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Organochlorine Chemical Residues in Northern Cardinal (*Cardinalis cardinalis*) Eggs from Greater Washington, DC USA

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Abstract

Northern Cardinal eggs from six neighborhoods near Washington DC were analyzed for organochlorine pesticides and PCBs. All compounds were detected more frequently and at higher concentrations in more heavily urbanized neighborhoods. DDT (mostly as *p,p′*-DDE) was detected in all neighborhoods. *p,p′*-DDT was typically 0.5–16 ng/g (ww) in most suburban neighborhoods but was not detected (< 0.1 ng/g) in more rural areas; however, *p,p′*-DDT was 127–1130 ng/g in eggs from two suburban Maryland nests and comprised 65.7% of total *p,p′*-DDT isomers in the most contaminated sample, indicating recent exposure to un-weathered DDT. Total chlordane (sum of 5 compounds) was 2–70 ng/g; concentrations were greatest in older suburban neighborhoods. Total PCB (sum of detected congeners) was < 5–21 ng/g. Congener patterns were similar in all neighborhoods and resembled those typical of weathered mixtures. Results indicate that wildlife remains exposed to low concentrations of legacy contaminants in suburban neighborhoods and that cardinal eggs can be used to monitor localized contamination.

Keywords Organochlorine pesticides · Polychlorinated biphenyls · Chlordane · Birds

The contamination of urban and suburban environments by industrial chemicals, consumer products, and pesticides is widely recognized. Anthropogenic chemicals were detected in 80% of 139 US streams draining urban, industrial, suburban, and agricultural areas (Kolpin et al. 2002), and polychlorinated biphenyls (PCBs) and pesticides were detected in streams of seven US metropolitan areas (Nowell et al. 2013). Birds inhabiting urbanized areas may be exposed to chemicals presently or formerly used in and around homes and other buildings, in transportation and utility corridors (including spills), or released from landfills and hazardous waste sites. Persistent organic pollutants (POPs) typically associated with urban and suburban environments include legacy organochlorine pesticides (OCPs) and PCBs, which are ubiquitous, bioaccumulative pollutants that are toxic to many species (Darnerud 2003; Chen et al. 2010; Beyer and Meador 2011).

Although most OCPs are no longer used and PCBs are not manufactured, large quantities of these compounds persist in many places as a result of historical use and disposal, especially near former sites of synthesis, formulation, and storage (e.g., Secord et al. 1999; Hinck et al. 2009; Bienkowski 2014; Mackintosh et al. 2016). OCPs were widely used in the United States to control insects in agriculture, forests, wetlands, and suburban neighborhoods and are present in and around homes (Bekarian et al. 2006; Stapleton et al. 2005). Suburban DDT uses in the United States from 1939 to 1972 included mosquito fogging of neighborhoods and spraying to control insects on fruit and ornamental trees (USEPA 2018a). Chlordane was incorporated into soils around buildings for ant and termite control from 1948 until its use was restricted in 1988 (ATSDR 1994). PCBs were used in many applications including dielectric

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fluids, plasticizers, and lubricating and cutting oils (USEPA 2018b). Masonry and concrete buildings erected from the 1940s through the 1970s commonly had PCBs in paints (at 5%–10% concentration) and in caulks and adhesives (10%–20%; e.g., Priha et al. 2005). PCBs also were used in home electrical equipment, including the ballasts of fluorescent light fixtures (USEPA 2018b).

