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Organic Chemical Concentrations in Eggs and Nestlings of Cavity Nesting Birds at and around Los Alamos National Laboratory

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Abstract

In 1943, Los Alamos National Laboratory (LANL) was established as part of the Manhattan project to design atomic weapons. LANL now operates as a multidisciplinary research institution. As part of an ongoing assessment of site-related ecological risk, organochlorine pesticides, their metabolites, polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents (TEQs) were evaluated in western bluebird (*Sialia mexicana*) and ash-throated flycatcher (*Myiarchus cinerascens*) eggs relative to a developed but non-industrial reference area; PCBs and TEQs were also evaluated in nestlings. Chemicals were below detection limits in the majority of samples. Western bluebird eggs collected from the study area had significantly lower concentrations of dieldrin, oxychlordane, and *trans*-nonachlor when compared with eggs from the reference area. No differences were observed in concentrations of dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethane (DDT), and heptachlor epoxide. Ash-throated flycatcher eggs contained higher total TEQ concentrations when compared with western bluebird eggs; however, no differences in concentrations of DDE, DDT, dieldrin, or total PCBs were observed. No differences were observed in total PCBs or TEQs in nestlings between the two species. Western bluebird eggs contained higher levels of total PCBs and TEQs when compared with nestlings; no differences were observed in total PCBs or TEQs between ash-throated flycatcher eggs and nestlings. Chemical concentrations detected in eggs of both species were below levels that are associated with adverse effects reported in the scientific literature, suggesting that concentrations of organic chemicals observed here appear to be at levels causing negligible risks to local bird populations.

Keywords: Biomonitoring; Organochlorine pesticides (OCPs); Polychlorinated biphenyls (PCBs); Polycyclic aromatic hydrocarbons (PAHs); Dioxin equivalents (TEQ)

Introduction

Los Alamos National Laboratory (LANL), located in Los Alamos, New Mexico, was established in 1943 as part of the Manhattan Project to design and build atomic weapons. LANL currently operates as a multidisciplinary research and development institution with research topics spanning widely across science and technology fields. Operations and management of the site have resulted in the release of nonradioactive chemicals such as organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). Sources of these organic chemicals include usage and storage of transformers, power and water treatment plant outfalls, storm drains, asphalt paving, roofing tar, burning of materials, and routine pesticide spraying and storage [1-3]. Detectable concentrations of OCPs, PAHs, and PCBs have been recorded in soil samples collected from material disposal areas and from canyon bottoms across the current and historical boundaries of LANL; some of these levels exceed concentrations in locations not impacted by LANL operations, including PCBs, PAHs, and methoxychlor (OCP) [1-3]. Environmental monitoring at LANL is extensive and monitoring programs have been in operation since the 1970s. Organic chemicals and inorganic elements, including radionuclides, have been routinely monitored in soil and sediment, and in biota such as fish, small mammals, and large mammals (typically road killed deer and elk) [4]. These environmental media have been collected from LANL locations and from background locations for comparisons and to evaluate ecological risk. Dioxin-like congeners are routinely evaluated and include a dozen coplanar PCB congeners, which induce similar biological effects as those caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure [5]. Toxic equivalency factors (TEFs) have been developed for TCDD-like compounds [6] and can be used to determine the relative potency, or toxic equivalents (TEQs), of dioxin-like compounds [5]. Assessing TEQs may better determine dioxin-like risk [6].

Assessing concentrations of organic chemicals is of general interest as many are persistent in the environment, are lipophilic, have tendencies to bioaccumulate, and often biomagnify in the foodchain [7]. Specifically, evaluating organic chemical concentrations in avian tissues is of interest because of the known adverse effects these chemicals have on birds. Adverse effects include endocrine disruption, behavioral alterations, reduced reproductive success, and mortality [8-11]. Avian eggs are useful as bioindicators for several reasons: collection for analysis is generally nondestructive to populations when non-viable eggs are collected, bird species occupy many trophic levels, and many species live within close proximity to humans [12-14]. Avian eggs have sometimes been shown to reflect local contaminant exposure where a female was feeding during egg formation [12]; however, during egg formation when lipid stores are depleted, stored body burdens can become mobilized [15] and therefore can also reflect a previous exposure, such as those exposures during migration or at wintering grounds [14,16]. Additionally, as many organic chemicals are lipophilic, avian eggs are useful bioindicators of environmental conditions because of their high fat content [10].

The western bluebird (*Sialia mexicana*) and the ash-throated flycatcher (*Myiarchus cinerascens*) were selected as bioindicators of

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organic chemicals at and around the LANL area because these birds are common in the study area [17] and readily nest in manufactured nest boxes. Both species are primarily insectivorous during the breeding season; western bluebirds typically feed on ground dwelling insects while ash-throated flycatchers primarily feed on aerial insects; however, both feed on ants, wasps, and beetles [18,19]. During the winter, both species rely on fruits and western bluebirds also consume seeds [19-21]. These species have similar life histories; however, ash-throated flycatchers have a higher metabolism and their nestlings fledge approximately four to five days earlier than western bluebirds [22]. Ash-throated flycatchers are migratory and have a winter range spanning from Mexico to Costa Rica [23], while western bluebirds are known to reside year round in some locations [19], have been observed wintering in New Mexico, and are more abundant than ash-throated flycatchers during the breeding season.

