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Elemental and radionuclide exposures and uptakes by small rodents, invertebrates, and vegetation at active and post-production uranium mines in the Grand Canyon watershed

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HIGHLIGHTS

- First report of elements, radionuclides in biota at active, post-production uranium mines near Grand Canyon.
- Biota take up mining-related radionuclides, uranium, other elements.
- Some element concentrations remain elevated after cessation of active mining.
- Uranium, copper, arsenic, molybdenum, nickel, and lead may serve as mining signatures in biota.
- Prevalence and severity of lesions in livers and kidneys not definitively linked to mining.

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ABSTRACT

The effects of breccia pipe uranium mining in the Grand Canyon watershed (Arizona) on ecological and cultural resources are largely unknown. We characterized the exposure of biota to uranium and co-occurring ore body elements during active ore production and at a site where ore production had recently concluded. Our results indicate that biota have taken up uranium and other elements (e.g., arsenic, cadmium, copper, molybdenum, uranium) from exposure to ore and surficial contamination, like blowing dust. Results indicate the potential for prolonged exposure to elements and radionuclides upon conclusion of active ore production. Mean radium-226 in deer mice was up to 4 times greater than uranium-234 and uranium-238 in those same samples; this may indicate a potential for, but does not necessarily imply, radium-226 toxicity. Soil screening benchmarks for uranium and molybdenum and other toxicity thresholds for arsenic, copper, selenium, uranium (e.g., growth effects) were exceeded in vegetation, invertebrates, and rodents (*Peromyscus* spp., *Thomomys bottae, Tamias dorsalis, Dipodomys deserti*). However, the prevalence and severity of microscopic lesions in rodent tissues (as direct evidence of biological effects of uptake and exposure) could not be definitively linked to mining. Our data indicate that land managers might consider factors like species, seasonal changes in environmental concentrations, and bioavailability, when determining mine permitting and remediation in the Grand Canyon watershed. Ultimately, our results will be useful for site-specific ecological risk analysis and can support future decisions regarding the mineral extraction withdrawal in the Grand Canyon watershed and elsewhere.

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1. Introduction

Solution-collapse breccia pipes in the Grand Canyon region host some of the highest-grade uranium (U)-bearing ore in the United States. Mineralized breccia pipes are located on or immediately adjacent to Federal, State, and Tribal lands both north and south of Grand Canyon National Park and the Colorado River. The U ores are intergrown with co-occurring sulfide and oxide minerals, often resulting in enriched concentrations of copper (Cu), lead (Pb), molybdenum (Mo), arsenic (As), and other elements (Alpine, 2010). Although more than 150,000 metric tons of ore have been produced

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in the region to date (Energy Fuels Inc, 2014; Otton and Van Gosen, 2010), uncertainty about the potential effects of U mining on environmental and cultural resources in the region remains.

Grand Canyon National Park (GCNP) was designated a United Nations Educational, Scientific, and Cultural Organization World Heritage Site in 1979 for its exceptional beauty, having a geological record spanning the Precambrian to Cenozoic eras, and having diverse ecosystems and biological environments consisting of five of seven life zones in North America within the canyon walls (WHC, 1979). The Grand Canyon is also a refuge for dwindling ecosystems (e.g., desert riparian) and is home to endemic, rare, or endangered species (e.g., Tusayan fameflower, *Phemeranthus validulus*; Kaibab swallowtail, *Papilio indra kaibabensis*). Archaeological sites in the Grand Canyon watershed preserve ancient Native American structures and artifacts; and the region remains culturally important, serving as habitat for ceremonial and subsistence plants and animals (e.g., sagebrush, *Artemisia* spp.; pinyon pine, *Pinus edulis*; elk, *Cervus elaphus canadensis*). However, culturally-significant lands and resources are not limited to those immediately within the Grand Canyon walls. Rather, the larger regional landscapes (e.g., lands extending north into Utah and south of GCNP) also play significant roles in Native American creation stories and other religious and subsistence activities (e.g., Tikalsky and Euler, 2010). One example is Red Butte (located approximately 32 km south of GCNP), a sacred site for Native Americans, including the Havasupai Tribe. Red Butte and nearby areas are designated as a Traditional Cultural Property (TCP); and the TCP includes the Canyon Uranium Mine (35°52′59.3″ N, 112°05′46.1″ W).

In 2012, the Secretary of the U.S. Department of the Interior placed a 20-year limit on mineral extraction on federal lands in the Grand Canyon watershed (USDOI, 2012) to permit further study of the environmental effects of U mining. The public is often focused on radiation concerns of U mining, but chemical exposure to U and its co-occurring ore body elements may pose a greater toxicological risk to biota (Hinck et al., 2010). The chemical toxicity and effects of U exposure, particularly on the terrestrial food web, is a significant data gap in the scientific literature. Further, the region's semi-arid climate, seasonal storms, and wildfire potential may mobilize U and co-occurring ore body elements beyond the mine perimeters (e.g., Sims et al., 2013).