Comparatively little is known about the health or long-term survival of birds residing in urban and suburban areas, where they are exposed to a wide variety of potentially harmful anthropogenic contaminants. To date, lead from gasoline combustion has been the primary focus of most studies investigating the effects of urban pollution on birds (e.g., Roux and Marra 2007; Scheifler et al. 2006). Studies of organic chemicals in birds inhabiting urban areas have mainly been focused on larger, upper trophic level species (e.g., Chen et al. 2010; Gao et al. 2009; Elliott et al. 2015). Studies completed in Europe, India, and South Africa have documented organohalogen concentrations in the eggs of several resident passerine species (Bouwman et al. 2013 and references therein), and European Starling (*Sturnus vulgaris*) eggs have been proposed for international monitoring (Eens et al. 2013). Eens et al. (2013) sampled starlings at one site in Illinois, but studies of organic chemicals in the eggs of North American passerine birds have mainly focused on releases from hazardous waste sites and pesticides in agricultural areas, and most have been conducted in wetlands and riparian corridors (e.g., Bishop et al. 1995; Hebert et al. 1994; Harris et al. 2000; Reynolds et al. 2001; Secord et al. 1999; Echols et al. 2004). Exposure of passerine birds inhabiting urban and suburban areas of the United States to bioaccumulative organic chemicals has received less study, and exposure to contemporary concentrations is unknown.

The Northern Cardinal (*Cardinalis cardinalis*; henceforth cardinal) is a non-migratory species indigenous to most of eastern and central North America. Cardinals commonly inhabit urban and suburban areas, where they forage primarily on fruits and seeds augmented with insects and other invertebrates during the spring-summer nesting season. Their diet may include sunflower and other seeds from bird feeders. Pairs establish and defend relatively small (0.21–2.60 ha) home ranges during the nesting season but may forage over several kilometers during the winter (Halkin and Linville 1999). Contaminant exposure of cardinals can therefore be considered localized compared to wider-ranging species.

In this paper we report the concentrations of OCPs, PCBs, and several other chlorinated industrial compounds in cardinal eggs collected from nests in the greater Washington, DC, USA (WDC) area. The study was conducted as a pilot for future avian monitoring of urban and suburban neighborhoods. Objectives were to document contemporary exposure and accumulation of selected POPs by birds residing in such areas and to establish a benchmark against which to compare future data. A secondary objective was to document lipid content, wet egg mass, and egg length and width as potential indicators of biological effects of chemical exposure.

Materials and Methods

Cardinal eggs were collected in May 2008 from 31 nests located within 6 neighborhoods in greater WDC chosen to represent a gradient of urbanization and population density (Fig. 1). The neighborhoods were located in Arlington, VA (AR, densely populated suburban); Takoma Park, MD (TP, densely populated suburban); Greenbelt, MD (GB, managed suburban); Dunkirk, MD (DK, suburban); West Friendship, MD (WF, rural suburban); and White Hall, MD (WH, rural). Within each neighborhood, 5 or 6 first-brood nests separated by at least 100 m were located and checked every 2–3 days. One recently laid egg with intact shell was collected from each nest and transported to the laboratory on ice in clean, labeled containers. Fresh egg length, width, and mass were measured and the egg contents were dispensed into chemically cleaned, tared jars (I-CHEM® 340-0120) and confirmed to be in an early developmental stage (i.e., no visible embryo). All samples were immediately chilled (0 °C), shipped overnight on ice to the analytical laboratory, and stored frozen (− 16 °C) until thawed for analysis.

A 1-g aliquot of each egg sample was extracted with a pressurized liquid extraction system (Dionex ASE 200; Echols et al. 2013). A 0.1 g-equivalent portion of the extract was removed for gravimetric lipid determination. Post-extraction

