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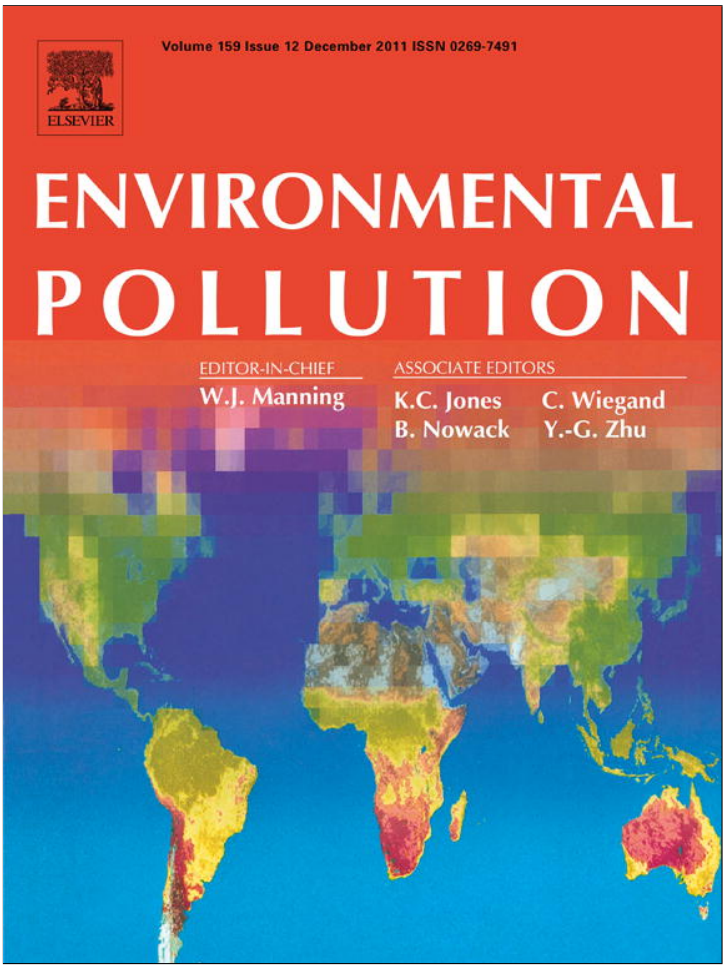

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Environmental Pollution 159 (2011) 3433–3438

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

PBDEs, PCBs, and DDE in eggs and their impacts on aplomado falcons (*Falco femoralis*) from Chihuahua and Veracruz, Mexico

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ARTICLE INFO

Article history:
Received 4 May 2011
Received in revised form
8 August 2011
Accepted 15 August 2011

Keywords:
Aplomado falcon
PBDEs
PCBs
DDE
Mexico

ABSTRACT

Eggs from aplomado falcons (*Falco femoralis septentrionalis*) nesting in Chihuahua and Veracruz, Mexico, were analyzed for organochlorine pesticides, PCBs, and PBDEs. p,p′-DDE was the only organochlorine found in all eggs at concentrations ranging from 0.13 to 7.85 μg/g wet weight. PCBs ranged from 0.04 to 2.80 μg/g wet weight and PBDEs from 62 to 798 ng/g lipid weight. DDE concentrations in eggs were not significantly different among regions; however, PCBs were significantly greater ($P = 0.015$) in Tinaja Verde, Chihuahua than in the other three regions. Also, PBDEs were significantly higher ($P < 0.0001$) in eggs from Veracruz than in those from Chihuahua. DDE concentrations in eggs were much lower than those associated with eggshell thinning. PBDEs and PCBs were lower than those reported in raptors from industrialized countries. Overall, contaminant concentrations observed suggest no likely impact on hatching success. The PBDE concentrations are among the first to be reported in raptor species in Mexico.
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1. Introduction

The northern aplomado falcon (*Falco femoralis septentrionalis*) was once a common raptor species in the southwestern United States, but disappeared in the 1940s due in part to habitat loss, agricultural use of pesticides, and egg collection (Keddy-Hector, 2000). Currently, the aplomado falcon is considered endangered in the United States and threatened in Mexico. The species is slowly recovering in the United States primarily due to a reintroduction program, under which, over the last 20 years approximately 1765 individuals have been released in southern Texas, west Texas, and New Mexico (The Peregrine Fund, unpublished data). Over the course of the recovery program, we have monitored the accumulation of environmental contaminants in aplomado falcons by collecting and analyzing addled eggs from breeding populations in southern Texas and northern Mexico in Chihuahua (Mora et al., 1997, 2008).

