Risk Assessment of Organohalogenated Compounds in Water Bird Eggs from South China

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Organohalogen compounds, the pesticides aldrin, dieldrin, endrin, hexachlorobenzene, mirex, ΣchIordanes, ΣDDTs, Σheptachlors, Σtoxaphenes, and the industrial chemicals ΣPCBs and ΣPBDEs, as well as total dioxin-like equivalents ($TEQ_{H4IIE\text{-}luc}$), were measured in eggs of two Ardeid species, the little egret (*Egretta garzetta*) and black-crowned night heron (*Nycticorax nycticorax*) from three port cities along the South China coast: Hong Kong, Xiamen, and Quanzhou. ΣDDTs was the predominant and most abundant residue, occurring at concentrations ranging from 2.7×10^3 to 8.7×10^4 ng/g lipid wt. The greatest concentrations of the studied compounds were observed in eggs collected from Hong Kong, with the exception of ΣDDTs, which occurred at the greatest concentrations in eggs from Quanzhou Bay and Xiamen Harbor. Concentrations of ΣPBDEs were greater in eggs from Quanzhou Bay and Xiamen Harbor, possibly because of rapid industrialization in these areas. Total concentrations of dioxin-like PCB toxic equivalents (TEQs), measured as the aryl hydrocarbon receptor (AhR)-mediated responses of the H4IIE-*luc* bioassay ($TEQ_{H4IIE\text{-}luc}$), were greatest in Hong Kong samples. A risk assessment of the relatively great concentrations of ΣDDEs and dioxin-like (coplanar) PCBs in the eggs (threshold levels: 2.8 μg/g wet wt. and 5 pg/g wet wt., respectively) predicted that concentrations of these compounds would be expected to affect some proportion of the Ardeid populations studied.

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Introduction

Global concern over releases of persistent organic pollutants (POPs) in the environment has been increasing for several decades (*1–3*). The 12 organochlorine POPs that are internationally regulated by the Stockholm Convention, which came into effect in China in 2004, are associated with pernicious effects to humans and wildlife because of their persistence, bioaccumulation through the food chain, and toxicity. Recent surveys of these contaminants in coastal areas of China have found relatively great concentrations of certain organohalogen compounds, including polybrominated diphenyl ethers (PDBEs), in mussels, fish, sediment, and human tissues (*4–7*) and water birds (*8*).

Parts of the southern coast of China urbanized rapidly after economic reforms in the 1970s, particularly in areas designated as Special Economic Zones (SEZs) where special management is allowed in order to encourage economic development. Examples of these intensively industrialized areas of South China include Shenzhen and Xiamen, which were designated as SEZs in the 1970s and 1980s, respectively. Foreign investments as well as rapid urbanization are changing the coastal environment of these areas, and pollutants generated by activities in the municipal, industrial, and agricultural sectors have released POPs into the coastal environment (*4*). However, there have been few comprehensive assessments of these pollutants, particularly in organisms at higher trophic levels.

Water birds have frequently been used as sentinels for determining status, trends, and spatial and temporal differences in concentrations of persistent contaminants and as species of concern in ecological risk assessments (*9–12*). Water birds, particularly piscivorous birds, can accumulate relatively great concentrations of POPs, making them useful in studies of organic pollutants and possible effects of POPs on wildlife in aquatic environments (*13–16*). In the present study, two species of Ardeid birds were chosen as indicators of toxicant exposure in several aquatic environments in South China. Ardeids inhabit coastal ecosystems and integrate exposure to pollutants over relatively wide areas. These contaminants may be transferred into the birds' eggs, where the developing embryo is thought to be the life stage that is most sensitive to contaminant effects. Therefore, bird eggs can be used as a site-specific, integrative measure of the status and trends in concentrations of POPs in the environment. The use of eggs offers the advantage of being easier to sample than adults, and Ardeids will lay additional eggs if egg collection is done properly, resulting in minimal impact on the population (*11, 15*). Concentrations of persistent organochlorines and ΣPBDEs were measured in Ardeid eggs by use of a combination of instrumental and bioanalytical techniques, and the potential for adverse effects of these chemicals in these water birds was evaluated. Residue concentrations were compared to compiled toxicity reference values (TRVs), and site-specific TRVs were developed for the predominant residues by use of a novel statistical procedure.

