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Title: 2019 Results for Avian Monitoring of Inorganic and Organic Element Concentrations in Passerine Eggs and a Nestling Collected from Technical Area 16 Burn Grounds, Technical Area 36 Minie, and Technical Area 39 Point 6 at Los Alamos National Laboratory

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2019 Results for Avian Monitoring of Inorganic and Organic Element Concentrations in Passerine Eggs and a Nestling Collected from Technical Area 16 Burn Grounds, Technical Area 36 Minie, and Technical Area 39 Point 6 at Los Alamos National Laboratory

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Prepared for: U.S. Department of Energy, National Nuclear Security Administration, Los Alamos Field Office

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ACRONYMS AND TERMS

ALS	Australian Laboratory Services
EPA	Environmental Protection Agency
LANL	Los Alamos National Laboratory
LOAEL	slowest observable adverse effect levels
mg/kg	milligrams per kilogram
pg/g	picograms per gram
PCBs	polychlorinated biphenyls
RSRLs	regional statistical reference levels
TA	Technical Area
TCDD	tetrachlorodibenzodioxin-2,3,7,8
TEF	toxic equivalent factors
TEQ	toxic equivalents
WHO	World Health Organization

1.0 SUMMARY

In 2019, non-viable avian eggs and one nestling were opportunistically collected at Los Alamos National Laboratory (LANL) near open detonation sites located at Technical Area (TA) 16 burn grounds, TA-36 Minie, and TA-39 Point 6. These samples were evaluated for inorganic elements (mostly metals), polychlorinated biphenyls (PCBs), dioxins, and furans. A total of 26 western bluebird (*Sialia mexicana*) and five mountain bluebird (*Sialia currucoides*) egg samples and one non-viable ash-throated flycatcher (*Myiarchus cinerascens*) nestling were collected among the three locations of interest. Concentrations of inorganic elements observed in this study were compared with the regional statistical reference level (RSRL) which is the upper-level bounds of background concentrations (mean + three standard deviations = 99% confidence interval). Several inorganic elements were not detected in avian eggs and the majority of inorganic elements detected were below the RSRL. The few elements that exceeded the RSRL were below the lowest observable adverse effect level (LOAEL), when available. One nestling collected from TA-16 contained detectable concentrations of some dioxin and furan congeners. Octachlorodibenzodioxin-1,2,3,4,6,7,8,9 concentration exceeded the RSRL, but did not exceed the calculated tetrachlorodibenzodioxin-2,3,7,8 (TCDD) toxic equivalent LOAEL. PCBs were also detected in the nestling sample and were above the RSRL but below the LOAEL. These data suggest that inorganic and organic element concentrations in eggs and nestlings are not of ecological concern. More data are needed to make a robust assessment and to evaluate trends over time.

2.0 INTRODUCTION

In support of the Resource Conservation and Recovery Act (RCRA) permit process, Los Alamos National Laboratory (LANL) began annual avian monitoring in 2013 around TA-16 burn grounds and at two firing sites, TA-36 Minie and TA-39 Point 6. Biomonitoring is an important tool for assessing environmental contamination by analyzing chemicals or their metabolites from biological tissues (Becker 2003). Avian eggs and nestlings are useful as bioindicators because different species occupy many trophic levels. Additionally, the collection of non-viable eggs and/or nestlings that die of natural causes is noninvasive and is nondestructive to populations. Inorganic elements and organic chemicals can pose risks of adverse effects to birds if exposed at high enough concentrations (Jones and de Voogt 1999). Levels of some constituents in biological tissues can also indicate whether adverse effects could be expected (Gochfeld and Burger 1998). Examining population parameters along with tissue concentrations provides a more comprehensive and robust assessment of potential impacts caused by environmental pollution.

Several congeners of PCBs, dioxins, and furans elicit similar toxic effects (i.e., immunotoxicity, carcinogenicity, and endocrine disruption) as those caused by tetrachlorodibenzodioxin-2,3,7,8 (TCDD), the most potent in this class of chemicals (Van den Berg et al. 2006). These congeners, like TCDD, have a high binding affinity to the aryl hydrocarbon receptor (Van den Berg et al. 2006). The World Health Organization (WHO) developed toxic equivalency factors (TEFs) for TCDD-like compounds that can be used to determine the relative potency, or toxic equivalents (TEQs), of dioxin-like compounds for different classes of animals (i.e., fish, birds,

and mammals), as well as to facilitate risk assessment for TCDD-like exposure (Van den Berg et al. 2006).