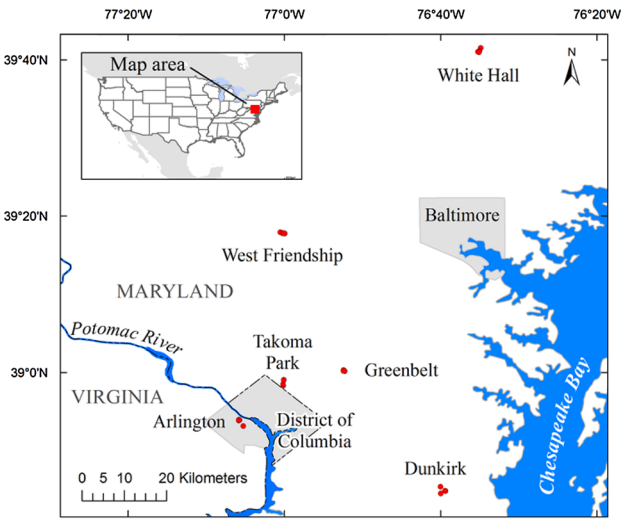

Fig. 1 Map of greater Washington, DC, USA showing the locations of sampled nests (red symbols) in Arlington, VA ($n=6$), and Takoma Park, Greenbelt, Dunkirk, West Friendship, and White Hall (all MD, $n=5$)

cleanup included flash gel permeation chromatography and fractionation with flash alumina chromatography (Peterman et al. 2006, 2009). PCB congeners (n = 141) and the least polar OCPs were analyzed with a gas chromatography/quadrupole mass spectrometry (GC/QMS) system with selected ion monitoring (SIM) and cool injection technique (Thermo Trace MS system). Limits-of-detection (LODs) were 0.3–5.5 ng/g wet-weight (ww) for PCB congeners, 0.9 ng/g for hexachlorobenzene (HCB), and 1.0–3.1 ng/g for pentachlorobenzene, octachlorostyrene, heptachlor, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, and mirex. More polar OCPs (e.g., dieldrin, heptachlor epoxide) were analyzed by GC/high-resolution (HR) MS (GC/HRMS; Waters Autospec M) with HRSIM (Echols et al. 2013). LODs were 0.2 ng/g for methoxychlor, 0.1 ng/g for *p,p′*-DDT and *p,p′*-DDD, and 0.01–0.10 ng/g for all other compounds. Quality assurance measures for all analyses included procedure blanks and fortified egg matrices. All samples contained method surrogate quantification standards. Recoveries were considered acceptable for all analyses and were typically 40%–110%. Endosulfan-1 and α- and γ-benzene hexachloride (BHC) were not quantified due to low recovery. Analytical results are reported in ng/g wet-weight (ww) and were not corrected for recovery. Chemical identities, additional information about analytical methods, QA, and LODs for all analytes are available online (Schmitt et al. 2018) along with raw data for detected compounds and lipid content; shell thickness; and fresh egg length, width, and mass for each sample.

Statistical analyses were conducted with V. 9.4 of the Statistical Analysis System (SAS Institute, Cary, NC). For residue concentrations that were > LOD in > 75% of the samples (few), 50% of the LOD was substituted for censored values and in OCP group totals. Total PCB and chlordane concentrations are reported and were analyzed as the sum of detected congeners. Differences among neighborhoods were analyzed with the nonparametric Kruskal–Wallis test and Dwass, Steel, Critchlow-Fligner post-hoc comparisons (SAS PROC NPAR1WAY) because most variables failed to meet the distributional and other assumptions of parametric methods. A p-value of < 0.05 was used to judge the significance of all statistical tests unless otherwise indicated.

Results and Discussion

Among the biological variables, only lipid content differed significantly among neighborhoods (p = 0.044). Mean lipid content was 5.8%–5.9% in eggs from AR and TP, the two most urbanized areas, and 8.2%–8.8% elsewhere. Lipid differences may reflect nutritional status/diet quality of the nesting females or egg age (Rattner et al. 2016); however, the latter is unlikely considering that all eggs were in early developmental stages. Among-neighborhood differences for fresh egg mass, egg length, and egg width were not significant (p = 0.16–0.96).

