

BURROWING OWLS AND AGRICULTURAL PESTICIDES: EVALUATION OF RESIDUES AND RISKS FOR THREE POPULATIONS IN CALIFORNIA, USA

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Abstract—We examined selenium, organophosphorus, and organochlorine pesticide residues in egg, footwash, and feather samples from burrowing owls in three populations in central and southern California. Eggs from all sites contained detectable levels of $p.p'$ -dichlorodiphenyldichloroethylene, with the San Joaquin Valley site containing up to 33 µg/g (geometric mean $\bar{x} = 7.52$). Only low levels of polychlorinated biphenyls were detected, however (geometric mean $\bar{x} = 1.98$, $n = 2$). Selenuim concentrations were low in all samples (geometric mean $\bar{x} = 0.426$, $n = 20$). Eggshells collected in 1996 were 22% thinner than eggs collected prior to 1937. In addition, feather samples contained low levels of dichlorodiphenyldichloroethylene, and footwash samples indicated exposure to the pesticide chlorpyrifos. Pesticide-use records indicated that one population might also be at risk from applications of aldicarb near nests during the breeding season.

Keywords-Athene cunicularia p, p' -dichlorodiphenyldichloroethylene Chlorpyrifos Organophosphorus pesticides Organochlorine pesticides

INTRODUCTION

Western burrowing owls (Athene cunicularia hypugea, formerly genus Speotyto) were once common and widespread in the western United States and Canada but recently have been declining over much of their range [1]. They are now listed as endangered in Canada and considered endangered or threatened in a number of states and provinces [1,2]. They are currently a species of special concern in California [3], where breeding populations are mainly near or adjacent to areas of intensive agriculture (D.F. DeSante, E.D. Ruhlen, and D.K. Rosenberg, unpublished data). One hypothesis for declining burrowing owl populations is that agricultural chemical exposure impairs their reproduction and survival.

Many of the pesticides both currently in use and previously used in the southern San Joaquin and Imperial Valleys have been found as contaminants in many species of wildlife and have been documented to have detrimental effects. Organochlorine compounds in particular are notorious for their effects on the survival and reproduction of birds, causing eggshell thinning and embryo toxicity [4,5], impaired development [6,7], and impaired nervous system function [8]. Both DDT and its analogs continue to be detected in the soils of California [9] and remain widespread as contaminants in wildlife, especially birds [10-13]. The contaminant concentration at which the reproduction and survival of many species are affected is not known, but in any case the concentrations found may bioaccumulate to dangerous levels in accipiters, falcons, and owls [14-16]. Contamination by dichlorodiphenyldichloroethylene (DDE) has been documented in Canadian burrowing owls [17].

Organophosphorus and carbamate compounds have been implicated in the direct mortality of a number of wildlife species $[18,19]$, including burrowing owls $[20,21]$.

Although selenium is a naturally occurring trace element, not an agricultural pesticide, it leaches from soils with irrigation and can bioaccumulate and cause wildlife mortality [22]. Because it is capable of injuring wildlife in both aquatic and terrestrial food webs $[22-24]$, it also has the potential to impact burrowing owl populations.

We undertook this study to explore the potential for agricultural contaminants to cause declines in burrowing owl populations in California, USA. We sampled owls breeding in areas of large-scale industrial agriculture in the southern San Joaquin and Imperial Valleys; these regions are among the most intensively farmed agricultural lands in the United States [25]. The Imperial and San Joaquin Valleys also include the breeding ranges of over 90% of the burrowing owls in California (D.F. DeSante, E.D. Ruhlen, and D.K. Rosenberg, unpublished data). In both areas, owls commonly nest within 1 km of agricultural fields receiving substantial chemical input [26]. We also collected reference samples from owls in the Carrizo Plain Natural Area southwest of the San Joaquin Valley. This site once supported agriculture of a much lower intensity and has not been farmed since the early 1980s.