As part of an ongoing assessment of site-related ecological risk at LANL [4] and to determine any risks associated with organic chemicals in avian species, organic chemical concentrations were assessed in eggs and nestlings of western bluebirds and ash-throated flycatchers at LANL. Here we 1) evaluate concentrations of organic chemicals in western bluebird eggs in the study area and hypothesize that eggs collected within the current and historic LANL boundary will have higher concentrations than those collected at a developed but non-industrial reference site, 2) evaluate concentrations of organic chemicals in eggs and nestlings of western bluebirds and ash-throated flycatchers and hypothesize that ash-throated flycatchers eggs will have differing concentrations due to their migratory behavior, but that no differences will be observed in nestlings, and 3) compare concentrations of organic chemicals in eggs with nestlings in each species and hypothesize that eggs will have higher concentrations than nestlings due to growth dilution.

Materials and Methods

Study location

LANL is situated on the Pajarito Plateau in north-central New Mexico and occupies approximately 104 km^2 (Figure 1). The region was formed by the eruptions of the Valles and Toledo volcanos approximately 1.4 and 1.1 million years ago [24] and consists of narrow mesa tops separated by steep canyons. The study area, which includes areas from within the current LANL boundary as well as areas with historic LANL operations (Figure 1), ranges in elevation from the Rio Grande at approximately 1,890 m to the Jemez Mountains at 2,400 m and has a southeastern drainage. The temperate mountain climate has four distinct seasons with an average annual precipitation of 47.9 cm and temperatures ranging from 2.7°C to 15.5°C [25]. The study area has been affected by other environmental perturbation not due to LANL operations such as wildfire, drought, and bark beetle infestation [26-29]. The predominant vegetation types on the Pajarito Plateau are pinyon-juniper woodland (*Pinus edulis-Juniperus monosperma*) and ponderosa pine forest (*Pinus ponderosa*) mixed with Gambel oak (*Quercus gambelii*); however, a wide range of vegetation types have been described ranging from willows (*Salix* spp.) and cottonwoods (*Populus* spp.) to aspen (*Populus tremuloides*) communities along the elevation gradient of the study area [30].

Nest boxes and egg collection

Nest boxes were first established at LANL and the surrounding area during the winter of 1997; the network now contains more than 500 boxes. Nest box locations do not necessarily overlap areas of organic chemical concerns. A developed but non-industrial reference site was selected upgradient (i.e., higher elevation/upstream of LANL) at a local cemetery and golf course (Figure 1). The majority of nest boxes were placed on ponderosa pine or pinyon pine approximately 1 m off of the ground and approximately 50 to 75 m apart. Field crews began nest box visits each May and continued throughout the breeding season. Deceased nestlings were collected (upon first observation) and nonviable eggs were collected. To ensure eggs were nonviable, eggs were only collected when nestlings in the same nest were ≥ 10 days old or when an entire clutch was abandoned. After collection, eggs were refrigerated and nestlings were frozen. Eggs were then processed, which consisted of rinsing (allowed to air dry), weighing, and separating egg contents from eggshells; egg contents were then frozen until samples were shipped to the analytical laboratory for chemical analyses. Moisture loss from eggs during storage was considered as a confounding variable; however, as egg moisture is lost, it results in higher measurements of element concentrations, which is a conservative error.

Chemical analyses

Eggs were analyzed for OCPs and/or their metabolites and PAHs at the Illinois Waste Management and Research Center, Champaign, Illinois, in 2003. PAH concentrations were determined by gas chromatography-mass spectrometry and OCPs by gas chromatography using an electron capture detector. A total of 23 western bluebird samples (study area=18; reference area=5) and nine ash-throated flycatcher samples (study area=9) were collected between 1997 and 2002 and submitted for analysis. Reporting detection limits (practical quantitation limit or PQL) were 2 ng/g for alpha-chlordane, beta-hexachlorocyclohexane (beta-BHC), dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), dieldrin, gamma-chlordane, lindane, methoxychlor, and *trans*-nonachlor; 4 ng/g for alpha-hexachlorocyclohexane (alpha-BHC), and heptachlor; 20 ng/g for acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(k)fluoranthene; and 40 ng/g for benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)perylene. All results are reported on a wet weight basis.