Concentrations of most residues generally were greatest in neighborhoods near WDC (TP, GB, and AR) and smallest in more rural neighborhoods. Residues of DDT (mostly as *p,p′*-DDE) were detected (> 0.2 ng/g) in eggs from 26 of 31 nests (84%); *p,p′*-DDT and *p,p′*-DDD were detected (> 0.1 ng/g) less frequently (55% and 29%, respectively). Total DDT (sum of *p,p′*-isomers) and *p,p′*-DDE differed significantly among neighborhoods (p = 0.028–0.036). Concentrations were greatest in GB and TP, but concentrations and isomer profiles varied greatly within and among neighborhoods (Fig. 2A). The *p,p′*-isomer composition was > 87% *p,p′*-DDE in most samples with detectable *p,p′*-DDT and *p,p′*-DDE, with two notable exceptions: The egg from TP Nest 100 contained 715 ng/g of total *p,p′*-DDT isomers comprising 76.5% *p,p′*-DDE and 127 ng/g of *p,p′*-DDT (17.8%); and GB Nest 49 contained 1719 ng/g of total *p,p′*-isomers comprising only 32.2% *p,p′*-DDE (Fig. 2A). This sample contained 1130 ng/g of *p,p′*-DDT (65.7%), which was confirmed by full-scan GC/QMS (Fig. 3). Total *p,p′*-isomer concentrations were lower (2.2–29.2 ng/g) in other samples with proportionally high concentrations of *p,p′*-DDT (Fig. 2A). *o,p′*-DDT, an impurity of technical DDT, was detected in two samples: TP Nest 100 contained 2.8 ng/g of *o,p′*-DDT, which is consistent with its 715 ng/g total *p,p′*-isomer concentration (Fig. 2A). In contrast, the egg from DK Nest 40 contained 21.7 ng/g of *o,p′*-DDT but did not contain high concentrations of any other analytes. The identity of this residue is uncertain because it was not confirmed by full-scan GC/QMS.

Technical DDT comprises 65%–80% *p,p′*-DDT, which is rapidly metabolized to *p,p′*-DDE by birds. Consequently, *p,p′*-DDE is typically the most abundant DDT homolog in bird eggs (Blus 2011 and references therein). As such, the presence of proportionally high concentrations of *p,p′*-DDT indicates relatively recent exposure of animals to DDT (Aguilar 1984). Although insecticidal use of DDT in the United States has been restricted since 1974, the GB and TP homolog mixtures indicate recent exposure to un-weathered DDT. Potential sources of technical DDT (and hence *p,p′*-DDT) include illicit use (including pesticide treatment of birdseed) or containers stored outdoors or in outbuildings. The latter could contaminate co-located bird seed or insects eaten by the cardinals. Residual DDT from historical use is unlikely given the proportionally high *p,p′*-DDT concentrations. We also note that GB and TP are near a hazardous waste site where elevated concentrations of DDT, other pesticides, and PCBs have been reported (USEPA 2017).

Eggshell thinning and reproductive effects in birds have been attributed to *p,p′*-DDE (Blus et al. 1997). In the field and laboratory studies reviewed by Blus (2011), the smallest effect concentrations were 1–2 µg/g (1000–2000 ng/g ww),