Aplomado falcons are widely distributed in eastern Mexico along the states of Tamaulipas, Veracruz, Tabasco, Campeche, and Chiapas (Keddy-Hector, 2000), with an isolated population in north-central Chihuahua (Montoya et al., 1997). Unfortunately, there is not much information about the potential impacts of environmental contaminants on the aplomado falcon in eastern Mexico or in tropical regions. It is widely speculated that aplomado falcons may be threatened by habitat loss and agriculture, but except for one study involving a small population in Chihuahua (Mora et al., 2008), no assessments of contaminant impacts have been conducted in Mexico in the last 20 years. The only contaminant data available from aplomado falcon populations in Veracruz were obtained during 1977 from seven addled eggs which had average p,p′-DDE [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene] residues of 297 μg/g lipid weight (approximately 14.85 μg/g wet weight, Kiff et al., 1980). DDE, is a metabolite of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], a persistent organochlorine pesticide used in Mexico in agriculture and for malaria control until about 2000 (Chanon et al., 2003). DDE has been associated with eggshell thinning and decreased reproduction in birds (Anderson and Hickey, 1972).

Similarly, information about the impacts of other contaminants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) on Mexican wildlife are lacking. PCBs were widely used throughout Mexico in transformers, capacitors, and fluorescent light bulbs; however, most current concentrations are in electric power substations, electric installations and industrial complexes (Commission for Environmental Cooperation, CEC, 1996). It is estimated that there are about 8000 tonnes of PCB oil

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doi:10.1016/j.envpol.2011.08.025

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in transformers and another 32,000 tonnes in storage (CEC, 1996). Polybrominated diphenyl ethers are commonly used as fire retardants in upholstery, foams, rubber, and circuit boards (Yogui and Sericano, 2009a; Chen and Hale, 2010). They have increasingly been found in the environment in both abiotic and biotic media. The vast majority of PBDEs enter the environment either directly from manufacturing sites or through leaching from treated products. Both PCBs and PBDEs are known as endocrine disruptors, embryotoxic, and as agents affecting reproduction in avian species (Rice et al., 2003; Chen and Hale, 2010).

Because of aplomado falcon's status as a threatened and endangered species in Mexico and the United States, respectively, it is important to monitor concentrations of persistent organic pollutants in eggs of this species to assess contaminant impacts on these populations. The compilation of contaminant data in Mexican populations will allow for comparisons of contaminant accumulation in individuals released in the United States. These data also will be useful for assessing contaminated sites throughout the species range in Mexico. The Commission for Environmental Cooperation, an official organization of the United States, Canada, and Mexico, has been leading a program for monitoring and assessing pollutants across North America, with particular emphasis on improving the capacity of Mexico to generate the "management of information needed to identify and assess trends and concerns related to contaminants and stressors that affect the environment and human health" (http://www.cec.org/). The CEC study also indicates that Mexico does not have an exhaustive PCB monitoring program and field studies of PCBs are lacking. Our study is in line with the CEC objectives and proposes to accomplish the following: 1) To evaluate concentrations of persistent organochlorine pollutants, including organochlorine pesticides, PCBs and PBDEs in eggs of aplomado falcons from Chihuahua and Veracruz Mexico; 2) to assess the potential impacts of such contaminants on aplomado falcon reproduction or any other sublethal effects on the population; and 3) to compare levels of PCBs, PBDEs, and OC pesticides between the aplomado falcon in Mexico, populations in the U.S.A., and raptors from other regions of the world.

