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Short Communication

ORGANOCHLORINE PESTICIDES, LEAD, AND MERCURY IN NORTHERN BOBWHITE (*COLINUS VIRGINIANUS*) AND SCALED QUAIL (*CALLIPEPLA SQUAMATA*) FROM THE ROLLING PLAINS ECOREGION OF TEXAS AND OKLAHOMA

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Abstract: Northern bobwhite (*Colinus virginianus*) and scaled quail (*Callipepla squamata*) from the Rolling Plains ecoregion in Texas and Oklahoma were evaluated for organochlorine pesticides, Pb, and Hg. Of all organochlorine pesticides analyzed, only *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) and *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD) were found in a few composite liver samples. Similarly, a small fraction of tissue samples had detectable levels of Hg (liver and breast) or Pb exceeding background concentrations (femur). Lead concentrations in a few individuals fell within the range associated with moderate toxicity. *Environ Toxicol Chem* 2015;34:1505–1510. © 2015 SETAC

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INTRODUCTION

Populations of northern bobwhite (*Colinus virginianus*) and scaled quail (*Callipepla squamata*) in the Rolling Plains ecoregion (TX and OK, USA) have been declining. Historical data suggest that, on a national level, this phenomenon may have begun as early as the 1800s [1]. In the Rolling Plains ecoregion specifically, data from the US Geological Survey's Breeding Bird Survey [2] show a –3.7% decrease for bobwhites for the years of 1966 to 2011, with a –5.8% decline for the years 2001 to 2011. For scaled quail, the trend is –3.8% for 1966 to 2011 and –8.0% for 2001 to 2011 [2]. Additional surveys from Texas Parks and Wildlife [3] corroborate these findings; data indicate that bobwhite populations have failed to rebound to long-term averages since at least 2007, and scaled quail have been in a state of decline since 1988 [3]. The latter phenomenon has been attributed anecdotally to a 1988 disease event [4]; overall, however, a comprehensive explanation for these population declines has yet to emerge. The more pronounced decreases in the past decade have raised concerns about the potential cause of these trends.

Bobwhite and scaled quail populations are thought to be influenced by many external factors, including habitat quality [1], weather [5,6], predation [7,8], parasites [9–11], and environmental contaminants [12,13]. Habitat quality often is cited as the ultimate cause of quail decline, with other factors being proximate causes [14]; furthermore, poor habitat is thought to increase quail susceptibility to these proximate causes [15]. Despite their known deleterious effects [16–23], organochlorine pesticides, Pb, and Hg have not been previously examined in quail inhabiting the Rolling Plains ecoregion. The goal of the present study was to quantify tissue levels of these pesticides and heavy metals in Rolling Plains quail and evaluate whether these concentrations reflect possible toxicity that could contribute to the observed population decline in bobwhite and scaled quail.

MATERIALS AND METHODS

Study sites and sample collection

Bobwhite and scaled quail were trapped on 35 ranches located in the Rolling Plains ecoregion (24 counties in Texas and 9 counties in Oklahoma; Figure 1). Birds were collected under a Texas Parks and Wildlife permit (SPR-1098-984; Austin, TX, USA) and Institutional Animal Care and Use protocols from both Texas Tech University (11049-07; Lubbock, TX, USA) and Texas A&M University (2011-93; College Station, TX, USA). Trapping took place during 2-wk sessions in August and October of 2011 and 2012. Funnel-style walk-in traps were baited with milo or birdseed and covered with local vegetation. At each ranch, traps were checked 4 times over a period of 2 d. Species, age, and sex were determined using plumage characteristics for all birds caught [24]. As part of the overall study design, and in agreement with participating landowners, only every third bird caught was euthanized (cervical dislocation). Following euthanasia, bird carcasses were refrigerated and transported to The Institute of Environmental and Human Health at Texas Tech University for necropsy within 48 h. During necropsy, breast tissue, liver, brain, and legs (containing thigh muscle, femur, and associated skin and feathers) were removed and frozen (–80 °C) until analysis. All tissues were catalogued according to collection year, age, sex, and species (Supplemental Data, Tables S1–S2). Because samples collected under this effort were divided among multiple studies and institutions, we were afforded only specific tissues from euthanized birds as available. Such distribution methods meant that we were unable to obtain equal sample sizes with regard to age, sex, species, collection year, or tissue type; we chose instead to evaluate as many samples as possible.