Eggs and nestlings were analyzed for PCB congeners by the US Environmental Protection Agency (EPA) Method 1668A at Cape Fear Analytical LLC, Wilmington, North Carolina, in 2014 (samples were collected between 1998 and 2012). The analytical laboratory reported total PCB concentrations as an additive of all 10 homologs, and when a homolog contained all nondetects, that particular homolog was assigned a value of zero. To meet minimum sample mass requirements for detection limits and to increase the probability of detecting chemicals in the samples (i.e., greater mass), eggs and nestlings were pooled to form composite samples. Nestlings were generally composited from the same nest; otherwise nestlings or eggs were composited from the same location (i.e., same reaches of a canyon). A total of 11 western bluebird composite egg samples, 33 western bluebird composite nestling samples, four ash-throated flycatcher composite egg samples, and three ash-throated flycatcher nestling samples (one single nestling and two composites) were submitted for analysis; all were collected at LANL (Figure 1). Weighted geometric means were used to represent composite samples (i.e., eggs collected from multiple nest boxes; Figure 1). Two egg and two nestling composite samples from the non-industrial reference area were also submitted for analyses but were not included in the statistical analyses due to the small sample size. The lowest method detection limit (MDL) range for individual dioxin-like PCB congeners

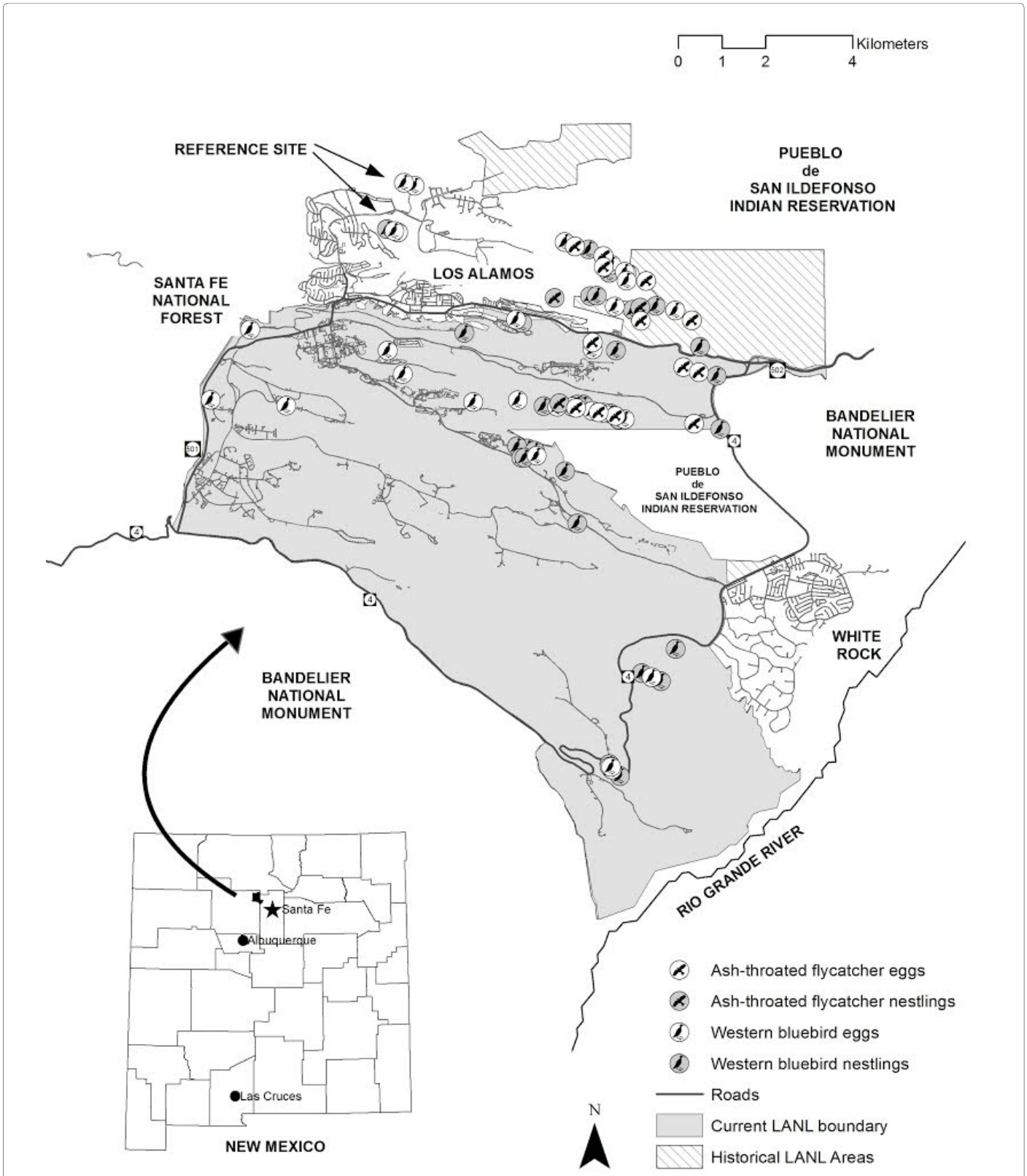

Figure 1: The study area displaying where western bluebird and ash-throated flycatcher eggs and nestlings were collected and underwent organic chemical analysis. Weighted geometric means were used to represent composite samples (i.e., eggs collected from multiple nest boxes). The study area includes areas within the current LANL boundary and historical areas. The non-industrial reference area is located at a cemetery and a municipal golf course and is upgradient (upstream) of LANL in the townsite. Samples were collected between 1997 and 2012.