Toxicity varies among elements (e.g., speciation, solubility), exposure pathways, and biological receptors (Hinck et al., 2014). Further, elemental toxicity and the priority of the exposure pathways can be dependent on the mining life stage. For example, surficial concentrations of elements and radioisotopes in soil can increase during U ore production compared to the pre-production stage; (Cleveland et al., 2019; Hinck et al., 2014, 2017; Otton et al., 2010), and the weathering of exposed waste rock during active production and post-production prior to reclamation can enhance the environmental mobility of elements and radioisotopes (Attendorn and Bowen, 1997; Lottermoser et al., 2005). Previous studies by the U.S. Geological Survey have established pre-mining elemental and radiological baselines in soil and biota (Hinck et al., 2017; Naftz and Walton-Day, 2016); and results for Se and As in amphibian and bird tissues indicated that further monitoring of breccia pipe sites as mining progressed could be warranted. Cleveland et al. (2019) found that biota have taken up mining-related elements following decades-long chronic exposure to surficial contamination mine wastes.

Building upon these results, we aimed to determine how elemental and radiochemical concentrations in biota change over the active and post-production mining life stages. Our study was designed to compare the active and post-production results to concentrations in similar species collected from a pre-production mine site (Hinck et al., 2017) and a non-mineralized reference site, to understand important pathways of exposure, uptake, and effect endpoints. We evaluated exposure and uptake by measuring elements and radioisotopes in biological tissues (above-ground vegetation, invertebrates, small rodents); and we examined rodent livers and kidneys for microscopic lesions as evidence of sublethal biological effects. Few studies at uranium mines include exposure and biological effects; most studies in the literature compare exposure or effects data at large, open-pit mines to reference sites. In contrast, our study design allows for comparisons throughout the mining life cycle because breccia pipe deposits have small footprints and are regionally co-located. We hypothesized that exposure to elements and radioisotopes would be greater at the active-production site compared to the post-production, pre-production, and reference sites, with the potential for increased adverse effects at active-production and post-production sites. We further hypothesized that post-production site elemental concentrations would remain elevated (e.g., Cleveland et al., 2019) relative to the pre-production and reference sites due to the high degree of surface disturbance at the active and post-production sites and subsequent aeolian transport of element- and radionuclide-laden dust into the mine surrounds. Aeolian transport and atmospheric deposition of surficial soil, dust, and weathered rock are important vectors for the movement of mining-related constituents off-site and into the surrounding landscapes (Pozolotina et al., 2000; Rickard and Garland, 1983).

An additional aim was to establish tissue concentrations across mining life stages to support site-specific ecological risk assessments within the Grand Canyon watershed. Elemental concentrations in tissues are a fundamental requirement to determine toxicity and risk to biota, but empirical data from historical or active U mines in the region are limited. However, it is important to note that this study was intended as an exposure assessment, and not an ecological risk assessment. Ultimately, data from this and other studies (e.g., Cleveland et al., 2019; Hinck et al., 2017; Minter et al., 2019) will be used to (1) determine whether changes in radiation levels and chemical concentrations from U mining result in greater uptake, exposure, and biological effects in biota inhabiting the mine surrounds (USDOI, 2012); and (2) inform risk assessments and decisions on ending, extending, or modifying the mining withdrawal on Federal lands in the Grand Canyon watershed. Moreover, our biological effects approach (i.e., severity scoring of lesions in small rodents as sentinels), combined with uptake assessments, could be applied at other sites to better evaluate the extent of the impacts of U mining.

2. Methods

2.1. Study areas

We collected biota from active, post-production, and non-mineralized reference sites in 2015 (Supplemental Information [SI] Figure S1). The active production mine site (36°30′11.16″ N, 112°43′57.08″ W; Pinenut Mine) is located north of the Grand Canyon in Mohave County, Arizona, approximately 56 km south of Fredonia, AZ USA and 6 km west of Kanab Creek, a tributary of the Colorado River. The active mine produced ore in the 1980's, was on standby from 1989 to 2013, and produced ore again from 2013 until 2015, when it was closed permanently. The site was actively producing U ore during our 2015 sampling.

The post-production site (36°30′27.04″ N, 112°48′22.11″ W; Arizona 1 Mine) is located in Mohave County, Arizona, approximately 56 km south of Fredonia, AZ USA and 13 km west of Kanab Creek. The mine produced ore from 2009 to 2014, when it went on standby status. Although production could be resumed in the future, this site was considered a post-production (pre-

reclamation) location in our study. Large piles of mine waste rock were present at the post-production site during our sampling, but all ore had been removed. No milling was performed onsite; ore was shipped to a mill near Blanding, Utah, USA during active operations at both sites.