Sources of inorganic elements include both anthropogenic and natural sources; birds can be exposed through a number of routes, including diet, ingestion of soil, drinking water, and inhalation. Inorganic elements (mostly metals), dioxins, and furans are of interest at open-detonation firing sites (TA-36 and TA-39) and at the burn grounds at TA-16 (Fresquez 2011).

3.0 OBJECTIVES

The objective of this study is to document chemical concentrations in eggs and nestlings collected near TA-16 burn grounds, TA-36 Minie, and TA-39 Point 6 and to compare concentrations of inorganic elements, polychlorinated biphenyls (PCBs), dioxins, and furans observed in this study with the upper-level bounds of background concentrations.

4.0 METHODS

4.1. Sample Collection

Eggs and nestlings were collected from nest boxes when they were determined to be non-viable, based on documented timing of known incubation periods for the species. In 2019, warm temperatures in the early spring and then a period of very cold temperatures led to higher than usual numbers of non-viable eggs. We collected a total of 31 non-viable eggs at LANL near the TA-16 burn grounds (Figure 1) and near open detonation sites TA-36 Minie (Figure 2) and TA-39 Point 6 (Figure 3). At TA-16, 22 non-viable western bluebird (*Sialia mexicana*) samples and one non-viable ash-throated flycatcher (*Myiarchus cinerascens*) nestling sample were collected and submitted as six composite samples and one individual sample, respectively. At TA-36, five non-viable mountain bluebird (*Sialia currucoides*) eggs were collected and submitted as one composite sample. At TA-39, four non-viable western bluebird eggs were collected and submitted as one composite sample. All samples were collected May through July of 2019. Concentrations of chemicals in eggs and nestlings have been monitored annually at these locations since 2014.