All Supplemental Data may be found in the online version of this article.

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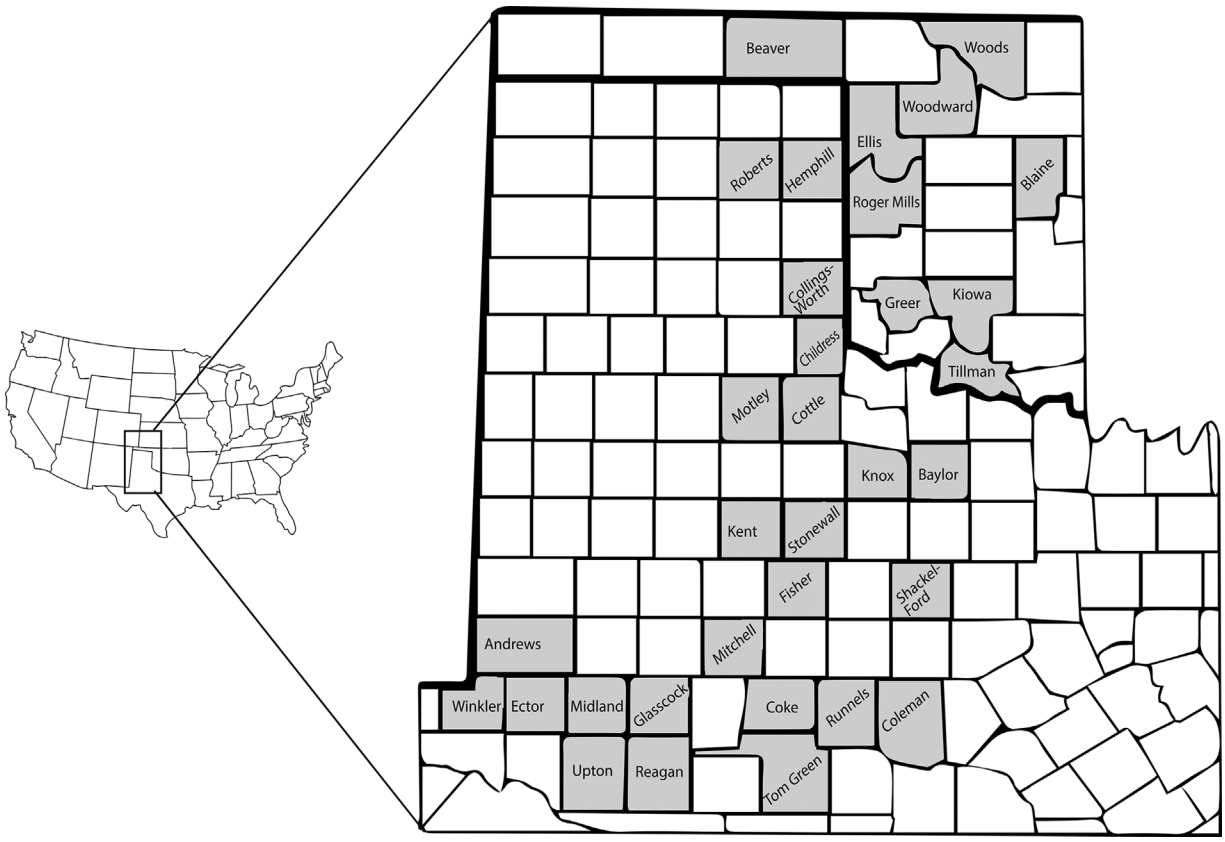

Figure 1. Map of Rolling Plains ecoregion in Texas and Oklahoma, USA. Shaded counties indicate where quail were collected. A total of 35 ranches and collection sites were located across these counties.

Thigh tissue samples were analyzed solely for organochlorine pesticides. Livers collected during 2012 were halved and used to make composites ($n = 19$, halves from 3–4 individuals per composite) for organochlorine pesticide analysis. The remaining 2012 livers and those collected in 2011 ($n = 192$) were analyzed for Hg. In a similar fashion, composites were made out of brain ($n = 17$) and out of skin and feather tissue ($n = 19$, from the thigh area) collected in 2012; these composites were analyzed for organochlorine pesticides only. All composites were made with the goal of grouping constituent tissues by collection location (ranch) and then by sex, age, and/or species whenever possible. Femurs ($n = 282$) were analyzed for Pb, and breast tissue samples ($n = 294$) were analyzed for Hg. Lipid percentages were determined for thigh tissues and liver composites. Insufficient samples of the other tissues were available to perform lipid determinations in addition to the organochlorine pesticide or metal analyses.