2. Materials and methods

2.1. Study areas

For the current study, the study area in Chihuahua was the same as described in Mora et al. (2008) and included the desert grasslands of north-central Chihuahua. The nesting sites were located in El Sueco and Tinaja Verde in the municipalities of Ahumada and Coyame (Fig. 1). The region consists primarily of grasslands and open halophyte grasslands interspersed with Chihuahuan desert scrublands. The land use includes ranching and some farming. The study areas in Veracruz were grouped in two main regions: Veracruz North (20°04′N, 96°45′W to 19°52′N, 96°29′W) in the municipalities of Nautla and Vega de Alatorre; and, Veracruz South (19°04′N, 96°06′W to 18°38′N, 95°18′W) in the municipalities of Alvarado, Medellin de Bravo and Tlacotalpan (Fig. 1). The northern region in Veracruz is dominated by farmlands, pastures interspersed with rolling hills and patches of second-growth forest. The southern region is similar in structure to the north, but flatter with more sugar cane plantations and pastures, and landscape dominated by palm-savannahs such as Mexican palmetto (*Sabal nexicana*) and royal palm (*Scheelea liebmannii*).

2.2. Sample collection

Twenty-three addled eggs were collected between 2004 and 2007 from four main regions in Chihuahua (El Sueco and Coyame) and Veracruz (North and South) (Fig. 1). Nests were located based on previous surveys and through monitoring efforts. Eggs were collected from nests located at different stages during the breeding season. Some nests were located from the very beginning in the incubation period and were monitored throughout the breeding season. In all cases, eggs were collected until one or more chicks hatched or the nest was abandoned. The nests were inspected with a telescoping pole and the intact, non-viable eggs were retrieved with the use of climbers. Eggs were wrapped in aluminum foil and taken to the lab where they were placed in clean glass jars and kept frozen until chemical