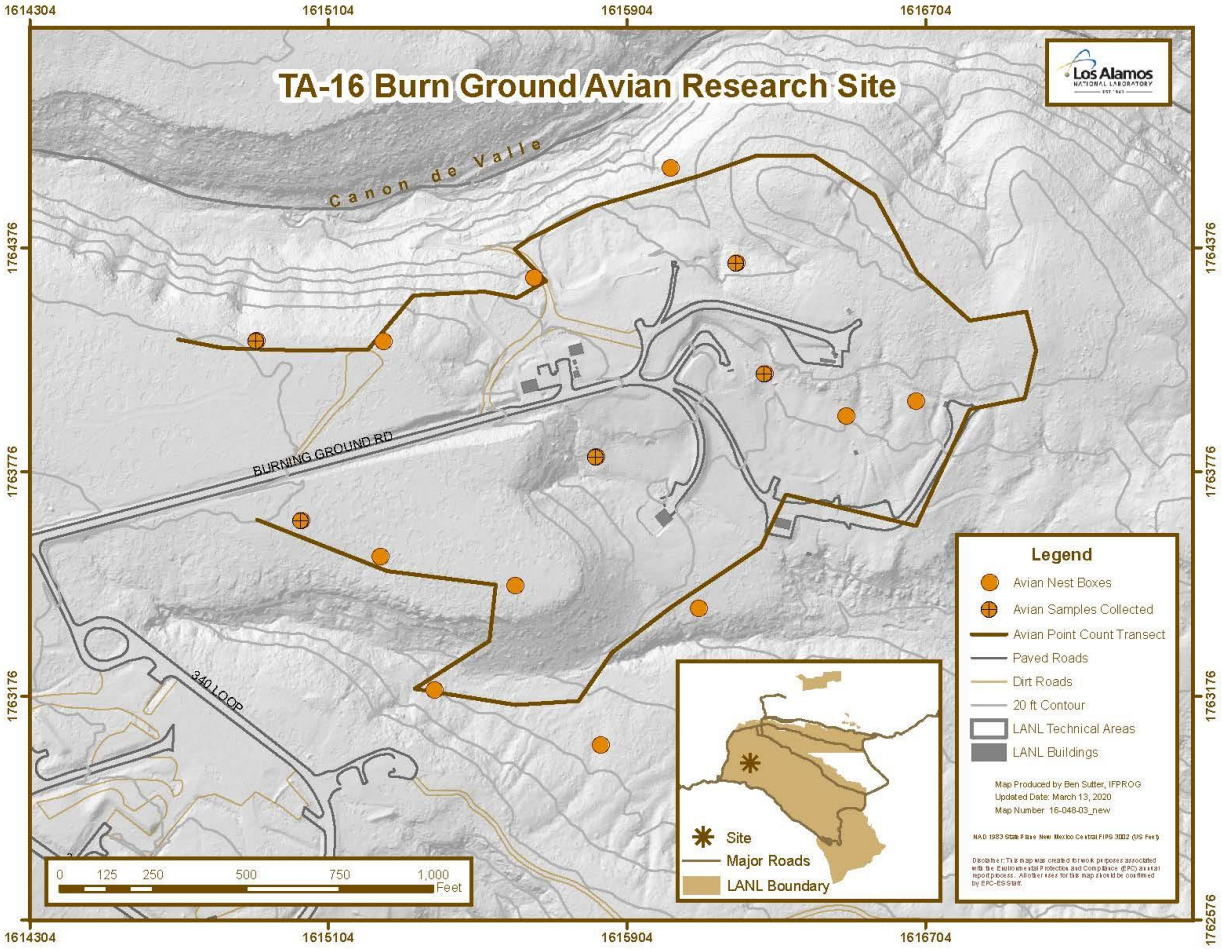

Figure 1. Avian nest box locations around TA-16 burn grounds.

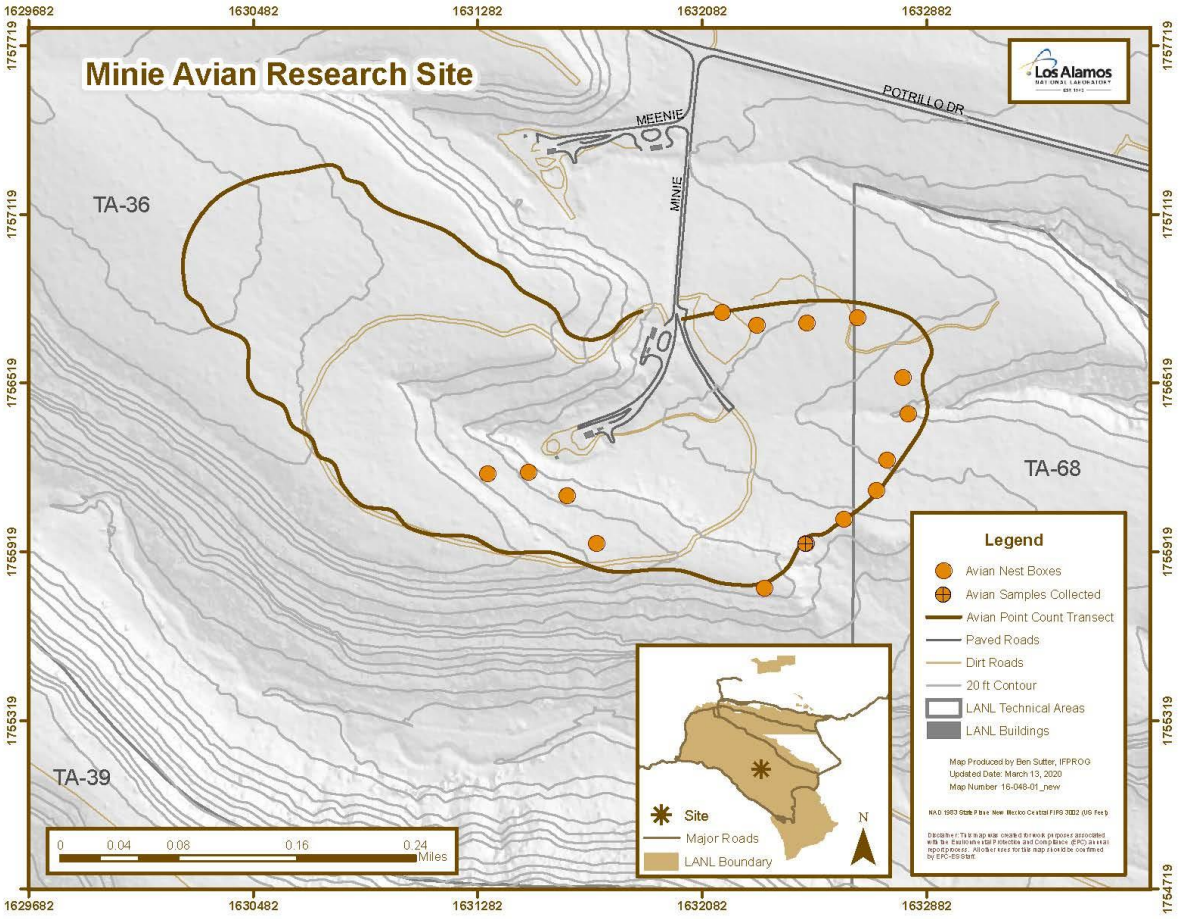

Figure 2. Avian nest box locations around TA-36 Minie.