77, 81, 114, 123, 126, 167, and 189 was 0.00374 to 0.0653 ng/g, 105 and 169 was 0.00562 to 0.098 ng/g, and 118 and 156/157 was 0.00749 to 0.131 ng/g. All results are reported on a wet weight basis.

TEQ calculations

TEQs were calculated for all 12 aryl hydrocarbon-binding PCB congeners; non-*ortho*-substituted PCB congeners 77, 81, 126, and 169 and mono-*ortho*-substituted PCB congeners 105, 114, 118, 123, 156, 157, 167, and 189. Each congener was multiplied by their respective avian-specific World Health Organization (WHO) TEF [6] and added for a total TEQ for each sample [5]. Congeners 156 and 157 co-eluted and therefore these two congeners were treated as one; the WHO TEFs are the same for both congeners.

Statistical analysis

Prior to statistical analyses, all OCPs and PAHs that were nondetects were assigned the PQL (MDLs were not provided by the analytical laboratory). To examine the influence of nondetects on dioxin-like PCB congener results, they were analyzed in two ways, 1) nondetects were assigned at the MDL (the MDL was used over PQL to prevent a high bias of unknown values) and 2) nondetects were assigned a value of zero. Datasets for any individual analyte did not undergo statistical analyses when approximately 80% or more of egg samples were nondetects for an individual analyte [31]. (Note: This rule was not applied to individual congeners to calculate TEQs since all 12 dioxin-like PCBs are needed to calculate TEQs.) For datasets containing nondetect values that did undergo statistical analyses, descriptive statistics (mean ± standard deviation) were calculated using the Kaplan-Meier approach (ProUCL 5.0 Statistical Software, 2013, US EPA) and comparisons between two groups were assessed with the Gehan-Wilcoxon test [32]; this included assessing differences in egg concentrations between the study area and the non-industrial reference site, between species, and between eggs and nestlings. For datasets that did not contain nondetect values, a Wilcoxon test was used to assess differences between the study area and the non-industrial reference site, between species, and between eggs and nestlings. All statistical analyses were performed using NCSS 9 Statistical Software 2013 (Kaysville, Utah).

Results

More than 80% of eggs were nondetects for alpha-BHC, alpha-chlordane, beta-BHC, DDD, gamma-chlordane, heptachlor, lindane, methoxychlor, and for all PAHs (Table 1); and approximately 80% of ash-throated flycatcher eggs were nondetects for heptachlor epoxide, oxychlordane, and *trans*-nonachlor (Table 1). PAHs phenanthrene, pyrene, and chrysene were each detected in three separate western bluebird eggs; all other PAHs were nondetects, including in all ash-throated flycatchers eggs. In both species, all nestlings had detectable levels of total PCBs and all eggs had detectable levels of total PCBs and DDE. Dieldrin and DDT were frequently (>33%) detected in eggs of both species and heptachlor epoxide, oxychlordane, and *trans*-nonachlor were frequently (>39%) detected in western bluebird eggs.

No ash-throated flycatcher eggs were collected from the non-industrial reference area; therefore, chemical concentrations in ash-throated flycatchers could not be compared between the sites. Several differences were observed in egg concentrations when western bluebird

	Western Bluebird			Ash-throated Flycatcher		
Organochlorine Pesticide	Obs	% ND	Detected Concentration(s)	Obs	% ND	Detected Concentration(s)
Alpha-BHC	23	100		9	100	
Alpha-chlordane	23	95.7	2.0	9	100	
Beta-BHC	23	100		9	100	
DDD	23	100		9	88.9	0.4
Gamma-chlordane	23	95.7	10.0	9	100	
Heptachlor	23	100		9	88.9	0.4
Heptachlor epoxide				3	100	
Lindane	23	95.7	0.4	9	100	
Methoxychlor	23	95.7	3.0	9	88.9	2.0
Oxychlordane				9	77.8	1.0–6.0
Trans-nonachlor				9	77.8	0.6–0.9
Polycyclic Aromatic Hydrocarbon						
Acenaphthylene	23	100		9	100	
Acenaphthene	23	100		9	100	
Fluorene	23	100		9	100	
Anthracene	23	100		9	100	
Phenanthrene	23	95.7	15.7	9	100	
Fluoranthene	23	100		9	100	
Pyrene	23	95.7	26.0	9	100	
Benzo[*a*]anthracene	23	100		9	100	
Chrysene	23	95.7	12.0	9	100	
Benzo[*b*]fluoranthene	23	100		9	100	
Benzo[*k*]fluoranthene	23	100		9	100	
Benzo[*a*]pyrene	23	100		9	100	
Dibenzo[*a,h*]anthracene	23	100		9	100	
Benzo[*g,h,i*]perylene	23	100		9	100	
Indeno[1,2,3-*cd*]perylene	23	100		9	100	

Table 1: Organic chemicals with ~80% or more of samples (Obs=observations) being nondetects (ND) in western bluebird and ash-throated flycatcher eggs collected from the study area and the non-industrial reference area. All values are reported in ng/g wet weight.