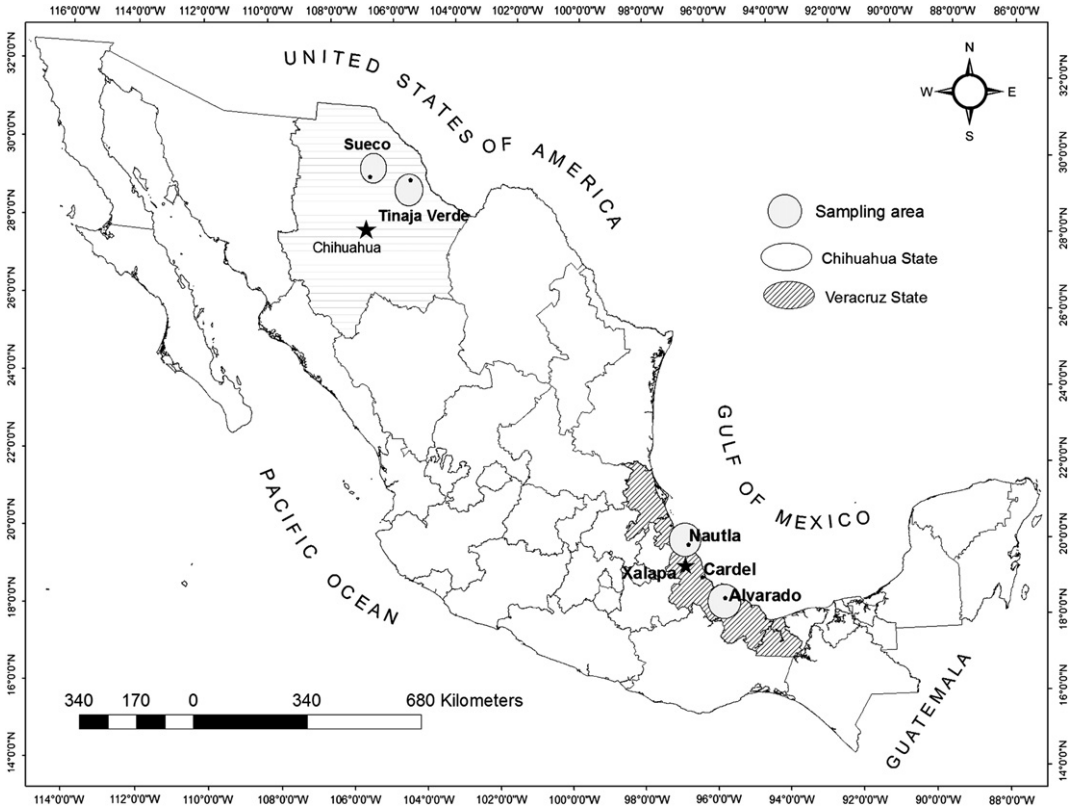

Fig. 1. Map of Mexico showing the collection locations in the states of Chihuahua and Veracruz, Mexico.

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analyses. In the laboratory, eggs were slightly thawed and, if the shell was not already broken, they were broken along the equator with an acetone-rinsed scalpel to remove the contents. Contents were transferred to clean glass jars with Teflon-lined lids and homogenized by stirring and/or grinding. Eggshells were rinsed with distilled water and acetone and saved for eggshell measurements. Eggshell thicknesses were measured with a micrometer (Starrett, Athol, MA) along the equator of each egg. The average of three measurements was recorded.

2.3. Chemical analysis

The analytical procedures used for the extraction, fractionation and cleanup of samples in the analyses of PBDEs, PCBs, and chlorinated pesticides in avian eggs follow GERG (Geochemical and Environmental Research Group, Texas A&M University) and others (Frank et al., 2001; Yogui and Sericano, 2009b; Taniguchi et al., 2009) standard operating procedures. Approximately 0.5–1 g of egg homogenate was mixed with anhydrous Na_2SO_4 and then extracted with methylene chloride using a homogenizer. Before extraction, 4,4′ dibromooctafluorobiphenyl, PCB 103 and PCB 198 were added as surrogate standards. Extracts were fractionated by partially deactivated silica:alumina column chromatography by eluting with a 1:1 mixture of pentane and methylene chloride. The fraction was further purified by high-performance liquid chromatography to remove excess lipids, and concentrated to a volume of 1 ml in hexane for gas chromatographic analysis.

PBDEs, PCBs and chlorinated pesticides were quantitatively analyzed by gas chromatography with mass spectrometric detection (HP-5890 and HP-5970-MSD) in the selected ion mode. All the sample extracts were injected in the splitless mode into a 30 m × 0.25 mm i.d. (0.25 μm film thickness) DB-5 fused silica capillary column (J&W Scientific) and temperature programmed for optimum resolution of analytes. For PBDEs, an initial temperature of 130 °C was held for 1 min and then ramped to 154, 210, and 300 °C at 12, 2, and 3 °C min^{-1}, respectively, with a final holding time of 5 min. For PCBs, an initial temperature of 75 °C was held for 3 min and then ramped to 150, 260, and 300 °C at 15, 2, and 20 °C min^{-1}, respectively. The oven was held at the higher temperature for 3 min. For chlorinated pesticides, the sample extracts were injected at an initial temperature of 100 °C and programmed to 200 at 10 °C min^{-1} and to 300 °C at 5 °C min^{-1}. The temperature was held at 300 °C for 3 min. In all cases, the temperature of the injector was 270 °C and the instrument was calibrated by injection of standard mixtures at four different concentrations prior to the analysis of the samples. Tetrachloro-*meta*-xylene was used as internal standard.

Method blanks, sample duplicates, and matrix spikes were processed with each sample preparation batch for quality control. The average percent recoveries of the internal standard used for quantitation (PCB 103) were 93.6 ± 33.1 and 71.7 ± 16.2 for PCBs and chlorinated hydrocarbons, respectively, and 85 ± 3.9 for surrogate compounds of PBDEs. The average recoveries of analytes in the spiked matrices were 100.8 ± 5.1 and 96.2 ± 10.3 for PCBs and PBDEs, respectively. The relative percent differences between the matrix duplicates were 4% for PCBs, 3.7% for PBDEs. The method detection limits ranged between 0.1and 0.2 ng/g ww for DDE, PCBs and PBDEs; they were calculated based on the lowest calibration solution and taking into consideration the sample weight and its final volume.