Materials and Methods

Sample Collection. Little egret (*Egretta garzetta*) and black-crowned night heron (*Nycticorax nycticorax*) eggs were collected from Hong Kong, Xiamen, and Quanzhou (Figure S1) during the breeding season (March−May 2004). Five eggs of each species from each sampling location were collected following conditions stipulated in a permit issued by the Hong Kong Agriculture, Fisheries, and Conservation Depart-

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ment. Detailed descriptions of the egretries from Hong Kong have been reported previously (*15*). Eggs from Xiamen were collected from Jiyu Islet in the Xiamen Egret Nature Reserve, which is located on the southern part of Xiamen Island and is also the most developed area of Xiamen. Eggs from Quanzhou were collected from rookeries near Quanzhou Bay, which has been undergoing rapid socio-economic growth and expanding resource use. Procedures for the collection of eggs have been described previously (*11, 15*). Briefly, a single egg was collected from each randomly selected nest and kept at 4 °C during transportation to the laboratory. The egg surface was cleaned with deionized water, and eggs were stored at −20 °C until analysis.

Identification and Quantification of Organohalogens. Quantification of organochlorines [hexachlorobenzene (HCB), aldrin, dieldrin, endrin, mirex, total heptachlor (Σheptachlors), total chlordane (Σchlordanes), total DDTs (ΣDDTs), total PCBs (ΣPCBs), and total toxaphene (Σtoxaphenes)] and ΣPBDEs was accomplished by use of previously established methods (*17, 18, 19*, respectively) with modifications. Eggs were analyzed individually. Instrumental analysis of organochlorines was conducted by use of GC-ECD with dual columns, except for Σtoxaphenes and ΣPBDEs, which were quantified by use of GC-MS in NCI and EI modes, respectively. Details of the extraction and chemical analysis, as well as recoveries and detection limits, can be found in Supporting Information.

H4IIE-*luc* Cell Culture and Bioassay. The H4IIE-*luc* transactivation assay, in which rat hepatoma cells that express the aryl hydrocarbon (AhR) receptor have been stably transfected with a luciferase reporter gene under the control of dioxin-responsive elements (*20*), was used to measure total concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs), which represent total AhR-mediated 2,3,7,8-TCDD-like activities in sample extracts. Cell bioassays were conducted as previously described (*21*); information on the cell culture and bioassay procedures can be found in Supporting Information.

Statistical Analysis and TEQ Determinations. Comparisons of residue concentrations among locations were made by use of one-way ANOVA (SigmaStat 2.03; Windows Version, SPSS, Chicago, IL). Data was tested for normality by use of a Kolmogorov−Smirnov test and for homogeneity of variance by use of a Levene's test. If data met assumptions of normality and homogeneity of variance, statistical analyses were conducted on the untransformed values. If the data were not normally distributed, nonparametric Kruskal−Wallis ANOVA on the ranks of the values was used for intergroup comparisons. Statistical significance was accepted at $p < 0.05$ for all statistical methods. Samples for which concentrations were less than the LOQ for instrumental analysis were assigned a value equal to half of the LOQ. Sensitivity analyses were conducted to determine the magnitude of bias that this action might contribute to the analyses.

The assumption of parallelism between the sample and standard dose−response relationships could not be tested for the results of the H4IIE-*luc* bioassay because a mixture was being compared to a single standard and efficacies (maximum response achieved) were not equal. Therefore, the point-of-departure method was used to determine the dioxin-like activities of sample extracts (*22*). In this procedure, a one-tailed Dunnett's test (Systat 11, SSI, Richmond, CA) was used in order to determine the first sample concentration that was significantly different from zero (*23*). This concentration was then related to the least standard concentration that was significantly different from zero (0.16 pg 2,3,7,8-TCDD/well) in order to estimate TEQs for the tested samples. The equivalent concentrations of 2,3,7,8-TCDD were expressed as $TEQ_{H4IIE\text{-}luc}$.

Two approaches were used to compare the TEQ concentrations contributed by instrumentally measured AhR-active compounds such as coplanar PCB congeners and $TEQ_{H4IIE\text{-}luc}$. The activities determined by use of the bioassay included all of the AhR-active compounds, such as PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). TEQ concentrations were calculated from measured coplanar PCB concentrations in two ways. The first way was used for risk assessment while the second was used for making comparisons between bioassay-derived $TEQ_{H4IIE\text{-}luc}$ values and those predicted from instrumentally derived concentrations of coplanar PCBs and in some cases PCDDs and PCDFs. The first approach ($TEQ_{WHO\text{-}Avian\text{-}TEF}$) was based on TCDD equivalency factors (TEFs) determined for birds by the World Health Organization ($TEF_{WHO\text{-}Avian}$) (*24*). $TEQ_{WHO\text{-}Avian\text{-}TEF}$ concentrations were calculated as the sum of the product of the concentration of each coplanar PCB congener by its corresponding $TEF_{WHO\text{-}Avian}$ value. Values for $TEF_{WHO\text{-}Avian}$ were derived for use in risk assessments and tend to overestimate the potential for effects, making them protective rather than predictive in nature and not appropriate for use in a relative potency balance. Therefore, a second method was used ($TEQ_{H4IIE\text{-}luc\text{-}ReP}$) to determine the contribution of coplanar PCBs to total TEQs by the use of relative potency factors (RePs) (*22*) to calculate $TEQ_{H4IIE\text{-}luc\text{-}ReP}$ from the measured concentrations of coplanar PCBs and PCDDs/DFs (*25*).