eggs collected from the study area were compared with eggs from a non-industrial reference area. Western bluebird eggs collected from the study area contained twofold less dieldrin [study area 1.81 ± 1.86 (M ± SD) ng/g, reference area 3.8 ± 1.47 ng/g; P=0.02], 4.5 times less oxychlordane (study area 4.14 ± 2.52 ng/g, reference area 18.80 ± 15.48 ng/g; P=0.01), and 6.6 times less *trans*-nonachlor (study area 1.45 ± 1.17 ng/g, reference area 9.60 ± 9.39 ng/g; P<0.01) when compared with eggs from a non-industrial reference area (Table 2). In western bluebird eggs, DDE, DDT, and heptachlor epoxide concentrations did not differ statistically between the study area and the non-industrial reference area (P>0.05; Table 2). Total PCBs and TEQs were not compared between sites due to a small sample size at the non-industrial reference site (n=2).

In eggs, DDE, DDT, dieldrin, and total PCB concentrations did not differ statistically between species (P>0.05; Table 3). When nondetectable dioxin-like PCB congeners were assigned the detection limit, ash-throated flycatcher eggs contained three times more TEQ concentrations (0.01 ± 0.008 ng/g) when compared with western bluebird eggs (0.003 ± 0.002; P=0.03; Table 3). When nondetectable dioxin-like PCB congeners were assigned a value of zero, ash-throated flycatcher eggs contained 31 times more TEQ concentrations (0.0093 ± 0.0083 ng/g) when compared with western bluebird eggs (0.0003 ± 0.0003; P<0.01; Table 3). No difference in total PCBs or TEQ concentrations in nestlings was observed between species (P>0.05; Table 3).

Total PCB concentrations were significantly higher in western bluebird eggs when compared with nestlings (eggs 152.65 ± 115.19 ng/g, nestlings 32.84 ± 54.34 ng/g; P<0.001; Table 4); western bluebird eggs contained on average 4.5 times more PCBs than nestlings. TEQ concentrations revealed a similar trend wherein TEQs were 1.5 times higher in eggs than nestlings in western bluebirds (eggs 0.003 ± 0.002 ng/g, nestlings 0.002 ± 0.002 ng/g; P<0.01; Table 4) when nondetects were assigned a value of the detection limit; and eggs contained ~twofold more TEQs than nestlings when the nondetectable dioxin-like PCB congeners were assigned a value of zero (eggs 0.0003 ± 0.0003 ng/g, nestlings 0.00016 ± 0.00059 ng/g; P<0.001; Table 4). Although ash-throated eggs contained higher levels of total PCBs and TEQs when compared with nestlings, this was not statistically significant (P>0.05; Table 4).

All western bluebird eggs contained detectable concentrations of congeners 105, 114, 118, 156/157, 167, and 189, while no eggs contained detectable concentrations of congeners 77, 81, or 126; congeners 123 and 169 were detected in 64% and 27% of eggs, respectively (Table 5). Most (≥ 67%) western bluebird nestlings contained detectable concentrations of congeners 105, 118, 156/157, 167, 189, while congeners 114 and 123 were detected in 36% and 18% in nestlings, respectively; 3% of nestlings contained congeners 77, 126, and 169. Congener 81 was not detected in any western bluebird nestlings (Table 5).

All ash-throated flycatcher eggs contained detectable concentrations of congeners 105, 118, 156/157, 167, 189, and 126, while congener 81 was not detected in any eggs. Congeners 77 and 169 were detected in 25% of eggs and congeners 114 and 123 were detected in 75% of eggs (Table 5). All ash-throated flycatcher nestlings contained congeners 105, 118, 156/157, 167, and 189 while congeners 81 and 169 were not detected in any nestlings. Congeners 114, 123, 77, 126, were observed in 33% of the nestlings (Table 5).

In western bluebirds, when nondetects values were assigned a value of the detection limit, non-*ortho*-substituted congeners 77, 81, and 126 comprised the greatest percentage of the total TEQ (Figure 2A) in both eggs and nestlings, even though these congeners were all nondetects in eggs and only detected in 3% of nestlings. However, when nondetectable congeners were assigned a value of zero, the greatest percentage of TEQs were mono-*ortho*-substituted congeners 156/157 for both western bluebird eggs and nestlings (Figure 2B).

All ash-throated flycatcher eggs contained congener 126, which comprised 73% of the total TEQ concentration when nondetects were assigned a value of the detection limit (Figure 2C). When the nondetectable congeners were assigned a value of zero, congener 126 comprised 96% of the total TEQ in ash-throated flycatcher eggs (Figure 2D). In ash-throated flycatcher nestlings, congener 126 comprised 44% of the total TEQ when nondetects were assigned a value of the detection limit (Figure 2C) and only 22% when nondetects were assigned a value of zero (Figure 2D).