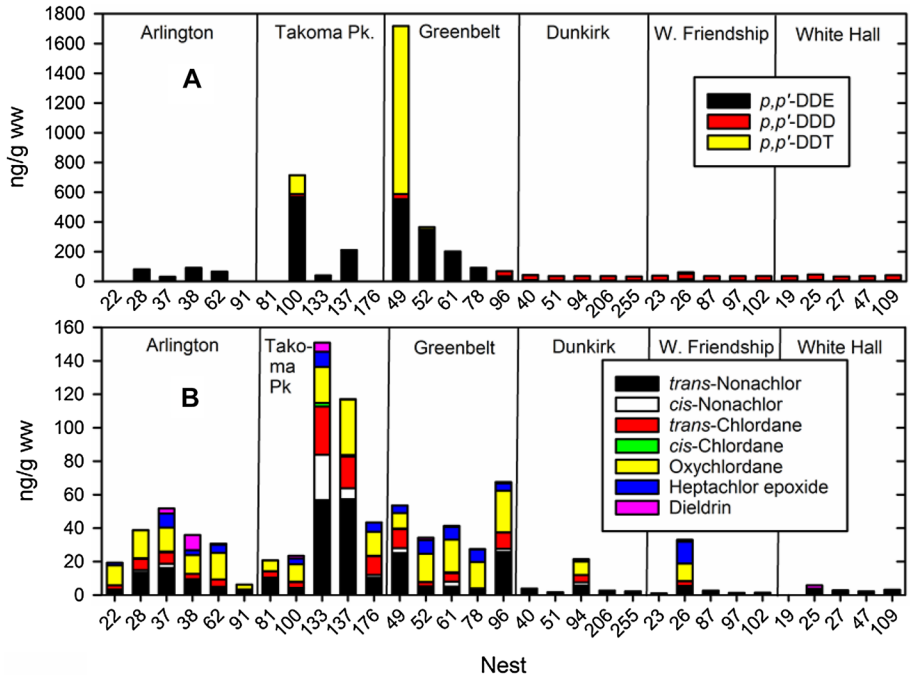

Fig. 2 Concentrations of DDT isomers (**A**) and cyclodiene pesticides (**B**) in Northern Cardinal eggs from greater Washington, DC, USA neighborhoods (censored values represented as 50% LOD)

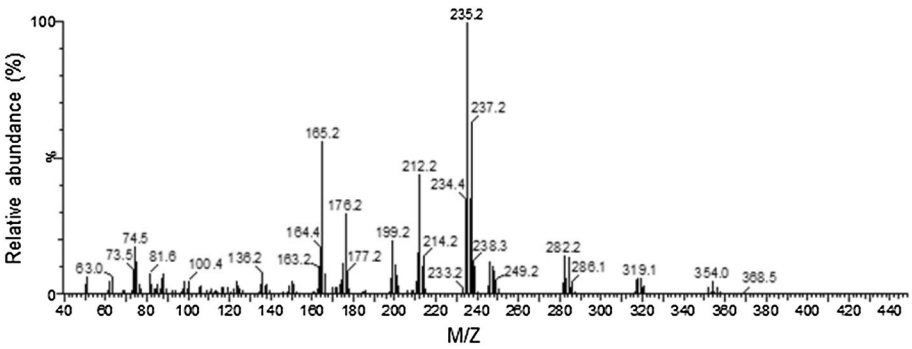

Fig. 3 Full-scan GC/QMS mass spectrum of compounds detected in the alumina fraction from Greenbelt Nest 49, which was a near-perfect match to a *p,p′*-DDT standard

but they varied by more than 10-fold among species and no passerine birds were evaluated. In our study, the greatest *p,p′*-DDE concentration (589 ng/g ww) was less than known thresholds (Blus 2011). Nevertheless, *p,p′*-DDE would have been 1720 ng/g ww if all the *p,p′*-DDT the GB egg had been metabolized to *p,p′*-DDE, which would exceed the lowest thresholds in species evaluated by Blus (2011). In addition, the residues in these eggs, together with those of related compounds not included in our analyses, could also be toxic to birds through mechanisms other than eggshell thinning (e.g., Mackintosh et al. 2016).

Cyclodiene pesticides were detected in all 31 samples (Fig. 2B). Total chlordane (sum of *cis*- and *trans*-chlordanes and nonachlors, oxychlordane, and heptachlor epoxide) differed significantly among neighborhoods ($p = 0.001$), as did concentrations of all components ($p < 0.001$–0.002) except heptachlor epoxide ($p = 0.093$). No un-metabolized heptachlor was detected. Although there were exceptions, total chlordane and all components were greatest in the urbanized neighborhoods near WDC (AR, TP, and GB; Fig. 2B). Most total chlordane concentrations were < 2.0 ng/g; however, TP Nests 133 contained 143 ng/g and Nest 137 contained 117 ng/g. Oxychlordane, a metabolite of *cis*-chlordane (the primary active insecticide in technical chlordane), was detected in all samples; *cis*-chlordane was detected in 25 samples (80.6%). *trans*-Chlordane and -nonachlor were also detected in all samples. Aldrin was not analyzed because it is rapidly metabolized (to dieldrin) and is seldom detected in vertebrates, but dieldrin was detected in 15 samples (48%) and in at least one sample from all neighborhoods. Most dieldrin concentrations were < 2.0 ng/g but were 9.1 ng/g in AR Nest 38 and 5.5 ng/g ww in TP Nest 133. This sample also contained the greatest total chlordane concentration (Fig. 2B). Traces of endrin (0.1 ng/g) were detected in one DK sample.