Samples were also collected from two nearby non-mineralized (i.e., no breccia pipe) reference locations. Little Robinson Tank (LRT; 36°30′00.96″ N, 112°50′30.96″ W), located approximately 2 km west of the post-production site, is an earthen stock tank and is open to grazing. The LRT reference site serves as a reference for all biological samples except Valley pocket gophers (*Thomomys bottae*). Only one pocket gopher was captured at LRT. Therefore, the reference pocket gophers (n = 5) were collected at Wild Band Reservoir (36°41′29.61″ N, 112°49′16.88″ W), which is located approximately 20 km north of both the post-production site and LRT. Wild Band Reservoir has three earthen stock tanks and is also open to grazing; the presence of trees surrounding the reservoir indicates a more permanent source of water at this location. A pre-production site (35°52′59.3″ N, 112°05′46.1″ W; Canyon Uranium Mine) has been described elsewhere (Hinck et al., 2017), but the data are included in the present paper for comparisons among mine life stages.

2.2. Sample collection

Biota were collected following the methods of Hinck et al. (2017); all collection, handling, and euthanasia procedures followed animal care and use guidelines approved by the U.S. Geological Survey (in accordance with guidelines from the National Institutes of Health under the auspices of the National Research Council, 2011) and allowed under Arizona Game and Fish Department's Scientific Collecting Permit (#SP715011). Digital photos were taken of all specimens to confirm and record species identification. Collection locations were georeferenced using hand-held global positioning system navigation units. All biota were collected outside of the fenced mine (active and post-production site) perimeters; collections were made within 200 m of the mine yards. There was no perimeter at the non-mineralized reference sites; however, collections were made using the same random approach as at the mine sites.

Vegetation (e.g., above-ground tissues: blades, stems, leaves) was collected using a random sampling approach, identified to species, and composited for elemental and radiochemical analyses by functional group (i.e., forb, grass, and shrub). Vegetation collection polygons at each site were similar to those used at the pre-production site (Hinck et al., 2017); vegetation samples were collected along three transects per polygon (Hinck et al., 2014; Mann and Duniway, 2020). Composited samples consisted of a mix of species that represented vegetation from the entire sampling polygon (LRT reference site: n = 24 each forbs, grasses, and shrubs; pre-production site: n = 3 forbs, n = 24 grasses, n = 24 shrubs; active production site: n = 33 each forbs, grasses, shrubs; post-production site: n = 30 each forbs, grasses, shrubs).

Ground-dwelling terrestrial invertebrates were collected using unbaited pitfall traps; invertebrates were removed from the traps every 1–3 days and sorted to family or order (e.g., Orthoptera, Coleoptera, Araneae, Hemiptera) using a stereo dissecting microscope. Aerial invertebrates were collected from the active and post-production sites using two insect light traps (Universal Light Trap, 12-W black light, BioQuip, Rancho Dominguez, California, USA). Traps were deployed from dusk to dawn for 5 consecutive nights and were located near the containment ponds, but outside of the northern mine perimeter fences. Trap collection funnels contained 95% ethanol as a preservative. Samples were composited by site as needed to obtain adequate mass for elemental analyses (SI Table S1).

Rodent species having small home ranges and high site fidelity to the mine areas were targeted (similar to Cleveland et al., 2019; Hinck et al., 2014, 2017). Valley pocket gophers (n = 15), deer mice (*Peromyscus maniculatus*; n = 30), brush mice (*P. boylii*, n = 12), kangaroo rat (*Dipodomys deserti*; n = 9), and cliff chipmunk (*Tamias dorsalis*; n = 5) were collected using live traps (Sherman Traps, Tallahassee, Florida; Havaharts®, Lititz, Pennsylvania, USA) or kill traps (Victor® Gopher Traps, Lititz, Pennsylvania; Gophinators, East Granby, Connecticut, USA). Kill traps were checked every 1–2 h to collect fresh dead animals; animals captured by live trap were euthanized (carbon dioxide). Necropsies were performed after euthanasia or retrieval of fresh dead animals, and the livers and kidneys were harvested. The liver and left kidney were immediately preserved in 10% neutral buffered formalin for histopathological examination. Lungs were also removed from the deer mice, brush mice, and pocket gophers to study the effects of weathering on elemental bioavailability and toxicity (Lowers, 2018). The carcass-remainders (hereafter referred to as whole bodies; e.g., soft tissues, fur, bones, teeth, gastrointestinal tracts) and right kidneys were frozen separately for chemical analyses.