The following PCB congeners coeluted together **128**/162, 132/**153**/168, **138**/158, **146**/161, **160**/163/164, **170**/190, **180**/193, 182/**187**, **196/203** (the most predominant are shown in bold). The most commonly detected PBDE congeners were BDE-47, 99, 100, 153, 154, and 183; however, BDE-28, 49, and 138 also were detected in a few samples.

2.4. Statistical analysis

The regular data were not normally distributed as indicated by the PROC UNIVARIATE procedure and the Shapiro–Wilk's statistic, W; thus, contaminant data were log transformed to satisfy the assumptions of normality and equality of variance. The log transformed contaminant data were analyzed by PROC GLM ANCOVA with year as a covariate. Since year was not important, PROC GLM ANOVA was used to determine differences in concentrations of contaminants among regions. Type III statistics and Tukey comparison of means were used to determine which means were significant. Simple linear regression was used to determine the relationship between eggshell thickness and log p,p′-DDE. The level of significance was set at $P < 0.05$. All statistical analyses were conducted with the use of SAS® software (SAS Institute, Inc, Cary, NC, USA). To facilitate comparisons with the literature, concentrations of p,p′-DDE and PCBs are provided in μg/g wet weight, and PBDEs in ng/g lipid weight.

3. Results

Eggshell thickness, percent moisture, and geometric mean concentrations for p,p′-DDE, total PCBs, and total PBDEs in eggs are shown in Table 1. Of all the organochlorine pesticides analyzed only p,p′-DDE was detected at concentrations significantly above the detection limit in over 50% of the samples. Hexachlorobenzene, heptachlor, heptachlor epoxide, oxychlordane, γ chlordane, trans-nonachlor, mirex, and p,p′-DDT were all detected in a few samples (<than 50%), at levels near detection limits, with mean concentrations between 0.001 and 0.012 μg/g ww (not reported in Table 1). Mean geometric DDE concentrations were higher in eggs from the region of Tinaja Verde, Chihuahua; however, they were not significantly different from those from other regions in Chihuahua and Veracruz (Table 1). Geometric mean total PCB concentrations were significantly greater in Tinaja Verde, Chihuahua ($F_{3,19} = 4.5$, $P = 0.015$) than in El Sueco, but were not different from PCB concentrations in the Veracruz north and south regions. Fig. 2 shows the contribution of each PCB congener to the total PCB concentrations reported in eggs. The PCB congeners that contributed the most to total PCBs were, in order of predominance from higher to lower: 153, 180, 170, 138, 194, 196, 118, 182, 183, and 206. Congeners 153 and 180 combined contributed about 40% of total PCBs. Noticeably, the low molecular weight PCB congeners were not detected in eggs.

Mean total PBDE concentrations were significantly greater in falcon eggs from the two Veracruz regions ($F_{3,19}$, $P < 0.0001$) than in those from Tinaja Verde and El Sueco, Chihuahua. Eggs from Veracruz had, on average, twice as much total PBDEs as those from Chihuahua. Fig. 3 shows the contribution of each PBDE congener to the total PBDE concentrations reported in eggs. The most common PBDE congeners in eggs in order of predominance were: BDE-153, 99, 154, 47, 100, and 183. BDE congener 153 was the most abundant and contributed approximately 50% of the total BDEs. BDEs 28, 49, and 138 were detected in a few eggs at low concentrations.

Mean eggshell thickness ranged from 0.262 ± 0.014 mm for eggs from Veracruz North to 0.301 ± 0.041 mm for eggs from El Sueco, Chihuahua (Table 1). There was a poor ($R^2 = 0.04$) non-significant correlation between log-DDE and eggshell thickness from all the eggs collected in Mexico during 2004–2007.