Sample analyses

Organochlorines. Thigh, liver, brain, and skin and feather samples were extracted using a general Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method. Briefly, approximately 3 g wet weight tissue was freeze dried and added to a 50-mL centrifuge tube containing 4 g anhydrous magnesium sulfate and 1 g anhydrous sodium chloride (United Chemical Technologies). To this tube, 15 mL to 20 mL acetonitrile was added with 5 μL tetrachloro-*m*-xylene (TCMX; Accustandard) as an internal standard. After vortexing and centrifugation (3000 rpm for 10 min), the extract was decanted and transferred into a 15-mL QuEChERS cleanup centrifuge tube containing 900 mg anhydrous magnesium sulfate, 300 mg primary–secondary amine exchange material, and 150 mg endcapped C18 (United Chemical Technologies). The extract was vortexed and centrifuged, and the final extract was filtered (0.2 μm polytetrafluoroethylene [PTFE] filter, Fisher), reduced using a nitrogen evaporator, and brought to 1 mL. Extracts were then transferred to autosampler vials for analysis by gas chromatography–electron capture detector (ECD).

Samples were analyzed on a Hewlett-Packard 6890 gas chromatograph equipped with 2 Agilent columns (primary: DB-17 ms, 30 m × 0.32 mm × 0.25 μm; secondary: DB-XLB, 30 m × 0.32 mm × 0.50 μm) and 2 μECDs. Inlet temperature was 220 °C, and the temperature for both detectors was 300 °C. A volume of 2 μL was injected in pulsed splitless mode with a pulse pressure of 40.0 psi, pulse time of 0.20 min, purge flow of 44.8 mL/min and purge time of 1.00 min. Helium was used as the carrier gas (4.0 mL/min), with P5 serving as the makeup gas (5%, combined flow 60.0 mL/min). An initial column temperature of 60 °C was increased to 230 °C at 50 °C/min, increased at 25 °C/min to 235 °C, increased at 0.50 °C/min to 239 °C, increased at 5 °C/min to 250 °C, and then finally increased at 50 °C/min to a final temperature of 300 °C (held for 4 min). Peak identifications were made based on the average retention time in minutes (± 1.5%) of each pesticide in the entire set of matrix-matched calibration standards. Each standard was made by spiking a chicken breast extract (confirmed to be free of analytes) with a certified organochlorine pesticide mixture (Restek) and TCMX. The primary column was used to quantitate α-hexachlorocyclohexane (HCH), γ-HCH (lindane), α-chlordane, γ-chlordane, heptachlor epoxide, *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD), *p,p′*-dichlorodiphenyldichloroethylene

(*p,p′*-DDE), *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), methoxychlor, aldrin, dieldrin, endrin, endrin ketone, endosulfan I, endosulfan II, endosulfan sulfate, and TCMX. Because of interferences, β-HCH, γ-HCH, δ-HCH, and heptachlor were quantitated on the secondary column. Endrin aldehyde could not be quantitated because of poor recovery, perhaps as a result of removal by primary–secondary amine exchange material in the extraction step, which has been documented previously [25].

Samples were analyzed in batches of 20 at maximum, each batch with a blank, spiked blank, duplicate, matrix spike, and matrix spike duplicate. No blanks were found to have detectable concentrations of any analytes. Detectable analytes in the duplicate and matrix spike duplicate pairs agreed with each other on average within 20% relative standard deviation (RSD). Method detection limits (MDLs) were established using the method outlined by the Electronic Code of Federal Regulations [26]. The highest MDL determined was for methoxychlor at 70 ng/g; for the other organochlorine pesticides, MDLs ranged from 8.0 ng/g for heptachlor epoxide to 18 ng/g for aldrin and endosulfan sulfate. Recoveries from all tissue type matrix spikes and matrix spike duplicates (excluding endrin aldehyde) are as follows (mean ± standard deviation): 74 ± 18 ng/g with 24% RSD for TCMX, 91 ± 24 ng/g with 26% RSD for the hexachlorocyclohexanes, 95 ± 21 ng/g with 22% RSD for DDTs and methoxychlor, and 86 ± 21 ng/g with 25% RSD for the remaining chlordanes. All pesticide concentrations are reported as nanograms per gram wet weight.