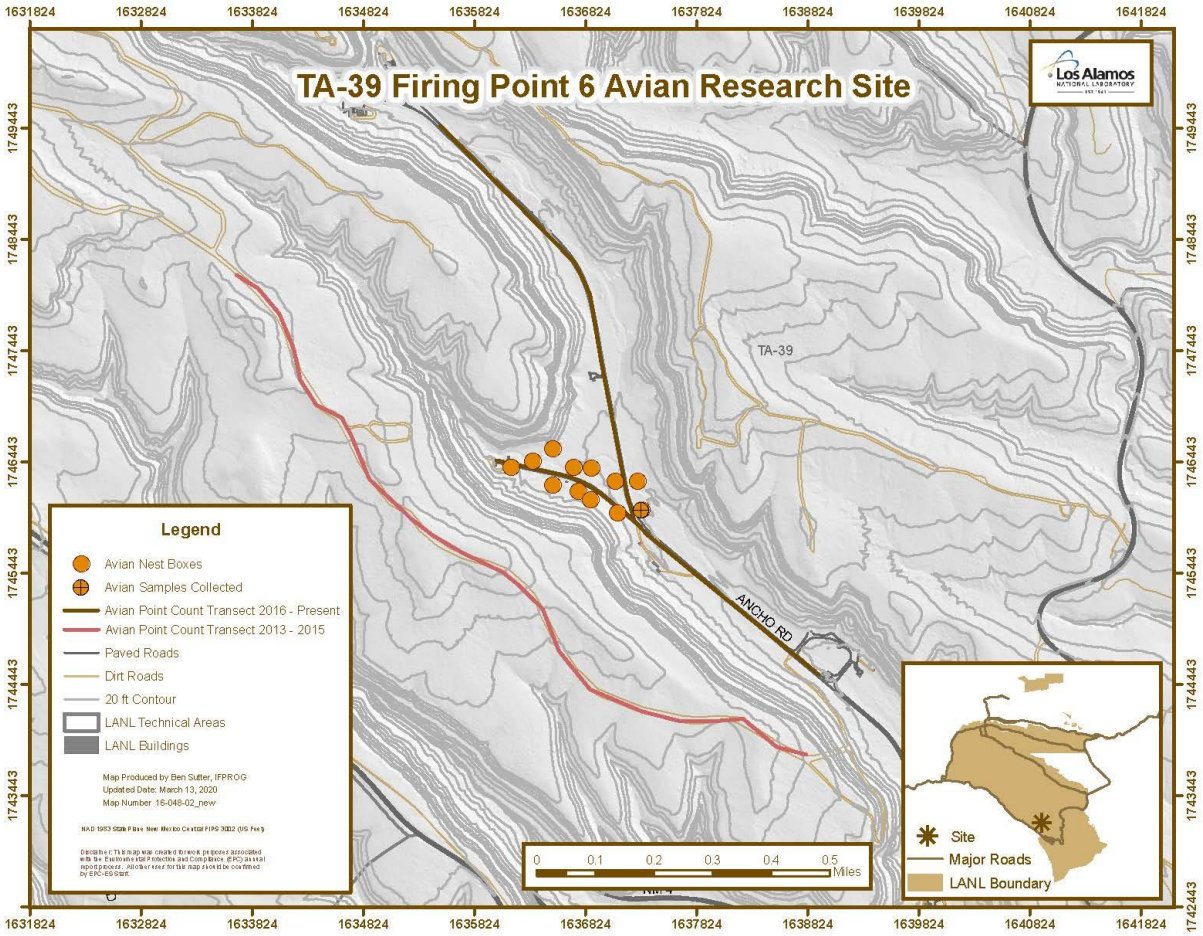

Figure 3. Avian nest box locations around TA-39 Point 6.