4. Discussion

The concentrations of p,p′-DDE in eggs of aplomado falcons collected during 2004–2007 in El Sueco and Tinaja Verde in the state of Chihuahua were 1.5–6 times lower than those observed during the period 1977–2003 in the same regions (Mora et al., 2008). These results provide additional support to the suggestion that the use of DDT has diminished or stopped altogether in these

Table 1
Eggshell thickness (mm) and percent moisture in aplomado falcons from Mexico (expressed as arithmetic means ± SD). Geometric mean concentrations and ranges for p,p′-DDE and total PCBs (listed as μg/g wet weight). Total PBDEs (as ng/g lipid weight).[a]

Location	*n*	% Moisture	Eggshell Thickness	p,p′-DDE	Total PCBs	Total PBDEs
Chihuahua, El Sueco	7	75.7 ± 2.3	0.301 ± 0.041	0.55^{A} (0.19–5.15)	0.06^{B} (0.04–0.09)	101^{B} (62–197)
Chihuahua, Tinaja Verde	7	77.6 ± 2.2	0.293 ± 0.032	1.39^{A} (0.25–7.85)	0.39^{A} (0.06–2.80)	116^{B} (76–165)
Veracruz, North	4	80.2 ± 0.9	0.262 ± 0.014	0.38^{A} (0.25–0.67)	0.19^{AB} (0.17–0.26)	304^{A} (221–429)
Veracruz, South	5	78.0 ± 3.8	0.280 ± 0.047	0.86^{A} (0.13–5.51)	0.33^{AB} (0.16–1.33)	430^{A} (249–798)

[a] Within columns, means not sharing the same letter are significantly different.

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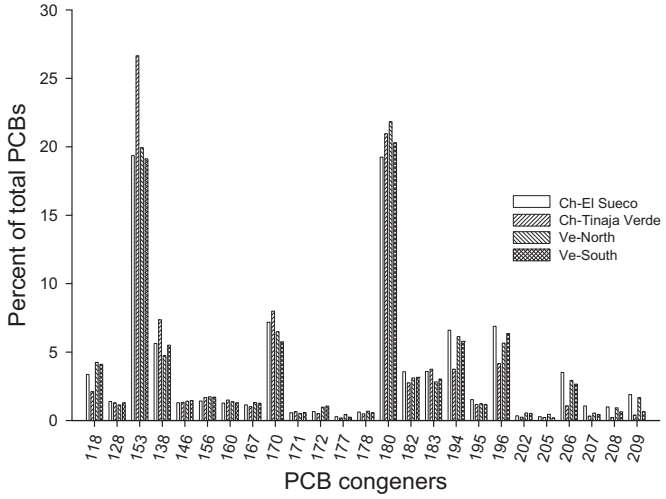

Fig. 2. PCB congener patterns in eggs of aplomado falcons from Chihuahua and Veracruz, Mexico.

regions (Chanon et al., 2003); consequently, an environmental decrease of DDE in biota should be expected. Surprisingly, mean DDE values in the region of Tinaja Verde were somewhat higher (although statistically non-significant) than in the El Sueco region; the opposite of what we observed in a previous study (Mora et al., 2008). This is also surprising because there is more agricultural development in the El Sueco region than in Tinaja Verde (Macías-Duarte et al., 2009). Approximately 30,000 ha of grasslands have been converted to agriculture since 2005 in the El Sueco area. Nonetheless, Macias-Duarte et al. (2004) found that aplomado falcon productivity was lower in the Tinaja Verde region (0.53 fledglings per occupied territory) than in El Sueco (0.94 fledglings per occupied territory). Mean DDE concentrations in eggs of aplomado falcons from Veracruz were similar to those from Chihuahua. The only contaminant data available for this species from Veracruz was reported by Kiff et al. (1980). DDE residues in 20 eggshells collected in Veracruz between 1957 and 1966 averaged 297 μg/g lipid weight (Kiff et al., 1980), approximately 14.8 μg/g wet weight. A similar DDE average was reported for seven eggshell fragments collected in 1977 (Kiff et al., 1980). These DDE values reported by Kiff et al. (1980) are 17–40 times greater than the values observed