Hazard and Risk Quotient Derivation. The potential of organochlorine residues in eggs to cause adverse effects was evaluated by several methods, including point estimate comparisons and probabilistic approaches. In the point-estimate approach, concentrations of individual residues measured in eggs were compared to published, consensus toxicity reference values (TRVs) for each compound. The hazard quotient (HQ) was calculated as the quotient of the concentration in the egg divided by the appropriate TRV. In addition to comparison to global TRVs, TRVs were determined by use of a regression of percentage of young fledged as a function of $\log_{10}$-transformed residue concentrations measured in eggs in this study. The method used to derive TRV values was similar to that described previously (*11, 15*). A stepwise increment of 0.05 log concentration units was used, followed by a one-sample *t*-test comparing the percentage of young fledged with the 100% fledging success level at which a stable population for many avian species can be sustained. A 95% confidence interval was calculated for the regression line so that the discrimination power of the relationship could be made. TRVs derived in this manner include the potential effects of other co-occurring toxicants and thus tend to be protective. However, since the absolute and relative concentrations of the co-occurring residues vary among locations, the correction may not be appropriate for all locations. For calculating the probability of risk quotients (RQs) exceeding unity, Monte Carlo simulation using Crystal Ball (Oracle, Denver, CO) was carried out.

Results and Discussion

Residue Concentrations in Eggs. Among the pollutants analyzed, concentrations of ΣDDTs, ΣPCBs, and Σchlordanes were significantly greater than concentrations of other residues measured (Figure 1). Concentrations of ΣDDTs accounted for 52%, 93%, and 95% of all trace organic pollutants in little egret eggs from Hong Kong, Xiamen, and Quanzhou, respectively (Table S1). These results are similar to those of a study of the same species on Tai Lake in the delta of the Yangtze River (*8*). Concentrations of organochlorine residues in Ardeid eggs from Hong Kong were greater than those in eggs from the other two port cities, with the exception of DDTs and HCB (Figure 1).

ΣDDTs. The greatest mean concentration of ΣDDTs (5.8×10^4 ng/g, lipid weight [lw]) was found in eggs from Quanzhou. This concentration was approximately 2- and

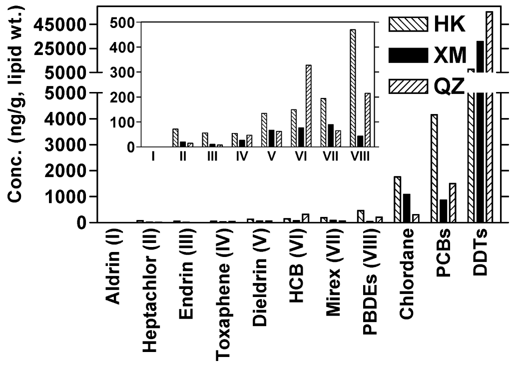

FIGURE 1. Concentrations of trace organic pollutants in little egret eggs from Hong Kong (HK), Xiamen (XM), and Quanzhou (QZ).

4-fold greater than those in eggs from Xiamen and Hong Kong, respectively. The relative proportion of ΣDDTs that was comprised of *p,p'*-DDT in eggs from Quanzhou was significantly greater than at the other two locations (Figure S2), with *p,p'*-DDT accounting for approximately 30% of the ΣDDTs in eggs from Quanzhou compared to only 3% and 10% in eggs from Hong Kong and Xiamen, respectively. These differences are likely due to more recent inputs of DDT into the Quanzhou environment. There has been no comprehensive monitoring of chlorinated pesticides in Quanzhou, so it is difficult to determine potential sources of DDTs. Nevertheless, Quanzhou Bay is one of the most seriously polluted coastal areas in China, and quality of seawater in this area was found to be poorer than the grade IV standard according to a 2002 report on the State of the Environment in China (http://www.zhb.gov.cn/english/SOE/soechina2002/). Recent investigations have indicated relatively great concentrations of ΣDDTs in coastal environments of China (*2, 6, 26, 27*) and in areas near the coast, such as in Tai Lake, located upstream from Shanghai on the Yangtze River (*8*). Relatively great concentrations of ΣDDTs were also detected in fish and mussels from southeast China and a high proportion (40–60% of ΣDDTs) was found to be *p,p'*-DDT in these samples (*28*). These results are consistent with our study, suggesting recent inputs of this insecticide.