MATERIALS AND METHODS

Study sites

We sampled owls from two sites in the San Joaquin Valley, from a single site in the Imperial Valley, and from within the Carrizo Plain. Naval Air Station Lemoore (NAS Lemoore),

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located 50 km southeast of Fresno, California, USA, was chosen to represent the San Joaquin Valley. The majority of the land on the base is leased to surrounding farms, and the owls nest in small. grassy areas surrounded by intensively farmed fields. Cotton is the major crop grown both on the base and within the region [26], and the birds forage in the fields and along drainage ditches. A second San Joaquin Valley study site near the Pixley National Wildlife Refuge (NWR), 10 km southwest of Pixley, California, USA, was sampled in 1998 following analysis of the 1996 NAS Lemoore samples. The crops grown in the rcgion surrounding this second site include alfalfa and winter wheat [26]. The samples from the Imperial Valley were obtaincd frorn owls breeding at the Salton Sea National Wildlife Refuge, 40 km north of El Centro, California, USA. Much of the refuge is also under agricultural production, although pesticide use is restricted on the refuge itself. The lands surrounding the refuge are intensively farmed with a variety of crops, including carrots, lettuce, melons, and suentity of the owls use artificial nest burrows placed on the roadsides and forage in the surrounding agricultural fields both on and off the refuge. The Carrizo Plain Natural Area is a 73,000-ha reserve located 100 km west of Bakersfield, CA, USA. Carrizo once supported nonirrigated grain production but has not been farmed for nearly two decades (A. Kuritsubo, personal communication). It served as our nonagricultural reference site.

Sample collection

We located nests by walking transects in likely areas and flushing owls from burrow entrances. We used a Christensen Designs (Manteca, CA, USA) infrared video camera (location) probe to examine burrow contents and removed eggs by digging access holes into the burrow tunnel ncar thc nest chamber. We collected eggs from artificial nest burrows within the refuge by removing the tops of the nest boxes and removing one egg. Eggs were stored unfrozen on ice or refrigerated until delivered to the laboratory for storage, processing, and subsequent analysis.

We captured adult owls to collect footwash and feather samples with a combination of one-way door and tomahawk traps set at the entrance to the nest burrow [27]. Owls were held in traps for no more than 2 h, and captured owls in the trap came into contact with only the soil at the entrances of their burrows.

We washed the feet of each captured owl using a solution of 95% ethanol and a solvent-rinsed toothbrush and collected the rinse solution in an acetone-rinsed, prelabeled glass jar that we placed on ice immediately after sample collection. The tips of 30 body feathers were removed from the breast, sides, and back of each owl. We stored feather samples in acetone-rinsed, prelabeled glass jars placed on ice.

We collected regurgitated pellets and noted prey remains for each burrow during each visit: pellets were later examined for prey species composition. Pellets were collected from burrows at the Salton Sea National Wildlife Refuge in 1997.

Sample analysis

All chemical analyses were performed by the California Diagnostic Veterinary Laboratory in Davis. California, USA. A list of the analytes tested for and the detection limits are listed in the Appendix; full details of laboratory methods are given elsewhere [28]. Whole eggs and feather samples were homogenized prior to laboratory analysis. A11 sample matrices

were analyzed similarly. For organophosphate compounds. samples were extracted with 5% ethanol in ethyl acetate and cleaned up using automated gel permutation chromatography (model 1002A, Autoprep GPC, ABC Laboratories, Columbia, MO, USA). Samples were then analyzed using gas chromatography with flame photometric detector (Hewlett-Packard Model 5890. Hewlett-Packard, Avondale, PA, USA). For organochlorine contaminant determination, including polychlorinated biphenyls, samples were again extracted with 5% ethanol in ethyl acetate and cleaned up using automated gel permutation chromatography. Gas chromatography was then used with an electron capture detector (Perkin-Elmer Model Sigma 2000, Perkin-Elmer, Norwalk, CT, USA). All confirmations were done using mass spectrometry. Selenium in egg samples was analyzed by digesting samples in nitric acid, followed by oxidation using perchloric acid. Samples were then analyzed using inductively coupled plasma mission spectrometry (Applied Research Laboratories, Austin, TX, USA) with a hydride generation system. For all sample matrices, every fourth sample was duplicated, and all sample runs were bracketed with control solutions with known analyte concentrations. Spike recoveries for analytes ranged from 70 to 110% .