Chlordane, heptachlor, and aldrin/dieldrin were used to control ants and termites in and around buildings and in agriculture to control soil insects. Heptachlor is also a

minor component of technical chlordane. The residue patterns reflect residential uses; concentrations were generally greatest in densely populated neighborhoods located nearest to WDC. The profiles were dominated by longer-lived components and metabolites (e.g., oxychlordane and nonachlors; Fig. 2B). The presence of cyclodiene residues in less densely populated neighborhoods could also reflect recent urbanization of agricultural areas.

Cyclodiene insecticides (especially endrin, dieldrin, and oxychlordane) are highly toxic to birds (Elliott and Bishop 2011). Because they are neurotoxins, many toxicity thresholds for OCP residues are based on brain concentrations; there is comparatively little information about toxic effects associated with residues in eggs for many compounds, especially in passerine species. An additive model of pesticide toxicity ("dieldrin equivalents") for cyclodiene pesticides and DDT has been proposed (Elliott and Bishop 2011). Using the equivalency factors of this model and a brain:egg ratio of 0.27 [based on data from gulls (Vermeer and Reynolds 1970)], the maximum estimated dieldrin-equivalent concentrations in cardinal brains were 20.3–30.7 ng/g (0.020–0.031 µg/g), which are two orders of magnitude lower than the lethal brain concentrations in the studies reviewed by Elliott and Bishop (2011). However, the authors caution that the brain:egg conversion factors and the additive model are based on data from few species, and that these compounds may be toxic to birds via other mechanisms (e.g., Mackintosh et al. 2016).

Traces of mirex, which was formerly used as a flame retardant and as a soil insecticide, were detected (> 2.0 ng/g) in 11 samples (36%) and in at least one sample from all neighborhoods at 2.1–2.6 ng/g. Other compounds detected at low concentrations included pentachloroanisole, a metabolite of the wood preservative pentachlorophenol (n = 8); the herbicide Dacthal© (n = 1); and the insecticide β-BHC, the most persistent BHC isomer (Gao et al. 2009). Endosulfan (-2 and sulfate), δ-BHC, pentachlorobenzene, hexachlorobenzene, and octachlorostyrene were not detected in any samples.

At least one PCB congener was detected in 24 of 31 samples (77%), but no sample contained more than 17 chromatographic peaks (Fig. 4). Concentrations were greatest in neighborhoods near WDC (AR, TP, and GB) and smallest in more rural neighborhoods (Fig. 4), and all samples with no detections were from the more rural neighborhoods (three each from WH and WF, one from DK). Of the 122 chromatographic peaks representing 141 PCB congeners included in our analysis, only 25 peaks representing 31 congeners exceeded LODs (0.6–1.3 ng/g). No congeners containing fewer than five chlorines were detected in any sample. The most frequently detected congeners were PCBs 153/132 (71%), 187 (68%), 118 (48%), 138/158 (39%), 180/193 (39%), and 199 (22%), which collectively accounted for 62%–100% of total detected PCBs in samples with at least one detected congener (Fig. 4). The number of detected congeners increased with total PCB concentrations, which ranged from < LOD to 43.6 ng/g. Although total PCB concentrations varied substantially among nests, differences among neighborhoods were nevertheless significant (p = 0.001). Congeners detected at the greatest concentrations were PCBs 153/132 (13.4 ng/g), 138/158 (7.4 ng/g), 118 (5.9 ng/g), 172 (5.8 ng/g) and 180/193 (5.8 ng/g ww). PCB 118 (2,3′,4,4′,5-pentachlorobiphenyl) is one of only two dioxin-like congeners resolved by our analysis; it was detected in most samples from neighborhoods located near WDC (Fig. 4). The other was PCB 105 (2,3,3′,4,4′-pentachlorobiphenyl), which was detected (0.62–1.47 ng/g) in two samples from GB. The largest concentration of PCB 105 occurred in the sample containing the greatest concentration of PCB 118 (5.9 ng/g). Together these concentrations represent the toxic equivalent of 0.21 pg/g (ww) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin based on consensus avian toxic equivalency factors (van den Berg et al. 1998), which is very low.