Lipids. Thigh muscles and liver composites were analyzed for lipid content using a modified Folch method [27–29]. Approximately 0.5 g to 1.0 g of wet tissue was extracted with 20 mL of 2:1 dichloromethane:methanol and filtered. A 3-mL volume of filtered extract was placed in a preweighed aluminum pan and allowed to dry. The lipid composition of the tissue was then determined by gravimetric evaluation of the residue.

Mercury. Breast muscle and liver were tested for Hg using a modified US Environmental Protection Agency (USEPA) method SW-846 7471B [30]. These samples were analyzed using a CETAC M-6100 cold vapor atomic absorbance mercury analyzer. Absorbance for Hg was measured at 253.7 nm. Method blanks were found to contain less than 2 μg/kg Hg. Each calibration standard had a recovery between 97% and 105%. For the breast tissue samples, the recovery for the matrix spike and matrix spike duplicate sample ranged from 101% to 106%. For liver samples, the recovery for the matrix spike and matrix spike duplicate was between 98% and 104%. Sample detection limits (SDLs) for breast tissue were between 2.24 ng/g and 50.0 ng/g. In livers, SLDs ranged from 1.43 ng/g to 50.0 ng/g. All Hg concentrations are reported as nanograms per gram dry weight.

Lead. Femur samples were prepared for Pb analysis using a modified version of USEPA method SW-846 3050B [31]. Analysis of femurs for lead followed USEPA method SW-846 6010C using a Perkin Optima 8300 duel view inductively coupled plasma–optical emission spectrometer. Lead was measured at a wavelength of 220.353 nm in axial mode. Method blanks were found to contain less than 0.260 ng/kg Pb. Each calibration standard had a recovery between 96% and 108%. For the femur samples, the recovery for the matrix spike and matrix spike duplicate ranged from 99% to 112%. The SDLs for femurs ranged from 0.637 μg/g to 1.430 μg/g. All Pb concentrations are reported as micrograms per gram dry weight.

Statistical analyses

Statistical tests were performed using R statistical software (Ver 2.15.0) [32]. Pesticide concentrations that fell below the MDL were considered censored (nondetects) and were not evaluated further. Because of the very small number of values above MDLs, no statistical comparisons could be performed between tissue types, sexes, ages, species, or collection locations. Lipid data from thighs and livers were log transformed to meet assumptions of normality and homogeneity of variance (confirmed by Shapiro-Wilks and Bartlett's tests) and analyzed using a two-way analysis of variance (ANOVA) with type III sum of squares. For Pb and Hg data, values that fell below the SDLs were treated as zeros (nondetects). Because relatively low concentrations were found for Hg and Pb, we chose to do statistical evaluation only of the detection frequencies. To evaluate Hg and Pb detection frequencies, Fisher's exact test was used to find any significant differences in age (adult and juvenile), sex (male and female), species (scaled and bobwhite) and collection year (2011 and 2012) for each metal. A $p \leq 0.05$ level of significance was set a priori for all tests.

RESULTS AND DISCUSSION

Organochlorines

The only pesticides found above MDLs were *p,p′*-DDD (2 at 21 ng/g wet wt and 1 at 27 ng/g wet wt) and *p,p′*-DDE (1 at 43 ng/g wet wt and 1 at 56 ng/g wet wt), all from liver composite samples. The 3 samples containing *p,p′*-DDD were all from scaled quail, and both containing *p,p′*-DDE were from bobwhites (Supplemental Data, Table S3). There were no pesticides found above MDLs in thigh muscle samples, brain composites, or skin and feather composites.