Residue levels in seven of the 20 egg samples collected in 1996 and all five egg samples collected in 1998 were confirmed. All footwash and seven of 15 feather samples with initial pesticide contamination detections were confirmed.

All eggshells in this study were measured by the same investigator using a Starrett digital thickness indicator (Model 2500, Athol, MA, USA) mounted on a Federal bench comparator. Five measurements were made around the equators of the collected shells, and the mean of these measurements for each egg was used in the analyses. To examine the degree to which burrowing owl eggshells have thinned since the advent of the widespread use of synthetic pesticides, we also measured 142 archived burrowing owl eggs from a total of 45 clutches that had been collected from San Diego, Riverside, Fresno. San Luis Obispo, and Imperial Counties, California, USA, between 1878 and 1936. These eggs are now at the Western Foundation of Vertebrate Zoology in Camarillo, California. The archived eggshells were also measured along the equator, but the tiny size of the single blowhole in each egg prevented measurements from being taken at more than one point. It also prevented us from determining whether membranes had separated from the eggshells. Three measurements of this point on each egg were taken, and these were averaged. We measured at least two eggs from each of the 45 clutches; the means of the eggs measured from each clutch were used for analysis.

We scored the collected burrowing owl pellets on the occurrence of prey species or upper taxonomic levels on the basis of a list generated from all pellets combined. A sample was considered to be the collection of pellets made on a certain date at a specific burrow. We identified vertebrates to species when possible, but invertebrates were identified only to order with a few exceptions.

Records of 1996 pesticide use for Kings and Fresno Counties were obtained from the California Department of Pesticide Regulation [26]. From these data, we summarized which compounds were applied within 1 km of active burrowing owl nests at NAS Lemoore after January 1, 1996.

Data analysis

For statistical analyses, half the minimum detectable limit value was assigned to samples falling below the detection limit. When more than one sample was taken from the same individual. the average value was used.

We adjusted contaminant concentrations in eggs to reflect fresh-wet concentrations [29] by estimating egg volume with the equation $K \times L \times B^2$, where K is a dimensionless constant 0.00051 and L and B are length and width of the egg in millimeters, respectively.

We tested for differences in selenium and pesticide concentrations in cggs and eggshell thicknesses among sites using one-way analysis of variance. Selenium and p, p' -dichlorodiphenyldichloroethylene $(p, p-\text{'DDE})$ data were log-transformed to meet assumptions of normality and equality of variances prior to analysis. We also performed a regression analysis using eggshcll thickness as the dependent variable and the log-transformed concentrations of p, p' -DDE as the independent variable. The eggshell measurements of the eggs laid prior to 1937 and those collected for this study were compared using a t test. Most of the archived eggs were from nests in San Diego County; we compared these eggs to archived eggs from other counties using a t test to ensure that they did not reflect any geographic differences.

RESULTS

No sampled nests showed signs of subsequent disturbance or abandonment, and all but three nests produced at least one crnergent chick. Subsequent sitc visits included observations of chicks present above ground, but we did not attempt to determine fledging success.

Although many chemicals were applied in 1996 to agricultural fields surrounding our study site at Lemoore NAS, the two compounds most relevant to the work presented here in terms of usage and potential hazard to wildlife were aldicarb and chlorpyrifos. Aldicarb was applied to fields within 1 km of active nest burrows on 15 occasions in 1996 and within 3 weeks prior to our sample collecting on four occasions. Chlorpyrifos was not used until after the nesting season but was applied nine times within 1 km of active burrows in July and August.

We collected whole eggs from nine nests and an eggshell from a 10th nest at Lemoore NAS and four eggs from Carrizo Plain. Five eggs were collected from near Pixley NWR in 1998. At Salton Sea NWR, seven nests had one fresh egg removed, and an abandoned nest had two eggs collected. Six fresh eggs and one of the addled eggs were used for contaminant analyses. All eggshells except the 1998 eggs from Pixley were measured.