Overall, PCB concentrations were low and comprised recalcitrant congeners containing five or more chlorines. The profiles are typical of weathered PCBs in birds (Harris and Elliott 2011 and references therein; Maldinado et al. 2016), and the maximum total concentrations (46.3 ng/g)

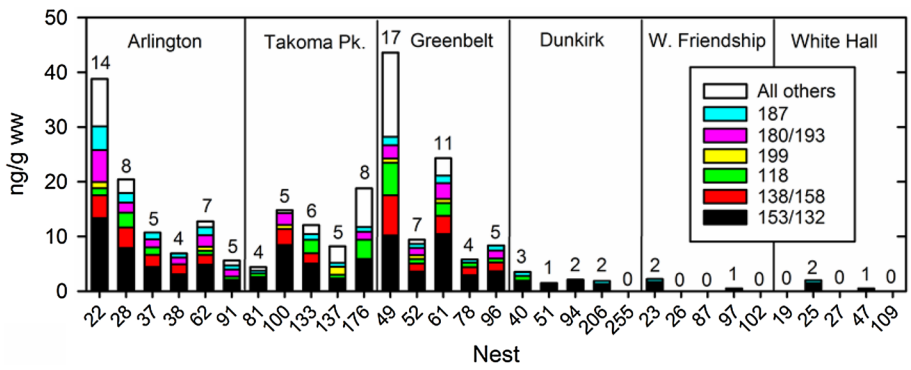

Fig. 4 Numbers and concentrations of detected polychlorinated biphenyl congeners in Northern Cardinal eggs from greater Washington, DC, USA neighborhoods

and dioxin-equivalent concentrations (0.21 pg/g) are less than known toxic thresholds for birds and concentrations in bird eggs from contaminated sites (Secord et al. 1999). However, we cannot discount the potential contribution of other unmeasured congeners or structurally similar compounds (e.g., chlorinated dioxins and furans) that may be present at very low concentrations. In addition, the consensus avian TEFs are dated, and more recent research has revealed substantial among-species differences in sensitivity to dioxin-like compounds (e.g., Farmahin et al. 2012; Hwang et al. 2016).

In summary, OCP and PCB concentrations were greater in cardinal eggs from urbanized areas near WDC than rural areas, but concentrations were generally low. These results are consistent with recent reports (Mora et al. 2016; Maldinado et al. 2016) indicating that concentrations of OCPs and PCBs in passerine and other resident and migratory birds have declined in recent decades. Nevertheless, concentrations were higher than most in some nests and the within-neighborhood variability was much greater than we expected, indicating that the neighborhoods and nearby areas are not uniformly contaminated. Samples with elevated concentrations of *p,p′*-DDT were especially noteworthy in this regard. Within-clutch differences may contribute to this variation, but the differences are probably diet-related (Van den Steen et al. 2006). These localized differences, which no doubt reflect the cardinal's small home range and feeding site fidelity, support its use for spatial and geographic trend assessments and the identification of "hot spots". Concentrations may also be greater in species occupying higher trophic levels or that are more closely associated with soil. Our results and those of others (e.g., Bienkowski 2014) indicate that birds inhabiting these areas remain exposed to DDT and other POPs. Future studies might therefore consider measuring eggshell thickness.

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