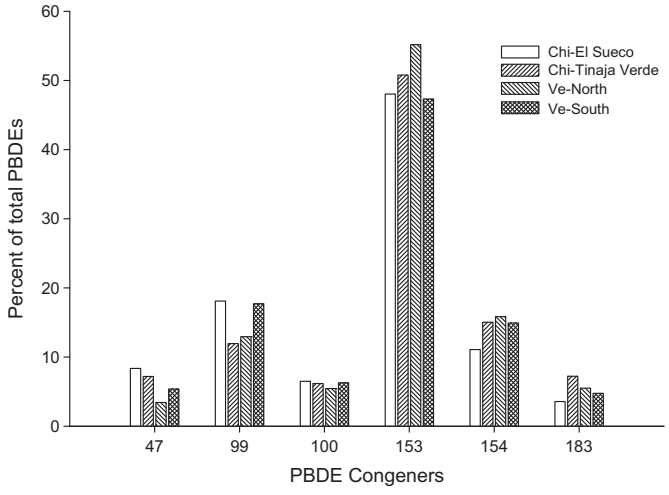

Fig. 3. PBDE congener patterns in eggs of aplomado falcons from Chihuahua and Veracruz, Mexico.

in the eggs we collected 30 years later in the same regions in Veracruz.

Except for the mean eggshell thickness values from Veracruz North, which were lower, all the other mean values were above the mean thickness of 0.279 mm reported for 20 aplomado falcon eggshells from pre-DDT clutches (Kiff et al., 1980). The mean eggshell thickness values reported in our study also were over 20% thicker than those reported in 1977. The above suggests that the currently observed levels of p,p′-DDE in eggs of aplomado falcons in Chihuahua and Veracruz are probably not affecting thickness or reproduction of the species. This is further supported by the poor and non-significant correlation between log-DDE and eggshell thickness from all the eggs collected in Mexico during 2004–2007. The lowest dietary DDE concentrations at which adverse effects have been observed in raptor species range from 1 to 3 μg/g ww (Wiemeyer and Porter, 1970; Enderson et al., 1982; Mendenhall et al., 1983). Enderson et al. (1982) indicated that peregrine falcons (*Falco peregrinus*) feeding on prey with DDE levels as low as 1 μg/g ww were expected to produce thin-shelled eggs. Unfortunately, we did not collect potential prey of the aplomado falcon to analyze for contaminants; however, the lowest observed DDE concentrations in eggs of raptors associated with eggshell thinning are close to 7 μg/g ww (Enderson and Wrege, 1973), about 5 times greater than the highest mean concentrations reported in our study.

PCBs were notably low reflecting minimal exposure to such contaminants, given that the study locations were away from industrial sources, although the birds from Veracruz were presumably closer to such sources. Kiff et al. (1980) also reported low levels of PCBs in two eggs of aplomado falcons collected in Veracruz in 1977. Unfortunately, there is basically no information about PCB concentrations in eggs or other tissues of wildlife in eastern Mexico. Thus, we were unable to compare the PCB residues with other local species. Nonetheless, the PCB levels reported in our study were much lower than those reported in eggs of raptors from the United States, Canada, and Europe. Total PCBs in two aplomado falcon eggs collected in 1995–1996 in the Lower Rio Grande Valley, Texas, ranged from 0.50 to 1.52 μg/g ww (Mora et al., 1997) within the range of the values observed in our study. The PCB values were also similar to those reported in eggs collected in Chihuahua during 1997–2003, but 2–3 times lower than those observed in the Lower Rio Grande Valley during 1999–2003 (Mora et al., 2008). Overall, the PCB levels were much lower than those observed in eggs (4–18 μg/g ww) of experimental raptors at which no adverse effect levels were observed (McLane and Hughes, 1980).