Of the nine pesticides listed under the Stockholm Convention, four are still being produced, used or released in China—DDT, chlordane, mirex, and HCB—which were among the seven most prevalent pollutants (DDTs, PCBs, chlordane, PBDEs, mirex, HCB, and dieldrin) found in the eggs (Figure 1). Production of DDTs in China was 0.4 million tons between the 1950s and 1980s, accounting for 20% of total world production (*28*). Technical DDTs have been banned in China for almost two decades, but recent studies have indicated that impurities in technical mixtures of the insecticide dicofol could possibly be contributing to current ΣDDTs pollution in China (*29, 30*).

Concentrations of ΣDDTs measured in little egret and black crowned night heron eggs were compared to those measured in eggs of the same species from Tai Lake in the Yangtze River Delta near Shanghai (*8*) by correcting the reported dry weight values to lipid weight (an average lipid content of 6.7% and moisture content of 80% was assumed). After this correction, ΣDDT concentrations in little egrets from Hong Kong (range: $4.32-11.4 \times 10^3$ ng/g lw) were found to lie within the range of the same species at Tai Lake (range: $1.17-40.2 \times 10^3$ ng/g lw (*8*)). ΣDDT levels in both little egret and night heron eggs from Quanzhou (range: $2.89-7.93 \times 10^4$ ng/g lw) and Xiamen (range: $1.50-5.94 \times 10^4$ ng/g lw) were greater than those from Tai Lake.

ΣPCBs. Unlike the distribution of ΣDDTs observed in eggs from Quanzhou (Figure 1), ΣPCBs in eggs from Hong Kong were significantly greater ($p < 0.05$) than concentrations at the other two sites, which suggests the presence of local sources of PCBs in the Pearl River Delta (PRD) region. Concentrations of $TEQ_{H4IIE\text{-}luc}$ and $TEQ_{WHO\text{-}avian\text{-}TEF}$ were also greatest in eggs of little egrets from Hong Kong (Figure S3). Concentrations of $TEQ_{WHO\text{-}avian\text{-}TEF}$ were greater than $TEQ_{H4IIE\text{-}luc}$, probably because of differences in contaminant sensitivities between birds and mammals (*31*), differences between *in vivo* and *in vitro* systems, as well as potential interference or antagonism among compounds in the bioassay. Concentrations of $TEQ_{H4IIE\text{-}luc}$ were greater than $TEQ_{H4IIE\text{-}ReP}$ in eggs from Hong Kong (Figure S3). The results of the mass balance comparison suggested the presence of other dioxin-like compounds such as PCDDs/DFs, so comparisons were made to PCDD/DF concentrations measured in eggs collected in 2006. Although these data were two years younger than the egg data presented in this study, the persistence of PCDDs/DFs makes it reasonable to use these concentrations as an estimate to determine the contribution of PCDDs/DFs to $TEQ_{H4IIE\text{-}luc}$ in Hong Kong. The results of the mass balance for dioxin-like compounds in Hong Kong egg samples further suggest that PCDD/DFs are more prevalent in the PRD region than the other two sites.

The PRD region, located northwest of Hong Kong, was one of the first areas in South China to be industrially developed when there was a rapid change of land use from agricultural to industrial purposes starting in the late 1970s. Great demand for electricity and extensive industrial activities required huge amounts of insulating and cooling agents in transformers and capacitors, and PCBs were extensively used during this period. Despite the phasing out since the early 1980s of the use of PCBs in new products in China, large quantities of PCBs have not been disposed of effectively and leaking has been reported in some storage sites (http://english.people.com.cn/200411/11/eng20041111_163497.html). A recent study by the European Union (EU) measured trace organic pollutants in little egret eggs from three locations in China, including the PRD (*32*). Concentrations of ΣPCBs reported in the EU study (20–94 ng ΣPCBs/g, wet weight [ww]; 299 and 1403 ng ΣPCBs/g lw) were similar to those in eggs from Xiamen and Quanzhou. Differences in PCB concentrations in eggs from the PRD, as reported in the EU study, and in Hong Kong samples in the present study, are possibly due to the dissimilar local pollution status of the two sampling sites. Eggs from the PRD analyzed in the EU study were collected from upstream areas, whereas eggs from Hong Kong in the present study were collected from locations in the PRD estuary, which is likely to be more contaminated due to the accumulation of organochlorine residues from the different streams of the PRD.