We collected footwash samples from 17 owls; four were recaptured and resampled, resulting in a total of 21 footwash samples (15 owls from Lemoore NAS, four from Carrizo). We also collected feather samples from 21 owls (17 from Lemoore, four from Carrizo). Blood samples were collected from the tibiotarsal joint of 21 owls, but these samples were lost when a freezer malfunctioned and thus were never analyzed. Most of the owls captured were females because of the type of trap used. Because female owls spend much of the incubation period underground, feather and footwash testing for external contaminants probably represents a conservative estimate of exposure for male owls.

The most frequently detected contaminant within the egg samples was p, p' -DDE, a metabolic product of DDT. All eggs collected from Lemoore NAS, Pixley, and Salton Sea NWR had detectable levels of p, p' -DDE. Two of the four eggs from Carrizo Plain had no detectable p, p' -DDE, and the remaining two had low levels (Table 1). Hexachlorobenzene was detected

Table 1. p, p' -DDE and sclenium concentrations (μ g/g fresh weight) and eggshell thicknesses (mm) of burrowing owl eggs

Variable	Site				
	Lemoore	Salton Sea	Carrizo Plain	Pixley	
DDE					
Mean	7.52	0.62	0.10	1.19	
Range	$1.50 - 33.00$	$0.20 - 3.40$	$0.09 - 0.30$	$0.70 - 2.82$	
n	9	7	$\overline{4}$	5	
Selenium					
Mean	0.404	0.365	0.423		
Range		$0.335 - 0.840 - 0.319 - 0.476$	$0.383 - 0.487$		
n	9	7	4		
Eggshells					
Mean	0.169	0.183	0.183		
SE.	0.004	0.004	0.003		
Range	$0.150 - 0.183$	$0.170 - 0.194$	$0.175 - 0.187$		
\boldsymbol{H}	10	O)	4		

^a Geometric means are given for contaminants, and the arithmetic mean is given for shell thickness. The minimum detectable level of $p.p'$ -DDE and selenium were 0.1 and 0.005 μ g/g fresh weight, respectively. Eggs collected from Pixley were not analyzed for selenium, and eggshells were not measured. Selenium levels that were considered normal ranged from 0.2 to $1.5 \mu g/g$ wet weight.

at $0.11 \mu g/g$ in one egg from Lemoore, and polychlorinated biphenyls were detected in two eggs from Lemoore (1.4 μ g/g and 2.8 μ g/g). These same eggs contained 32.82 μ g/g, 4.16 μ g/g, and 4.33 μ g/g p,p'-DDE, respectively. Selenium was detected in all eggs but in small quantities (Table 1). Sites did not differ in selenium concentrations ($F = 0.54$, $p = 0.59$). No organophosphate compounds were detected in eggs.

Mean eggshell thicknesses varied among sites (Table 1). Sites differed in both eggshell thicknesses ($F = 5.17$, $p =$ 0.02) and p, p' -DDE concentrations ($F = 24.94, p < 0.01$). Eggshell thickness was negatively related to p, p' -DDE concentrations ($p = 0.03$), but DDE concentrations explained only 22.1% of the variance in eggshell thicknesses (Fig. 1). Eggs collected from Lemoore NAS had the thinnest shells and contained the highest concentrations of p, p' -DDE.

Eggshells collected in 1996 were, on average, 22.6% thinner than those collected prior to 1937 (arithmetic mean, \bar{x} = 0.177 mm, SE = 0.0026, range = 0.150 - 0.198 mm, $n = 23$, and $\bar{x} = 0.229$ mm, SE = 0.0027, range = 0.171-0.265 mm,

Fig. 1. Relationship between p, p' -DDE concentration and eggshell thickness. $r = 0.2394$, $p = 0.02$.