Concentrations of organochlorine pesticides found in the present study are not likely to be causing direct toxic effects in Rolling Plains quail. Low organochlorine pesticide residues are likely the result of both species' tendency to eat at lower levels of the food chain (i.e., insects, seeds, and vegetation) and a lack of any substantial organochlorine pesticide reservoirs in the region (e.g., sediments from nonephemeral bodies of water). It is not entirely unexpected for the liver to have the most (or the only) detections compared with the other tissues, given its metabolic function. The fact that only DDE and DDD were detected, rather than DDT, suggests past, rather than recent, use of the pesticide [19]. The presence of DDE more than 40 yr after the banning of DDT was expected, as *p,p′*-DDE is both persistent in abiotic media and difficult for birds to remove from their bodies [33]. It is possible that the DDE in quail could have been the result of metabolism of environmental DDT or the uptake and storage of DDE transformed previously in the environment [34]. Limited data from controlled exposure studies for doses of *p,p′*-DDE and *p,p′*-DDD causing major toxic effects suggest that concentrations of concern usually fall in the parts per million range; brown-headed cowbirds (*Molothrus ater*), American robins (*Turdus migratorius*), and clapper rails (*Rallus longirostris*) that died from dietary exposure had liver DDE residues that exceeded 3000 μg/g wet weight and DDD residues exceeding 1000 μg/g [35]. For additional comparison, birds of prey suffering from *p,p′*-DDE poisoning had 100 μg/g wet weight concentrations of DDE in their livers [36]. Common egrets (*Ardea alba*) with thinning, breakage-prone eggs had liver DDE levels of 124 300 ng/g wet weight [37].

The 3 liver samples with DDD concentrations greater than the MDL likely are a result of postmortem anaerobic conversion of DDE or DDT [38], which seemed to be more likely to occur in scaled quail than bobwhites. We do not believe that the DDD we

observed would be found in living birds, and even if it were, it would be unlikely to remain in the body long enough to cause damage [33]. It is also worth noting that the DDE samples were from Oklahoma, whereas the DDD samples came from the southwestern reach of our study region in Texas. This finding may be something of an artifact of species ranges; scaled quail have a more westerly range even when sympatric with bobwhites [39].

There are some older studies on organochlorine pesticide residues in Texas birds to which we can compare our results. Attwater's greater prairie chickens (*Tympanuchus cupido attwateri*) sampled in 1966 from the southern coast of Texas had levels of *p,p′*-DDE below 0.6 mg/kg, and birds from 1975 to 1980 lacked any detections [40]. In the same study, 3 bobwhites from Refugio County were found to have heptachlor epoxide ranging from 4.8 mg/kg to 14 mg/kg. Closer to our study region of the Rolling Plains, blue teal (*Anas discors*) tissue and pheasant (*Phasianus colchius*) eggs collected in the early 1980s in Castro County, Texas, had DDE ranges of 0.03 mg/kg to 18.45 mg/kg and 0.045 mg/kg to 0.175 mg/kg, respectively (B.M. Wallace, 1984, Master's thesis, Texas Tech University, Lubbock, TX, USA). One would expect that the present study, occurring more than 40 yr after the ban of DDT, should have lower concentrations. More recent research on gallinaceous birds from North America—namely, willow and rock ptarmigans (*Lagopus lagopus* and *Lagopus mutus,* respectively), ruffed, spruce, and sharp-tailed grouse (*Bonasa umbellus, Dendragapus canadensis,* and *Tympanuchus phasianellus,* respectively) from Canada—found extremely low organochlorine pesticide concentrations, with medians <0.001 ng/g wet weight in breast tissue [41], suggesting that dietary habits of gallinaceous birds may preclude significant organochlorine pesticide bioaccumulation.

Thus, organochlorine pesticides do not seem to be a problem for quail in the Rolling Plains on a direct toxicity level. There still exists the possibility that organochlorine pesticide residues could predispose birds via immunosuppression to increased disease and parasite incidence.

Lipids

No significant relationships were found in the thigh or liver lipid data. In thighs, percent lipid composition ranged from 1.3% to 9.5%, with an average of 2.5% ($n = 298$). In livers, the range was 3.6% to 6.5%, with an average of 4.9% ($n = 19$). Lipid percentages for thigh muscles were within the range previously cited for birds from the family Phasianidae [42]. The lack of any significant differences for either thigh or liver lipid data implies that fat accumulation in all birds was similar. Body conditions for almost all birds were considered lean (probably because of an ongoing drought in the Rolling Plains), and so fat would be unlikely to be deposited in these 2 tissues in any case.