Similar patterns of relative concentrations were observed between the two industrial pollutants ΣPCBs and ΣPBDEs, but concentrations of both total ΣPCBs and ΣPBDEs in eggs from Hong Kong were greater than those from the other two locations. The observed differences can possibly be attributed to dissimilar industrial developmental phases among the three cities. Areas adjacent to Hong Kong, including the Shenzhen Special Economic Zone (SEZ) have been subjected to intense economic and industrial activities since the 1980s, while Xiamen and Quanzhou started large-scale industrial activities only in 1986 and 1991, respectively. It is likely that the longer history of extensive industrial development in the region around Hong Kong has resulted in greater concentrations of these contaminants in the Hong Kong environment compared to the other two sites.

Mirex and Toxaphene. Mirex and toxaphene are seldom studied in the Asia-Pacific region and there is a lack of comprehensive information reported on concentrations of these compounds in China. Mirex concentrations in bird eggs from some European and North American countries were found to be either decreasing or stable over time after mirex use was banned (*12, 33–35*). Comparison of mirex in eggs of piscivorous birds from different nations indicated that concentrations in eggs from South China were equal to or greater than those determined in other studies (Figure S4), with the exception of samples from Lakes Erie and Ontario that were collected during the 1990s (*33, 36*). However, most of the studies cited (Figure S4) were conducted in the 1990s and thus may not reflect the current status of mirex in the environment. Concentrations of mirex in blubber of Hong Kong cetaceans reported previously (*37*) were significantly greater than those in specimens from Brazil, Japan, India, and the Philippines. This result and the greater residue concentrations in eggs from South China compared with other studies (Figure S4) both suggest heavy usage in the past and/or recent inputs. This trend in mirex concentrations deserves further investigation, particularly in relation to the major degradation product of mirex, photomirex, which is persistent in the environment and is considerably more toxic than mirex itself (*38*).

Toxaphene has rarely been reported in avian species, possibly because of different relative concentration patterns of chloro-bornanes in tissues relative to technical toxaphene (*10*). Concentrations of Σtoxaphenes in birds include a value of 2600 ng/g lw in osprey (*Pandion haliaetus*) from Sweden (*39*), while guillemots collected in the 1970s from the Baltic Sea contained an average of 6500 ng/g lw and a sample of muscle from guillemot collected in the Barents Sea (Spitzbergen) in the early 1980s contained 4100 ng/g lw (*40*). The fact that mirex and toxaphene were detected in all samples in the current study indicates that these chemicals are widespread and ubiquitous in the environment in South China. To our knowledge, this is the first report of concentrations of mirex and toxaphene in bird eggs from China. To facilitate comparisons between the three sampling locations, little egret eggs were used and residue concentrations of both mirex and Σtoxaphenes were comparatively greater in Hong Kong, although the greatest mirex concentration (707 ng/g lw) was measured in the eggs of black-crowned night herons from Xiamen. Concentrations of Σtoxaphenes observed in this study were similar to or less than those reported historically or from other locations, with the exception of concentrations reported in bird eggs from North America (Figure S4). Concentrations of Σtoxaphenes in eggs of fish-eating birds from South China were generally similar to those measured in Germany but relatively less than those reported for birds in Europe and North America (Figure S4). The greatest concentration of Σtoxaphenes was found in samples from the North Pacific Ocean. This finding was consistent with results reported previously (*10*), which suggested that the Northern Atlantic Ocean and eastern North America were more polluted with toxaphene based on the results of avian and cetacean studies in different nations. Toxaphene use in the USA comprised up to 40% of the world market and was concentrated in the southeastern part of the country (*41*), so it is reasonable that toxaphene residues are generally present at greater concentrations in the North American region than in Asian countries.

Toxicity Reference Values for Birds and Risk Characterization. In the present study, the potential for adverse effects on the birds of interest was determined by comparing concentrations of individual residues or integrated measures of classes of compounds to toxicity reference values (TRVs). In some cases, such as for the cyclodiene insecticides and HCB, concentrations in the eggs were compared to existing screening values for risk assessment. In the case of ΣPBDEs, there was insufficient toxicological information in the literature to develop a TRV. TRVs can be species-specific or more universal, being developed from probabilistic relationships to protect populations or communities (*42*). Furthermore, TRVs can be global, with general applicability at all locations or they can be site-specific. In this study, additional TRVs were derived for a compound or mixture, such as for ΣDDTs, $TEQ_{H4IIE\text{-}luc}$ or ΣPCBs, in the presence of other relevant toxicants. The advantage of this process is that it integrates the effects of all of the other contaminants into the assessment and is more likely to be predictive of effects and more protective in a site-specific risk assessment. However, the limitation of the method is the inability to generalize to other locations or situations.