The PBDE values reported in eggs of aplomado falcons, to our knowledge, are among the first to be reported in raptor species in Mexico. Mean PBDE concentrations in aplomado falcon eggs from the four Mexican regions were lower than those observed in cliff swallows (*Petrochelidon pyrrhonota*, mean = 3582 ng/g lw) from the U.S.–Mexico border at El Paso, Texas, during approximately the same time period (Mora, unpublished data). This difference may be explained by the fact that the swallow samples were collected from locations in or near the cities of El Paso and Ciudad Juarez, along the U.S.–Mexico border; thus, the swallows were much closer to industrial sources than the falcons. The PBDE congener pattern in eggs of aplomado falcons was similar (though not identical) to those found in other raptors (most notably peregrine falcons), some terrestrial mammals, seabirds, and insectivorous songbirds (Chen and Hale, 2010). The PBDE concentrations in aplomado falcon eggs were much lower than mean PBDE values reported in eggs (2800 ng/g lw) of peregrine falcons in Sweden (Johansson et al., 2009) and in various other raptors from Europe (Voorspoels et al., 2006; Chen and Hale, 2010). Mean PBDE residues in eggs of aplomado falcons from Mexico were also approximately 10–33 times

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lower than mean residues in eggs of peregrine falcons from Chesapeake Bay (median = 2300 ng/g lw; Potter et al., 2009; Chen et al., 2010), the northeastern U.S. (mean = 7400 ng/g lw, Chen et al., 2008), and California (mean = 7850 ng/g lw; Holden et al., 2009). Unfortunately, we did not analyze for BDE-209 in our samples, thus, we are unable to compare concentrations of this congener (Deca mixture) with data from other species.

Our PBDE results suggest that the aplomado falcons in Veracruz were closer to PBDE sources than the populations in Chihuahua. It has been indicated that a more piscivorous diet may result in higher PBDE levels due to the propensity of bioaccumulation of PBDEs in fish (de Wit, 2002; Johansson et al., 2009); however, aplomado falcons are not known to feed on fish. The particular abundance of BDE-153 (about 50% in aplomado falcon eggs) seems to be rather common over many different species and habitats, which is surprising since it is not a major constituent of any of the popular PBDE technical formulations (La Guardia et al., 2006). It has been suggested that elevated concentrations of BDE-153 are most likely the result of metabolic breakdown of BDE-209; BDE-153, is disproportionately retained in the bodies of raptors and perhaps other organisms that they may feed on (Lindberg et al., 2004; Van den Steen et al., 2007; Johansson et al., 2009). Variation between congeners in this and other studies could be the result of differing levels of PBDE contamination as well as the differential presence of metabolized PBDE products. As indicated in Fig. 3 it appears that overall the four regions the proportional relationship between detected PBDE congeners is generally the same. Overall, the levels of PBDEs in eggs of aplomado falcons from Mexico were much lower than the reported lowest-observed-effect-level (LOEL), approximately 32 μg/g egg lw (McKernan et al., 2009); although Henny et al. (2009) have suggested that a much lower concentration, 1 μg/g ww (approximately 17.5 μg/g lw) could affect reproduction.

5. Conclusion

The aplomado falcon populations in north and eastern Mexico currently are not exposed to elevated concentrations of POPs. Despite concerns about the recent and past use of DDT in Mexico, the data indicate that, during the study period, DDE did not accumulate in eggs at levels that could be associated with eggshell thinning or reproductive effects. PCBs and PBDEs were much lower than those observed in raptors from industrialized countries. While the amounts found in these eggs are less than those found in the tissues of cliff swallows from Texas, the differences in total PBDE levels between eggs from Chihuahua and Veracruz merits some further studies into the local sources of PBDEs in these regions. BDE congeners found in these aplomado falcon eggs seem to show some isomers of the penta-formulation in addition to prominent amounts of BDE-153, which may be a product of metabolization of other, heavier PBDEs like BDE-209. This pattern of congeners is not uncommon in North America or Europe and has been recorded in numerous species. Overall, this study contributes to the CEC monitoring program for assessing pollutants across North America and provides information about biomagnification of persistent organic pollutants in raptor species in Mexico. The PBDE data are about the first to be reported in a raptor species from Mexico. This information should be useful to further identify trends and concerns regarding potential impacts of POPs on wildlife in Mexico.

Acknowledgments

This research was supported in part by the U.S. Geological Survey, the U.S. Fish and Wildlife Service, and the Peregrine Fund, Inc. We appreciate the assistance of A. Macias-Duarte and D. McCauley for locating nests and egg collection. This manuscript has benefited from comments by G. Hunt and two anonymous reviewers.

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