4.2. Chemical Analyses

Due to limited sample mass, non-viable eggs were analyzed for total analyte list (mostly inorganic metals) only and were analyzed at ALS (Australian Laboratory Services, formerly Paragon Analytics, Inc.) in Fort Collins, Colorado. Antimony, arsenic, cadmium, lead, selenium, silver, and thallium concentrations were measured in egg samples by inductively coupled plasma mass spectrometry (Environmental Protection Agency [EPA] SW-846 Method 6020A), and aluminum, barium, beryllium, calcium, chromium, cobalt, copper, iron, magnesium, manganese, nickel, potassium, sodium, vanadium, and zinc were measured by inductively coupled plasma atomic emission spectrometry (EPA SW-846 Method 6010B). Mercury was measured by cold-vapor atomic absorption procedure (EPA SW-846 Method 7471A). All inorganic element results were reported on an mg/kg (milligram per kilogram) dry weight basis.

The non-viable nestling sample collected near TA-16 was analyzed for PCB congeners by EPA Method 1668A and dioxin/furan congeners by EPA SW-846 Method 8290 at Cape Fear Analytical LLC, Wilmington, North Carolina. All organic chemical results are reported on a wet weight basis.

4.3. Statistical Methods

The 2019 results were compared with the regional statistical reference levels (RSRL), which represents natural and fallout levels of chemicals, and are the upper-level bounds of background concentrations (mean + three standard deviations = 99% confidence interval). Regional statistical reference levels were calculated from non-viable eggs of western bluebirds and ash-throated flycatchers collected from Bandelier National Monument from 2016 through 2019 (n = 23). Non-viable egg results are also compared with the lowest observable adverse effect levels (LOAEL) from peer reviewed literature, when available.

In the nestling, TCDD-like TEQs were calculated for all aryl hydrocarbon–binding PCB, dioxin, and furan congeners; these include:

- non-*ortho*-substituted PCB congeners 77, 81, 126, and 169
- mono-*ortho*-substituted PCB congeners 105, 114, 118, 123, 156, 157, 167, and 189
- tetrachlorodibenzodioxin-2,3,7,8
- pentachlorodibenzodioxin-1,2,3,7,8
- hexachlorodibenzodioxin-1,2,3,4,7,8
- hexachlorodibenzodioxin1,2,3,6,7,8
- hexachlorodibenzodioxin-1,2,3,7,8,9
- heptachlorodibenzodioxin-1,2,3,4,6,7,8
- octachlorodibenzodioxin-1,2,3,4,6,7,8,9
- tetrachlorodibenzofuran-2,3,7,8
- pentachlorodibenzofuran-1,2,3,7,8
- pentachlorodibenzofuran-2,3,4,7,8
- hexachlorodibenzofuran-1,2,3,4,7,8
- hexachlorodibenzofuran-1,2,3,6,7,8
- hexachlorodibenzofuran-1,2,3,7,8,9
- hexachlorodibenzofuran-2,3,4,6,7,8
- heptachlorodibenzofuran-1,2,3,4,6,7,8
- heptachlorodibenzofuran-1,2,3,4,7,8,9
- octachlorodibenzofuran-1,2,3,4,6,7,8,9

Each congener was multiplied by its respective avian-specific WHO TEF (Van den Berg et al. 2006), and added together for a total TEQ for the nestling sample. PCB congeners 156 and 157 co-eluted, and therefore were treated as one; the WHO TEFs were the same for both PCB congeners.

Nestling sample results of PCBs, dioxin, furans, and TEQs were compared with RSRLs and LOAELs, when available. The nestling RSRL was calculated from non-viable nestlings of western bluebirds and ash-throated flycatchers at background locations from Bandelier National Monument in 2018 and 2019 (n = 4 samples).

5.0 RESULTS AND DISCUSSION

Similar with previous years, many of the inorganic elements assessed in this study were not detected in passerine egg samples. Several elements are not (or very little is) maternally transferred into eggs or do not accumulate in eggs and include cadmium (Leach et al. 1979; Stoewsand et al. 1986), lead (Pattee 1984), vanadium (White and Dieter 1978), and silver (Schwarzbach et al. 2006; Seiler and Skorupa 2001), which may explain why these elements were mostly not detected.

Similarly, most dioxins and furans were not detected in the nestling sample collected from TA-16 burn grounds. Most constituents that were detected in eggs and the nestling were below RSRLs, and all constituents were below the LOAELs, when available.