Ideally, the TRV value can be used for protecting all individuals in a population. Since a certain number of young must fledge successfully in order to maintain a stable avian population, previous studies have used percent survival of young as an end point for risk assessment, even though decreased survival may not necessarily cause declines in the bird population if fledging success per breeding pair is not significantly affected. Using fledging success as an end point would therefore be protective of a population, and also would be more relevant to ecological risk assessment. However, populations are seldom stable and may fluctuate naturally in response to factors other than toxicants. Therefore, in this study, the predicted effects on populations should be considered to be only first approximations. The TRV values should be thought of a more protective than predictive and used as indicators of potential effects, rather than strict guidelines.

Mirex and Toxaphene. Hatchability of chicken (*Gallus gallus*) and Japanese quail (*Coturnix japonica*) eggs were not affected by concentrations of mirex or Σtoxaphenes as great as 14 and 90 mg/kg ww, respectively (*43, 44*). Assuming an average lipid content of approximately 6.7% (mean values ranged from 4.08% to 10.4%) for the eggs studied here these values would be approximately 209 and 1.3×10^3 mg/kg lw. Concentrations of mirex and Σtoxaphenes observed in the present study were far less than these TRV values, and the HQ values for both compounds were similarly far less than one. Thus, current concentrations of neither mirex nor Σtoxaphenes in eggs of Ardeids would be likely to cause reproductive impairment to these species in the areas of South China studied.

Cyclodiene Insecticides and HCB. When concentrations of dieldrin, endrin, and Σchlordanes were compared to NOAEL values (*45*), none of the HQs were greater than one. The aldrin concentrations measured in the water bird eggs were small (<1 ng/g lw), and aldrin degrades rapidly to dieldrin in the environment; thus, the risk due to this compound was considered to be quite low. HCB is known to be very toxic to birds, but the concentrations measured in the present study were lower than the NOAEL (1.5 μg/g ww) for mortality in herring gull (*Larus argentatus*) embryos in an egg-injection experiment (*46*). A consensus NOAEL value for birds was not available for Σheptachlors, so the measured levels were compared to previously reported concentrations known to affect reproduction ((*47*), and references therein), and the risk due to this group of compounds was found to be low. However, it should be noted that Ardeid-specific TRVs are not available for any of these compounds, and therefore this risk assessment is preliminary. HCB and Σchlordanes were associated with effects on thyroid function in glaucous gulls (*Larus hyperboreus*) at concentrations similar to those measured in this study (*48*), indicating that these compounds may still warrant concern.

DDTs. DDE is one of the major metabolites of technical DDTs and it is known to adversely affect avian reproduction by reducing eggshell thickness (*49*). The potential risk of DDE to cause thinning in Ardeid egg shells was recently reviewed, and threshold values were estimated (*15*). Using survival of young as the end point, the DDE effect threshold concentration at which there was a significant reduction in the fledged young was determined to be approximately 1.0 μg/g ww (*15*), which would be 14.9 mg/kg lw, assuming a lipid content of

6.7%. Comparison of TRVs based on threshold toxicity values for ΣDDTs in bald eagles (*Haliaeetus leucocephalus*; *50, 51*) and barn owls (*Tyto alba*; (*52*)) indicated that the TRV developed by Connell et al. (*15*) is the most conservative (protective).

In addition to these estimates based on laboratory studies, a TRV that would estimate the effect of DDT in the presence of the other contaminants was derived from literature values using a probabilistic assessment. The relationship between DDE concentrations in eggs of piscivorous birds and the percentage of young fledged for a sustainable population was used to establish a new threshold concentration for assessing DDE impacts to Ardeids of approximately 2.8×10^3 ng DDE/g ww egg (one-tailed $p = 0.031$) (Figure 2a; Table S2). The resulting TRV is approximately three-fold greater than the one estimated previously (*15*). While there may be several reasons for this difference, the most likely reason is the way in which the TRVs were derived. In the present study, the threshold for effects of DDT was determined using a different metric of breeding success, namely fledging success rather than survival of young.

A probabilistic assessment of DDE (ΣDDEs = sum of *o,p′* and *p,p′* isomers) exposure was conducted by comparing the probability of concentrations in eggs exceeding the TRV (*42*). In constructing the log-normal probability relationships (Figure 2b), ΣDDEs concentrations in eggs of little egrets and night herons from Xiamen and Quanzhou were combined to conduct a preliminary screening assessment of these contaminants. In the case of ΣDDEs, concentrations in little egret eggs measured in the present study and data reported previously (*15*) were combined because there was no statistically significant difference between the concentrations in the two data sets. The results indicated that 26% and 40% of ΣDDEs concentrations in eggs from Xiamen and Quanzhou were greater than the threshold associated with reproductive impairment of fish-eating bird populations. Assuming a log-normal distribution of the concentrations in eggs (Figure 2b), a Monte Carlo simulation was compared to the threshold value (2.8×10^3 ng ΣDDEs/g, ww, egg) and 95% CI (10965 ng ΣDDEs/g, ww egg) to calculate risk quotients (RQs). Using 10 000 simulation trials, the probabilities of RQs exceeding unity for Ardeids from Hong Kong, Xiamen, and Quanzhou were 9.9%, 31.6%, and 44.1%, respectively.