Mercury

In total, 29 breast samples and 26 liver samples were found to have levels of Hg exceeding SDLs. For breast tissue, the range was 2.4 ng/g to 82 ng/g Hg (mean of 12 ng/g). Livers had a range of 2.6 ng/g to 52 ng/g Hg (mean of 17 ng/g). A significantly higher number of breast and liver samples containing Hg belonged to adults rather than juveniles ($p < 0.001$, Fisher's Exact Test). This could be simply because adults have had a longer time than juveniles to accumulate Hg. All breast samples belonged to bobwhites ($p = 0.033$, Fisher's exact test), and all were collected in 2011 ($p < 0.001$, Fisher's exact test). Similarly, only 1 of the 26 liver samples was from a scaled quail ($p = 0.033$, Fisher's exact test), and only 3 were collected in 2012 ($p < 0.001$, Fisher's exact test).

Based on the low number of samples found to contain any Hg, and the low concentrations when detected, it seems unlikely that this is a contributing factor to the decline of quail the region. These values are similar to, or below, published background levels for total Hg in liver tissues (e.g., < 20 ng/g in control birds [*Coturnix coturnix*] [43]; 200 ng/g for birds raised in captivity [21]; 1000–10 000 ng/g, wild birds in general [21]). Concentrations associated with both acute and chronic effects are well above these values. It is important to note, however, that the toxic effects of Hg are highly dependent on the species of Hg present, organic Hg being much more toxic than inorganic Hg. Unfortunately, the form of Hg was not determined in the present study.

Lead

Only 16 femur samples were found to exceed SDLs for Pb. Values detected ranged from 0.640 μg/g to 151 μg/g (mean of 40.8 μg/g). The femurs were chosen for Pb analysis because bones are a better indicator of chronic lifetime exposure than internal organs [22]. It is worth noting, however, that Pb levels in bone are not useful in the cases of extreme acute toxicity, during which a bird may die before substantial quantities of Pb become deposited in the bone [44,45]. Past studies [21,46,47] have shown the background bone Pb level for wild birds to generally be below 10 μg/g. Specimens with bones containing 10 μg/g to 20 μg/g of Pb can be classified as having been exposed to higher than background levels of Pb and those specimens with bones containing over 20 μg/g of Pb to have been highly exposed to Pb. Consequently, the following 9 values would be considered elevated at ≥ 10 μg/g dry weight: 10.1 μg/g, 11.1 μg/g, 12.5 μg/g, 46.4 μg/g, 64.3 μg/g, 88.9 μg/g, 113 μg/g, 137 μg/g, and 151 μg/g. Surprisingly, there was no significant difference between males and females or any other significant differences between age, sex, location, or species.

Ingestion of Pb ammunition is a well-established exposure route to wildlife [22,23,44,46,47], especially in Galliformes, which can mistake the fragments for grit particles required to aid in the mechanical digestion of food [23]. Although we were not able to physically check the crop or gastrointestinal tract of the birds, because these organs were allocated to a separate study, the bone lead concentrations of the femur samples are certainly indicative of exposure.

Low concentrations of Pb can inhibit δ-aminolevulinic acid dehydratase (ALAD) activity [21,44]. Diets containing 5 mg/kg Pb have been found to inhibit ALAD in some birds [21]. In addition, it has been stated, because of the extremely sensitive nature of ALAD, there may not be a no-effect threshold level [44]. It is also possible that the birds found to have elevated Pb bone concentrations could have been anemic. Furthermore, elevated Pb levels may have caused other sub-lethal effects, including weight loss, impaired immune function, depressed hematopoiesis, and reduced reproduction [44]. Lead ingestion also can interfere with the nervous system, internal organ function, and behavior [21,22,44]. High Pb concentrations have been associated with thinning eggshells in some avian species [44].

CONCLUSIONS

Our findings revealed low organochlorine pesticide levels and background concentrations of mercury along with limited evidence of potentially toxic Pb accumulation in a small

proportion of quail. The scope of our project did not permit determination of the ultimate source of the Pb; without further analysis, we cannot rule out non-ammunition sources. Based on these data, we do not believe environmental contaminants are directly causing large-scale effects in Rolling Plains quail. Nevertheless, the potential for immunosuppression and similar alterations to quail physiology may warrant continued surveillance, because these issues may ultimately increase quail susceptibility to parasites, predation, and disease.

SUPPLEMENTAL DATA

Tables S1–S3. (25 KB DOC).

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Data availability—Data, associated metadata, and calculation tools are available on request from the Wildlife Toxicology Laboratory at Texas Tech University (ron.kendall@ttu.edu).

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