Discussion

The majority of the OCPs and/or their metabolites and PAHs were not detected in eggs of western bluebirds and ash-throated flycatchers and as a reminder, any potential moisture lost in eggs prior to chemical analyses would result in higher, or more conservative measurements, of chemical concentrations. PAHs are readily metabolized in birds and therefore are often not detected in tissues [10]. For example, an egg injection study in chickens (*Gallus domesticus*) [33] found that 94% of PAHs were metabolized within 14 days after injection. Rapid metabolism of PAHs may explain why very few PAHs were detected in this study. In eggs, DDE was detected in all samples; the largest source of DDT, the parent compound of DDE, in the study region is likely from the US Forest Service because they sprayed more than a million pounds of DDT across ~5,000 km^2 of the Santa Fe National Forest and surrounding area in the early 1950s and into the 1960s [34]. Finally, although PCBs have been detected at LANL [2], they have also been detected in the general study area [35], are ubiquitous in the environment, and unsurprisingly, were detected in all eggs and nestlings of both species.

Contrary to our predictions, western bluebird eggs collected from the study area contained significantly lower concentrations of dieldrin, oxychlordane, and *trans*-nonachlor when compared with eggs from the non-industrial reference site, and no differences in DDE, DDT, and heptachlor epoxide concentrations were observed between the two areas. The non-industrial reference area in this study includes both a cemetery and a municipal golf course where routine pesticide application is likely practiced; it is unknown what pesticides were historically used.

Dieldrin, DDE, DDT, and total PCB egg concentrations did not differ between species; however, ash-throated flycatcher eggs contained significantly higher levels of TEQs when compared with western bluebird eggs. Constituents in avian eggs generally reflect local contamination where the female is during egg formation. However, stored body burdens, particularly those with lipophilic properties, can become mobilized during egg formation when females lose lipid reserves [15]; therefore, egg concentrations can reflect previous exposure, such as those during migration and those at wintering grounds [14,16]. Ash-throated flycatchers are a migratory species and their wintering range spans from Mexico to Costa Rica; whereas western bluebirds may reside as year-round residents in many areas, including northern New Mexico [19,23]. The differences of TEQ levels in eggs between species could be explained by different PCB profile exposure (as evident by the presence of congener 126 in ash-throated flycatchers) during the