ΣPCBs. TRV values were determined for ΣPCBs based on *in ovo* exposures, since there is no clear benchmark TRV for effects of ΣPCBs on birds. TRVs were based on both the least and greatest NOAELs reported to cause reproductive impairment (*1*). TRVs derived from studies of the effects of ΣPCBs on the leghorn chicken and black-crowned night heron were 0.36 and 10.9 μg ΣPCBs/g ww, respectively (5.4 and 163 μg ΣPCBs/g lw, respectively, assuming an average lipid content of 6.7%). Additional TRVs based on the NOAEL and LOAEL values from a feeding study of screech owls (*Otus asio*) exposed to Aroclor 1248 were determined to be 7 and 21 μg ΣPCB/g egg ww, respectively (104 and 313 μg ΣPCB/g egg lw, respectively) (*53*). Concentrations of ΣPCBs in Ardeid eggs from Xiamen and Quanzhou were all less than the least TRV, but concentrations in 80% of Ardeid eggs from Hong Kong were greater than the least NOAEL. However, none of the egg concentrations from any of the locations exceeded the greater TRV of 163 μg ΣPCB/g lw. Thus, it is unlikely that current concentrations of PCBs are sufficient to cause observable adverse effects on Ardeids.

$TEQ_{WHO\text{-}avian\text{-}TEF}$. Due to the limitations of the ΣPCBs approach for risk assessment, congener-specific PCB analysis in combination with an *in vitro* bioassay was employed in an attempt to further evaluate the potential risk of various planar polychlorinated hydrocarbons to birds. In the present study, two species sensitivity distributions (SSDs) derived from both field and laboratory avian toxicity data were used

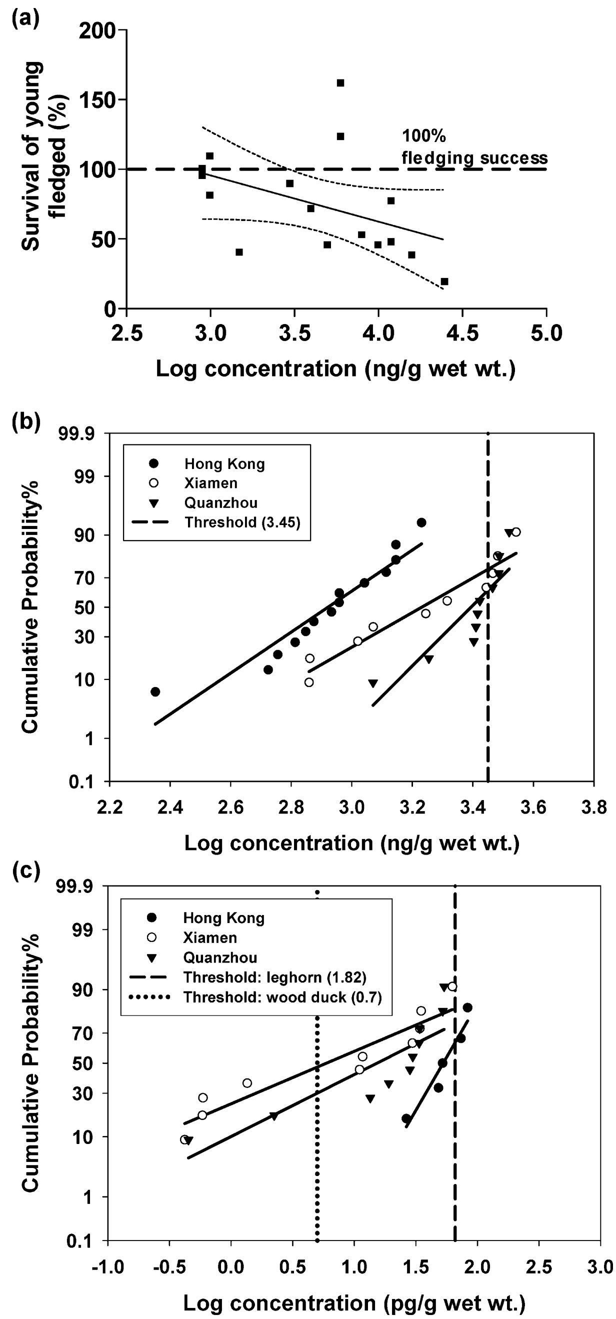

FIGURE 2. (a) Relationship between percentage of young fledged and ΣDDE concentrations in eggs [based on literature values] (Table S2) showing the regression line and 95% confidence intervals. (b) Cumulative probability distribution of ΣDDE concentrations in eggs of the combined data set of little egret and black-crowned night heron (Table S1) from Hong Kong, Xiamen and Quanzhou with ΣDDE TRV indicated (c) Cumulative probability distribution of $TEQ_{WHO\text{-}avian\text{-}TEF}$ concentrations in eggs of the combined data set of little egret and black-crowned night heron (Table S1) from Hong Kong, Xiamen, and Quanzhou with TEQ TRV indicated.