Table 2. Prey items most frequently identified in burrowing owl pellets^a

Item	Carrizo	Lemoore	Salton Sea
Coleoptera	100	87.6	79.1
Orthoptera	100	90.7	81.4
Rodentia	82.9	89.7	58.1
Microtus	8.6	52.6	9.3
P/M/P ^b	31.4	30.9	37.2
Thomomys	20.0	2.1	14.0
Dipodomys	11.4	θ	θ
Araneae (spiders)	62.9	42.3	48.8
Amphibia	5.7	40.2	9.3
Reptilia	11.4	θ	2.3
Solifugae	28.6	19.6	23.3
Birds	20.0	4. I	18.6
Scorpions	8.6	θ	4.7
Rocks	37.1	13.4	18.6
Sand and vegetation	14.3	39.2	2.3
Plastic	11.4	5.2	7.0
Dermaptera	θ	18.6	60.5
Chilopoda	2.9	4.1	θ
Aquatic organisms ^e	θ	θ	11.6

^a Percentages refer to the proportion of pellets containing the remains of at least one individual of each prey category. Values for Carrizo Plain are based on a total of 35 samples from 12 nests, the values for Lemoore Naval Air Station are based on 97 samples from 17 nests, and Salton Sea data represent 43 samples from 39 nests.

^b Remains of the rodents Peromyscus maniculatus gambelli, Mus musculus, and the Perognathonae were not differentiated from each other and are combined into the category P/M/P.

^c Aquatic organisms included snails, bivalves, and fish scales.

 $n = 45$, respectively; $t = 12.06$, $p < 0.001$, $df = 66$ adjusted for unequal variances). Because the Lemoore NAS eggs were thinner than those collected from Carrizo Plain and Salton Sea NWR, we also compared the recent eggs excluding the Lemoore samples with archived eggs. The results were very similar, indicating that they were not dependent solely on eggshell thinning from Lemoore (recent eggs without Lemoore, \bar{x} = 0.183 mm, SE = 0.0029, range = 0.160–0.198 mm, $n = 13$; $t = 11.41$, $p < 0.001$, $df = 56$ adjusted for unequal variances). We also randomly subsampled the archived eggshell measurements to perform all analyses with equal sample sizes; the results were unchanged. The archived eggs did not differ in measurements among counties ($F = 1.03$, $p = 0.40$), so the sampling bias toward San Diego County did not affect the results.

Organophosphorus insecticides were not detected in feather samples in amounts greater than the minimum detectable levels. Most of the samples from Lemoore NAS had traces of p, p' DDE (geometric mean $\bar{x} = 0.214$ ppm, range 0.05–1.02 ppm, $n = 17$), but no traces were found in the Carrizo feather samples. The only compound detected in the footwash samples was chlorpyrifos, in samples from Lemoore (geometric mean \bar{x} = 24.39 ng/bird, 12.5–35 ng/bird, $n = 15$).

To evaluate burrowing owl diet, we collected a total of 175 samples comprised of over 1,100 pellets. Owls at all sites appeared to rely heavily on grasshoppers, crickets, beetles, and small rodents (Table 2). Owls at Lemoore NAS commonly consumed toads, which appeared to be abundant in the spring and persisted in drainage ditches throughout the spring and summer. Owls at the Salton Sea NWR frequently captured organisms from the irrigation canals, including bivalves, snails, and scavenged fish carcass (Table 2).

Table 3. Levels of DDE in eggs implicated in impaired reproduction in various bird species

	Fresh weight concentration		
Species	$(\mu$ g/g)	Comments	Source
Barn owl	16	Nest failure; $5 \mu g/g$ no-effects limit suggested	1141
Brown pelican	3	Total reproductive failure at 4 μ g/g DDE	[43]
Bald eagle	5.	Decreased reproduction	[44–45]
Osprey	14	Addled egg samples, associat- ed with decreased reproduc- tion	1461
Peregrine falcon	20	Correlated with 18% shell thinning, declining repro- duction	1471
Prairie falcon	2	Decreased reproduction	1481
Merlin	6	Decreased reproduction	[48]
Black-crowned	8	Broken shells, decreased re-	1491
night-heron		production	[50]
White-faced ibis	4	Decreased reproduction	[51]
Black duck	6	Decreased reproduction, thin- ner shells	1521