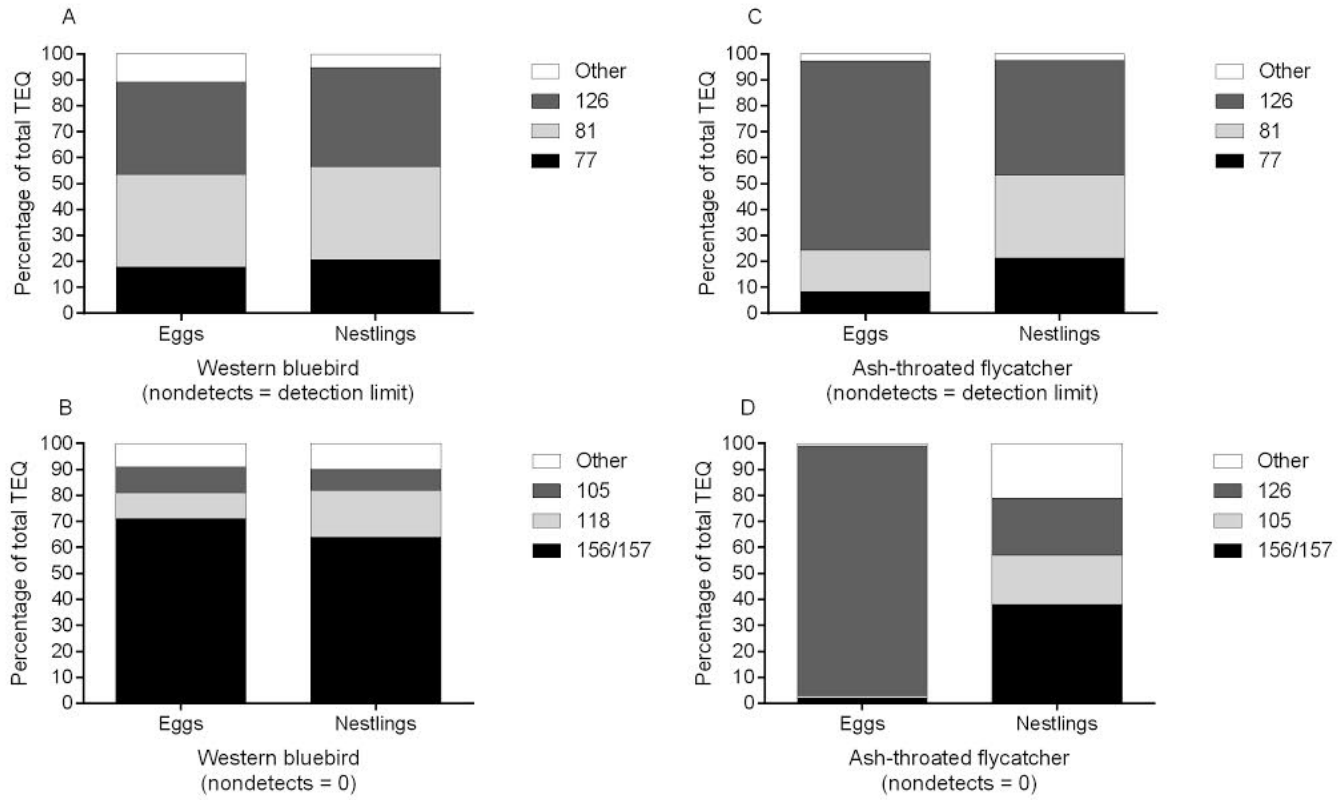

Figure 2: Contribution of non-*ortho*- and mono-*ortho*-substituted PCB congeners relative to the total TEQ concentration. A) Congeners contributing the greatest percentage of the total TEQ in western bluebird eggs and nestlings when nondetects were assigned values at the detection limit. B) Congeners contributing the greatest percentage of the total TEQ in western bluebird eggs and nestlings when nondetects were assigned a value of zero. C) Congeners contributing the greatest percentage of the total TEQ in ash-throated flycatcher eggs and nestlings when nondetects were assigned values at the detection limit. D) Congeners contributing the greatest percentage of the total TEQ in ash-throated flycatcher eggs and nestlings when nondetects were assigned a value of zero.

winter since these two species are in different locations, differences in metabolism between the species, or differences in their diets, especially their wintering diets, within the study region. Although TEQs differed in eggs between these two species, in support of our predictions, there was no difference observed in TEQs in nestlings between species. This observation could be explained by both species being exposed to similar PCB profiles while nesting at LANL due to overall very low TEQ concentrations in both species, or perhaps this observation is an artifact of a small sample size representing a large spatial span.

In support of our prediction, western bluebird eggs contained higher concentrations of total PCBs and TEQs than in nestlings. Although not statistically significant, a similar trend was observed in ash-throated flycatcher eggs; the lack of significance could be due to a small sample size. These observations are consistent with other studies [36,37] and may be explained by growth dilution; rapid growth of the nestlings typically dilute organochlorine concentrations or lipophilic contaminant levels, such as PCBs [38].

When nondetected dioxin-like PCB congeners were assigned values at the detection limit, non-*ortho*-substituted congeners 77, 81, and 126 were the greatest contributors to the total TEQ even though these congeners were largely nondetects, with the exception of 126 in ash-throated flycatcher eggs. Despite the low detection rate of these non-*ortho*-substituted congeners, they appear to make the greatest contribution of the TEQ because the avian WHO TEFs are the highest for these congeners when compared with TEFs for mono-*ortho*-substituted congeners [6]. When nondetectable congeners were assigned a value of zero, the greatest contributors to the total TEQ changed drastically; these data demonstrate the impact that statistical treatment of nondetects can have on the results and how the data are interpreted. In this study, no differences in statistical significance were observed when nondetects were assigned different values; however, the magnitude of significance differed in some comparisons. Since many of dioxin-like congeners are nondetects in this study, they may not be ecologically relevant. Congener 126 was detected in all ash-throated flycatcher eggs and consisted of a great percentage of the total TEQ, but was not detected in western bluebird eggs. The differences between species could again be explained by exposures to different PCB profiles during migration or on wintering grounds, metabolic differences, and/or dietary differences.

The organic chemicals detected in eggs in this study were well below levels typically associated with adverse effects in birds, or lowest observable adverse effects levels. Specifically, DDE-, dieldrin-, heptachlor-, and PCB-induced adverse biological effects have been associated with avian egg concentrations of 3,000 ng/g [8], 1,000 ng/g [9] 1,500 ng/g [39], and 1,000 to 4,000 ng/g [5], respectively. While these chemicals were detected in the majority of eggs examined in this study, all concentrations were well below, sometimes one to two orders of magnitude less than, the values associated with adverse effects. In

	Study Area			Non-industrial Reference Area			P
Compound	**Obs**	**% ND**	**M ± SD**	**Obs**	**% ND**	**M ± SD**	
DDE	18	0	52.7 ± 65.8	5	0	106.0 ± 67.4	0.06
DDT	18	55.6	7.72 ± 1.98	5	60	2.0 ± 0.0	0.90
Dieldrin	18	55.6	1.81 ± 1.86	5	20	3.8 ± 1.47	0.02
Heptachlor epoxide	7	57.1	0.57 ± 0.13	3	0	8.13 ± 7.80	0.06
Oxychlordane	18	50.0	4.14 ± 2.52	5	20	18.80 ± 15.48	0.01
Trans-nonachlor	18	61.1	1.45 ± 1.17	5	20	9.60 ± 9.39	<0.01

Table 2: Organochlorine pesticide concentrations [mean ± standard deviation (M ± SD)], ng/g wet weight in western bluebirds eggs (Obs=observations) collected from the study area compared with egg concentrations from a non-industrial reference area. For datasets containing nondetects (ND), M ± SD were calculated in ProUCL and comparisons between sites were evaluated with a Gehan-Wilcoxon test. The remaining comparisons between sites were examined with a Wilcoxon test.