as proposed by Suter (*50*) (Table S3) to characterize the risk due to dioxins and dioxin-like compounds. This approach uses the geometric mean of all available threshold concentrations for a given species, rather than just the most sensitive end point and species. The leghorn chicken has been determined to be among the most sensitive species of bird to the effects of TEQs with a mean NOAEL of 66 pg/g egg ww (*50*). However, there is a wide range in sensitivities among birds (*13, 14*), and therefore selection of the appropriate TRV has a large impact on the results of a risk assessment.

Log-normal probability plots of concentrations of $TEQ_{WHO-avian-TEF}$ in eggs from the three sites (Figure 2c) indicated that 45% of concentrations from Hong Kong were greater than the TRV based on the least NOAEL for leghorn chicken (*54*), while fewer than 10% of concentrations in eggs from Xiamen and Quanzhou exceeded the TRV. When the TRV based on the NOAEL for the wood duck (*Aix sponsa*) was compared to the distribution of concentrations in eggs, more than 50% of $TEQ_{WHO-Avian-TEF}$ exceeded the TRV. Assuming a log-normal distribution of the TEQ concentrations in eggs, the Monte Carlo simulation resulted in probabilities that risk quotient (RQ) values exceeded 1.0 of 11.5%, 2.7%, and 4.0% for Ardeids from Hong Kong, Xiamen, and Quanzhou, respectively, whereas probabilities for H4IIE bioassay-based TEQs were <0.1% for all sites.

Recently, it has been found that the relative sensitivity of birds to dioxin-like compounds is a function of binding of the ligand of interest (toxicant) to the ligand-binding domain (LBD) of the aryl hydrocarbon receptor (AhR) (*55*), and that differences in amino acid sequences in the LBD might also account for differences in dioxin sensitivity within the order Galliformes (*56*). The authors classified approximately 30 bird species based on their sensitivity to dioxin-like compounds into three categories: type I (chicken-like, very sensitive), type II (ring-necked pheasant/turkey-like, moderately sensitive), or type III (common tern-like, insensitive) (*56*).

To be protective of all avian species without knowing their sensitivity it would be necessary to use the minimal TRV. However, knowing which AhR LBD type is characteristic of a species would allow more refined risk assessments to be conducted because there would be less uncertainty about the potential effects of TEQs on the species for which population-level studies are not being conducted. On the basis of recent sequencing of the AhR in a range of bird species (unpublished data), the Ardeids studied here would be in the least sensitive group (Type III), and to date no other fish-eating birds have been found to be as sensitive as the chicken. Therefore, the species that are expected to be exposed to the greatest TEQ concentrations, predatory water birds, are likely to be the least sensitive species. Thus, even though the preliminary risk assessment using a TRV based on the white leghorn chicken indicated that Ardeids in Hong Kong could be expected to exhibit some adverse effects, an assessment using the TRV of the least sensitive species, the herring gull, produced HQ values less than 1.0, meaning that no adverse effects would be expected. Since the Ardeids are type III in terms of their AhR LBD structure, it is unlikely that current concentrations of TEQs would be likely to cause effects. In addition, the $TEF_{WHO-avian}$ values that were used to determine the total TEQ concentrations were meant to be conservative estimates of risk and tend to overestimate actual TEQ concentrations (*24*).

In this study, the HQ values for ΣPCBs were slightly greater than HQ values based on $TEF_{WHO-avian}$. PCBs can cause several types of toxicity, including that of the coplanar congers that would be measured as part of the $TEQ_{WHO-avian}$ (*31*). The greater HQ values for $TEQ_{H4IIE\text{-}luc}$ than those for $TEQ_{WHO-avian}$ were due to the fact that the $TEQ_{WHO-avian-TEF}$ were calculated from only the contribution of ΣPCB, while the concentrations of $TEQ_{H4IIE\text{-}luc}$ contained all of the dioxin-like compounds.

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Supporting Information Available

Details on extraction and instrumental analysis of organohalogen compounds and bioassay procedures; tables containing organohalogen concentrations and values used for risk assessment; figures showing contaminant proportions and global comparisons.This material is available free of charge via the Internet at http://pubs.acs.org.

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