5.1. TA-16 Burn Grounds

Western bluebird eggs collected from nest boxes at TA-16 burn grounds did not contain detectable concentrations of aluminum, arsenic, beryllium, cadmium, nickel, or vanadium. Of the elements containing detectable concentrations in eggs, only antimony, barium, and selenium were detected at concentrations above the RSRLs (Table 1). One sample out of the six collected contained slightly higher concentrations of antimony (0.27 mg/kg) compared with the RSRL (0.26 mg/kg). No reliable screening levels were available for antimony. One sample out of six collected contained higher concentrations of selenium (3.5 mg/kg) when compared with the RSRL (3.3 mg/kg dry weight). Selenium is an essential micronutrient and needed by living organisms. Additionally, the selenium concentration was far below the LOAEL of 10 mg/kg dry weight (Heinz et al. 1989).

Two samples out of six collected contained higher concentrations of barium (68 and 210 mg/kg) when compared with RSRL (31 mg/kg; Table 1). Legacy barium in the canyon sediment is known to occur in the area (Reid 2003) and has been detected in water samples near Cañon de Valle, which may suggest that birds may be exposed by direct drinking of water, and then the constituent is maternally transferred to their eggs. No reliable screening levels were available for barium; therefore, it is unknown at what concentrations adverse effects could be expected. However, of the non-viable egg samples collected at TA-16 burn grounds since 2016 ($n = 17$), only five of them contained barium concentrations above their respective RSRLs. Additionally, percentages of eggs hatched in nest boxes at TA-16 burn grounds ($n= 48$) compared with nest boxes at the background location ($n = 120$) were not statistically different (Mann-Whitney U test; $p > 0.05$). The hatching success in nest boxes at TA-16 burn grounds ($n = 48$) was 74.5% and was consistent with those reported previously for the area (Fair and Myers 2002). Barium did not have a negative impact on eggshell thickness when TA-16 burn grounds ($n = 40$) data was compared with background locations ($n = 54$; mixed-effects regression model; $p > 0.05$). These results suggest that adverse effects at the population level are unlikely to occur.

PCBs were detected in the nestling sample at 0.0126 mg/kg and were above the RSRL of 0.0045 mg/kg but well below the LOAEL in avian eggs of 3.0 mg/kg (Hoffman et al. 1996). Thus, even though the PCB concentrations were higher than the RSRL, these levels are not expected to negatively impact the bird population.

Most dioxins and furans were not detected in the nestling sample collected from TA-16 burn grounds. The nestling contained detectable concentrations of octachlorodibenzodioxin-1,2,3,4,6,7,8,9 of 7.65 mg/kg, which exceeds the RSRL of 2.42 mg/kg. Lowest observable adverse effect levels were not available for each dioxin and furan congener. However, the most potent dioxin congener, TCDD, is found to induce toxic effects in eastern bluebirds (*Sialia sialis*) when egg concentrations are between 0.001 and 0.01 mg/kg (Thiel et al. 1988). The TCDD toxic equivalency factor of octachlorodibenzodioxin-1,2,3,4,6,7,8,9 for avian species is 0.0001 (Van den Berg et al. 2006). Multiplying the detectable concentration of 7.65 mg/kg by the toxic equivalency factor yields a value of 0.000765 mg/kg, which was much less than the TCDD

LOAEL observed in eastern bluebird eggs. Total toxic equivalents (dioxin-like PCB, dioxin, and furan congeners) were 13.63 pg/g and were well below the RSRL of 23.51 pg/g.

5.2. TA-36 Minie

The one mountain bluebird egg sample, collected from TA-36 Minie, did not have detectable levels of several elements, including aluminum, antimony, arsenic, beryllium, cadmium, nickel, silver, or vanadium. Detectable concentrations of barium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, potassium, selenium, sodium, thallium, and zinc were all below the RSRL (Table 2). Mercury and selenium concentrations were well below LOAELs (Heinz et al. 1989, Thompson 1996); no other LOAELs were available.

5.3. TA-39 Point 6

One egg sample collected from a nest box at TA-39 Point 6 did not contain detectable concentrations of aluminum, arsenic, beryllium, cadmium, chromium, lead, nickel, silver, thallium, or vanadium. Detectable concentrations of antimony, barium, calcium, cobalt, copper, iron, magnesium, manganese, mercury, potassium, selenium, sodium, and zinc were all below the RSRLs (Table 3). Mercury and selenium concentrations were well below LOAELs (Heinz et al. 1989, Thompson 1996); no other LOAELs were available.