	Western bluebird			Ash-throated flycatcher			P
Compound/tissue	**Obs**	**% ND**	**M ± SD**	**Obs**	**% ND**	**M ± SD**	
Eggs							
DDE	18	0	52.7 ± 65.8	9	0	38.0 ± 25.9	0.88
DDT	18	56.6	1.72 ± 1.41	9	66.7	1.69 ± 2.23	0.21
Dieldrin	18	55.6	1.81 ± 1.86	9	33.3	1.83 ± 1.33	0.68
Total PCBs	11	0	152.65 ± 115.19	4	0	97.40 ± 85.26	0.27
TEQ (ND at DL)	11	–	0.003 ± 0.002	4	–	0.01 ± 0.008	0.03
TEQ (ND at 0)	11	–	0.0003 ± 0.0003	4	–	0.0093 ± 0.0083	< 0.01
Nestlings							
Total PCBs	33	0	32.84 ± 54.34	3	0	14.30 ± 7.03	0.91
TEQ (ND at DL)	33	–	0.002 ± 0.002	3	–	0.002 ± 0.001	0.46
TEQ (ND at 0)	33	–	0.00016 ± 0.00059	3	–	0.00099 ± 0.00168	0.23

Table 3: Organochlorine pesticides, total PCBs, and TEQ concentrations [mean ± standard deviation (M ± SD)] ng/g wet weight in eggs and nestlings (Obs=observations) of western bluebirds compared with ash-throated flycatchers collected from the study area. For datasets containing nondetects (ND), M ± SD were calculated in ProUCL and comparisons between species were evaluated with a Gehan-Wilcoxon test. The remaining comparisons between species were examined with a Wilcoxon test. TEQs were analyzed two ways in relation to treating NDs; NDs were set at the detection limit (DL) or to a value of zero. Note that percent nondetects for TEQs are not relevant as the TEQ value is a calculation based upon 12 congeners and a TEF, and not simply the number of samples nondetected for an individual analyte.

	Eggs		Nestlings		P
PCB/Species	**Obs**	**M ± SD**	**Obs**	**M ± SD**	
Ash-throated flycatcher	4	97.40 ± 85.26	3	14.30 ± 7.03	0.052
Western bluebird	11	152.65 ± 115.19	33	32.84 ± 54.34	<0.001
TEQ/Species					
Ash-throated flycatcher (ND at DL)	4	0.01 ± 0.008	3	0.002 ± 0.001	0.11
Ash-throated flycatcher (ND at 0)	4	0.0093 ± 0.0083	3	0.00099 ± 0.00168	0.11
Western bluebird (ND at DL)	11	0.003 ± 0.002	33	0.002 ± 0.002	<0.01
Western bluebird (ND at 0)	11	0.0003 ± 0.0003	33	0.00016 ± 0.00059	<0.001

Table 4: Total PCB and TEQ concentrations [mean ± standard deviation (M ± SD)] ng/g wet weight in eggs compared with nestlings (Obs=observations) in western bluebirds and ash-throated flycatchers. TEQs were analyzed two ways in relation to treating nondetects (ND); NDs were assigned a value of the detection limit (DL) or assigned a value of zero. Comparisons between species were examined with a Wilcoxon test.

	Western bluebird		Ash-throated flycatcher	
PCB congener	**Eggs (n=11)**	**Nestlings (n=33)**	**Eggs (n=3)**	**Nestlings (n=4)**
77	0	3	25	33
81	0	0	0	0
126	0	3	100	33
169	27	3	25	0
105	100	67	100	100
114	100	36	75	33
118	100	97	100	100
123	64	18	75	33
156/157	100	88	100	100
167	100	85	100	100
189	100	73	100	100

Table 5: The percentage of dioxin-like PCB congeners that were detected in western bluebird and ash-throated eggs and nestlings.

eastern bluebirds (*Sialia sialis*), TCDD-induced toxic effects were observed when egg concentrations were between 1,000 to 10,000 pg/g [40]. All eggs and nestlings of both species had TEQ concentrations at least two orders of magnitude less than a 1,000 pg/g adverse effect level. No reliable screening levels were found for DDT, oxychlordane, and *trans*-nonachlor [8,39,41].

Conclusions

This study is part of an ongoing ecological risk assessment at LANL. The organic chemicals evaluated in eggs and nestlings of western bluebirds and ash-throated flycatchers revealed that the majority of samples did not contain detectable concentrations. While all concentrations reported in this study are low, reporting low-level chemical concentrations in areas with potential environmental contamination is important as this will contribute to the understanding of the dynamics and behavior of chemicals in the environment as well as the bioavailability to avian wildlife. Concentrations of organic chemicals detected in western bluebird eggs from the study area were either not statistically different or were significantly less than those at the non-industrial reference area. Additionally, concentrations in both species were below levels associated with adverse effects in birds reported in the scientific literature. These findings suggest that concentrations measured in western bluebirds and ash-throated flycatchers are at levels associated with a negligible risk of adverse effects to the local bird populations.

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