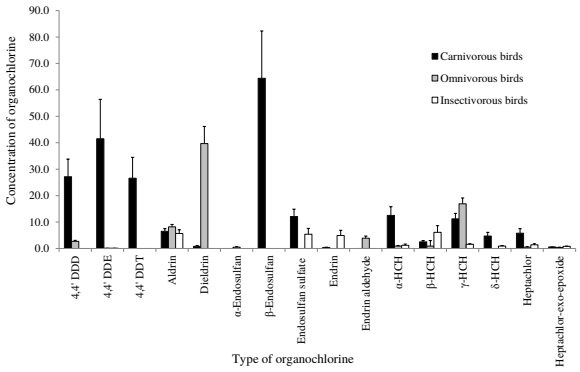

Fig. 2 Concentrations of different OCP isomers in each trophic level of birds in the Boraphet wetland.

RESULTS

OCPs were detected in the livers of all three groups of birds: carnivores, omnivores, and insectivores (Fig. 2). Total dichlorodiphenyltrichloroethane (ΣDDT: 4,4′-DDE, 4,4′-DDD, and 4,4′-DDT, 95 ± 19 ng/g) and total aldrin (ΣALD: aldrin, dieldrin, α-endosulphan, β-endosulphan, endosulphan sulphate, endrin, and endrin aldehyde, 84 ± 19 ng/g) accumulated most followed by total hexachlorocyclohexane (ΣHCH: α-HCH, β-HCH, δ-HCH, and γ-HCH, 30.9 ± 4 ng/g) and total chlordane compounds (ΣCHL: heptachlor and heptachlor-exo-epoxide, 5.9 ± 1.7 ng/g) successively (Fig. 2); but the concentrations of all OCP groups were not different between three bird types ($p > 0.05$). However, when all 16 OCPs analysed were considered individually, β-endosulphan, an ALD isomer, accumulated at the highest concentrations (Fig. 2). This suggested continual or extensive use of β-endosulphan in agriculture around the wetland[18], despite government restrictions on its use. However, the high β-endosulphan concentration in carnivorous birds (Fig. 2) suggested that biomagnification may also play a role.

The high ΣDDT accumulation was attributed to 4,4′-DDE, which mainly accumulated in carnivorous birds (Fig. 2). This indicated that biomagnification contributed, besides the extensive use of 4,4′-DDE in agriculture and malaria-control agent[19]. In the present study, the carnivorous yellow bittern, which mainly fed on fish, accumulated the highest 4,4′-DDE and β-endosulphan levels (Table 2). Omnivorous birds accumulated low concentration of ΣDDT, but high ΣALD concentrations. The highest ALD level accumulated was for dieldrin in the Purple Swamphen (Fig. 2 and Table 2). Omnivorous birds accumulated moderate concentrations of ΣHCH, but extremely low

ΣCHL concentrations (Fig. 2). In insectivorous birds no ΣDDT was detected, ΣALD and ΣHCH accumulation were low, and ΣCHL was barely detected (Fig. 2). Only aldrin, endosulphan sulphate, endrin and β-HCH were detected in these birds, all at relatively low levels (Fig. 2). This suggests insecticide usage around this area was modest or conducted carefully.

DISCUSSION

Variations in the accumulation of contaminants in birds are due to the differences in habitats, food preferences and the capacity to metabolize contaminants [16, 20]. Studies of the OCP accumulation in liver were carried out since this organ is an active site for contaminant biotransformation [21]. The OCP accumulation pattern observed was similar to that reported in previous studies elsewhere [20, 22–25], and reflected the extent to which each OCP was utilized in agricultural areas around the Boraphet wetland. ΣDDT and ΣALD were used extensively, ΣHCH only moderately, while, the use of ΣCHL was from mining. The illegal use of β-endosulphan to control the golden apple snail (*Pomacea* sp.) in paddy fields has been reported [10]. Predominant accumulation of 4,4′-DDE in carnivorous birds was consistent with other studies which found it to be the most frequent OCP detected in wild bird tissues due to its high persistence and lipophilicity [26–28]. ΣHCH contamination was moderate (Fig. 2). Among the four HCHs, the lindane (γ-HCH) isomer accumulated the most; similar to the findings of Sakellarides et al [29].

Nevertheless, concentrations of all OCPs detected in the present study are lower than those reported previously in the tissues of birds in the Boraphet wetland [30]. Additionally ΣDDT concentrations were lower than those reported in some waterbird species from Orissa, India; e.g., Eurasian Spoonbill, Northern Shoveler, and Ruff [31]. The decline in OCP contamination in the Boraphet wetland may be due to the increased of restrictions on nearby OCP agricultural use.

The risk of OCP contamination of birds depends on their trophic position. Carnivorous birds in both the present and previous studies [16, 32] were found much more contaminated with OCPs than omnivorous or insectivorous birds (Fig. 2). Similar results were also reported in the liver of birds collected from the coastal area of Campanis, Italy [28]. Birds that feed exclusively on fish have been shown to be at higher contamination risk than birds those feeding at lower trophic levels due to their low monooxygenase activity, resulting in lower biotransformation of xenobiotics and subsequent biomagnification [1, 33, 34].

CONCLUSIONS

The samples of a particular species from different locations were analysed and reported as one group. The present study indicated that birds in the Boraphet wetland are still contaminated with moderate levels of OCPs, with carnivorous birds being at greatest risk due to their high trophic level. Although OCP accumulation of birds was lower than previously reported, regular monitoring of OCP contamination remains essential to ascertain OCP usage in agricultural activities around the Boraphet wetland being properly conducted.

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