DISCUSSION

Only a very few pesticides were detected in our samples, and total polychlorinated biphenyls in eggs were not elevated in association with p, p' -DDE. However, the most significant finding is the high concentrations of p, p' -DDE in the owls' eggs. Despite a quarter-century ban on its use in the United States, DDT and its metabolites remain available for uptake and bioaccumulation in wildlife species in parts of the San Joaquin Valley. Burrowing owls appear to be less sensitive than other birds studied previously to the effects of p, p' -DDE on reproductive success, as the levels of DDE detected in the eggs of this study would cause reproductive failure in many other species of birds (Table 3). Because we were unable to closely follow reproductive success, we cannot determine whether the contamination levels we detected might be associated with lowered reproductive rates; however, given the 20.6% overall eggshell thinning since 1937 (but see [30] for other pre-1945 eggshell measurements) and the fact that the levels of p, p' -DDE we found have caused decreased reproduction in other raptors, it seems plausible that at least some owl pairs are being adversely affected.

The primary route of exposure to DDT and its metabolites for burrowing owls is likely to be through the food chain. Owls sampled in this study took a wide variety of prey, which in turn occupy many different trophic levels. Both DDT and its metabolites remain widely distributed in the agricultural soils of California statewide, and concentrations up to 3,750 ppb have been found in the San Joaquin Valley [9]. Our results suggest that the residues are patchily distributed; at the least, uptake by organisms is occurring at different rates at different sites within the same region. This pattern is further compounded by the variations in the owls' diets among sites.

Organophosphate and carbamate compounds pose a threat to wildlife through direct mortality and nonlethal impacts despite their lack of environmental persistence [18,31,32]. Organophosphorus pesticide exposure has been shown to alter avian behavior, making them more susceptible to predation or leading to neglect of their young [33,34]. The incidence of chlorpyrifos in the footwash samples of burrowing owls at Lemoore NAS and the documented use of aldicarb near active

nest burrows indicate that this population is at risk of exposure to organophosphate and carbamate insecticides applied to the local farm fields. Although other work has examined footwash contamination in relation to exposure [35], we found no data linking specific residue levels to physiological or behavioral effects. Rather, feather and footwash samples simply indicate that the bird was recently exposed to vegetation or soil with surface residues of the pesticides. The exception is the p, p' DDE found within the feather samples; p, p' DDE most likely was ingested in the form of contaminated prey, and feather loads probably represent excretion of the compound through the uropygial gland 1361, fiom which it would be spread over the feathers during preening. Further research into owls' use of agricultural fields and whether use shifts with spraying activity will help clarify the risk to local owl populations. Sampling done in conjunction with habitat use studies and timed to coincide with spray schedules will be necessary to assess the exposure risk of owls living near fields where organophosphorus compounds are applied, as behavior patterns influence the risk of exposure 137,381.

The risk posed by selenium is also difficult to assess directly. Selenium concentrations vary in the Imperial Valley, although concentrations potentially harmful to wildlife have been found in both sediments and biota [39]. Selenium levels available for biological uptake are variable through time, depending on a number of environmental factors, including soil profile, temperature, rainfall, and pH $[40-42]$. Given that the burrowing owls in the Salton Sea NWR include many aquaric organisms in their diet, they might be at risk from elevated selenium levels at various times of the year when more selenium is mobilized and available for uptake.

In conclusion, burrowing owls living in at least one site in the San Joaquin Valley are exposed to high levels of p, p' -DDE and may suffer impaired reproduction or survival as a result. In addition, the current load of p, p' -DDE may make the birds more susceptible to debilitating effects of pesticides still in use, such as chlorpyrifos and aldicarb, if exposure to these pesticides occurs when owls with high body burdens of p, p' -DDE are already compromised by mobilizing fat stores in response to stressors, such as breeding efforts. Further research on burrowing owl demography, combined with studies of habitat use and pesticide exposure, should prove helpful in determining whether the results presented here represent a substantial threat to the long-term survival of the species.

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APPENDIX

Organophosphate and organochlorine compounds tested for in egg, feather, and footwash analyses. Detection limits for each are listed in parentheses. Units are $\mu g/g$ for egg and feather analyses, and ng/bird for the footwa