6.0 CONCLUSIONS

The overall results indicate that the levels of constituents detected in the eggs and nestlings are not likely to cause adverse effects in breeding bird populations. Several constituents were not detected in the non-viable egg and nestling samples collected near TA-16 burn grounds, TA-36 Minie, and TA-39. Most constituents that were detected were below RSRLs and all were below the LOAELs, when available. These results suggest that the detectable concentrations observed here were not of ecological concern. More data from non-viable eggs and nestlings are needed to make a robust assessment and to examine trends over time. Evaluating avian nestling samples for high explosives are also of interest for future work as those data becomes available.

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Table 1. Inorganic element concentrations (mg/kg dry weight) detected in single or composite egg samples collected near TA-16 burn grounds compared with RSRL. The RSRL is the upper limit background concentrations (mean + three standard deviations) for passerine eggs based on data from 2016-2019 (n = 23).

Element	Western bluebird (n = 2) SFB-19-184785	Western bluebird (n = 5) SFB-19-184786	Western bluebird (n=1) SFB-19-184787	Western bluebird (n=4) SFB-19-184788	Western bluebird (n=4) SFB-19-184789	Western bluebird (n=4) SFB-19-184790	RSRL
Antimony	0.110	0.057	0.069	**0.270**	0.076	0.081	0.264
Barium	24	**68**	**210**	17	11	34	31
Calcium	2,700	2,500	3,800	3,800	2,900	2,600	5,637
Chromium	ND	0.20	ND	ND	ND	0.22	1.74
Cobalt	ND	0.089	0.077	0.046	0.041	0.063	0.354
Copper	3.10	2.30	2.80	3.90	3.30	2.80	4.85
Iron	180	170	100	130	120	150	274
Lead	ND	0.028	ND	ND	0.030	0.029	0.405
Magnesium	380	320	340	380	370	320	436
Manganese	2.80	2.70	2.00	1.20	2.40	3.40	4.47
Mercury	0.032	0.061	0.042	0.110	0.064	0.038	0.143
Potassium	7,400	6,700	6,800	8,200	7,000	6,200	11,035
Selenium	2.7	**3.5**	2.7	2.9	2.9	2.7	3.3
Silver	ND	ND	ND	ND	0.005	ND	0.040
Sodium	7,200	9,100	7,700	8,900	8,600	6,800	10,561
Thallium	ND	0.0076	0.0120	ND	0.0052	0.0090	0.0222
Zinc	65.0	65.0	43.0	62.0	51.0	52.0	95.8

ND = non-detect
Bold values indicate a detectable concentration that are higher than the RSRL.

Table 2. Inorganic element concentrations (mg/kg dry weight) detected in a mountain bluebird composite egg sample collected near the TA-36 Minie compared with RSRL. The RSRL is the upper limit background concentrations (mean + three standard deviations) for passerine eggs based on data from 2016-2019 (n = 23). No values were above the RSRL.

Element	Mountain bluebird (n = 5) SFB-19-184791	RSRL
Barium	15	31
Calcium	3,100	5,637
Chromium	0.20	1.74
Cobalt	0.049	0.354
Copper	2.90	4.85
Iron	140	274
Lead	0.042	0.405
Magnesium	330	436
Manganese	3.40	4.47
Mercury	0.011	0.143
Potassium	7,200	11,035
Selenium	2.0	3.3
Sodium	9,000	10,561
Thallium	0.0082	0.0222
Zinc	54.0	95.8

Table 3. Inorganic element concentrations (mg/kg dry weight) detected in a western bluebird composite egg sample collected near TA-39 Point 6 compared with RSRL. The RSRL is the upper limit background concentrations (mean + three standard deviations) for passerine eggs based on data from 2016-2019 (n=23). No values were above their respective RSRL.

Element	Western bluebird (n = 4) SFB-19-184792	RSRL
Antimony	0.130	0.264
Barium	10	31
Calcium	3,100	5,637
Cobalt	0.043	0.354
Copper	2.70	4.85
Iron	110	274
Magnesium	360	436
Manganese	2.20	4.47
Mercury	0.059	0.143
Potassium	6,900	11,035
Selenium	2.3	3.3
Sodium	7,200	10,561
Zinc	55.0	95.8