2.3. Elemental and radiochemical analyses

Samples were lyophilized and homogenized prior to elemental and radiological analyses. Rodent tissues were analyzed individually; no compositing was performed. One deer mouse whole body was lost during cryogrinding due to an equipment failure. Therefore, n = 12 for deer mice whole body samples at the active production site; the corresponding kidney was retained for analysis (n = 13). The rodents were not depurated, and furs were not washed prior to processing for analyses to better reflect rodent exposure pathways; whole body results include contributions from surficial dust and soil. Grooming, inhalation, dermal contact/fur adherence during burrowing and foraging, and incidental soil ingestion during feeding are important exposure pathways (Beyer et al., 1994; French et al., 1965). Vegetation and invertebrate tissues were also processed unwashed to reflect the dietary uptake of the rodents and other animals.

Elemental and radiochemical analyses have been described previously (Cleveland et al., 2019; Hinck et al., 2017). Briefly, inductively coupled plasma-mass spectrometry (ICP-MS; PerkinElmer Elan DRC-e; similar to Method 6020B, USEPA, 2014) following microwave-assisted nitric acid digestion (MARS 6 Xpress; similar to Method 3050B, USEPA, 1996) was used to quantify environmentally available (i.e., total recoverable) As, cadmium (Cd), Cu, Pb, Mo, nickel (Ni), thallium (Tl), thorium (Th), and U in the samples. The total recoverable digestion procedure approximates the bioavailable fraction (concentrations) of elements in the biota (USEPA, 2014). Selenium concentrations were determined using flow injection-hydride generation-atomic absorption spectrophotometry (FI-HG-AAS, PerkinElmer AAnalyst 400 with FIAS-400; similar to Method 7742, USEPA, 1994) following combination wet/dry ashing. All elemental concentrations are reported on a dry-weight basis (mg kg^{-1} dw). Analytical methods for elemental analyses were prioritized as ICP-MS > FI-HG-AAS; in this way, kidney sample weights and some invertebrate sample weights that remained after ICP-MS analyses were insufficient for Se analyses. Further, although Se is typically accessible by ICP-MS, at the time of analyses, the sensitivity of the laboratory ICP-MS was insufficient to accurately measure low-level Se. Therefore, kidney Se concentrations are not reported; and invertebrate Se concentrations are reported with reduced numbers of replicates (n).

Exposures of biota (vegetation and rodents) to naturally-occurring radioisotopes that may create biological health effects

were evaluated using standard radiochemical methods (e.g., Cleveland et al., 2019). Portions of the selected lyophilized rodent whole bodies (n = 29) and vegetation (n = 235) were screened for gross alpha and beta activities by gas flow proportional counting (Method 9310, USEPA, 1986). Rodent whole body samples (n = 9) having gross alpha activity >185 Bq kg^{-1} dw and vegetation samples (n = 43; 1 sample having the greatest mean alpha activity per polygon) were subsequently analyzed by alpha spectroscopy for isotopic U (U-234, U-235, U-238; USDOE, 1997), isotopic Th (Th-228, Th-230, Th-232; USDOE, 1997), and Ra-226 (rodents only; Maxwell and Culligan, 2012). Selection of samples for isotopic analyses were also guided by elemental Th and U concentrations; samples having Th and U concentrations <0.5 mg kg^{-1} dw had radioactivity results below the reporting limits, and are generally indicative of background (Kathren, 1984; and based on regional statistical reference levels for vegetation and field mice at Los Alamos National Laboratory, Fresquez and Gonzales, 2004; Fresquez, 2016). At least one sample per site was analyzed for radioisotopes to demonstrate background radioactivity; the sample with the greatest U concentration (or Th concentration when U was not detected) for that functional group or species at that site was selected. Pre-production site values for U-238 were obtained by gamma spectrometry instead of alpha spectrometry; no pre-production samples had positive U-238 detections (Hinck et al., 2017). Vegetation sampling at the pre-production site was focused on shrubs and grasses; radioactivity was not measured in the forbs because of limited sample size (n = 3).

The estimated method quantification limits (MQL) for total recoverable elements are in the SI, Table S2. Reporting limits (RLs) were 150 Bq kg^{-1} dw for gross alpha activity, 370 Bq kg^{-1} dw for gross beta activities, 37 Bq kg^{-1} dw for each U and Th isotope, and 18.5 Bq kg^{-1} dw for Ra-226. Quality control (QC) sample analyses indicated that the elemental and radiochemical analyses were generally in-control; a summary of the QC results is provided in the SI.

2.4. Hepatic and renal histopathology

Histopathological examinations were intended to characterize biological endpoints related to exposures to elements and radiation in small rodents. Formalin-fixed liver and kidney samples were dehydrated, embedded in paraffin, sectioned at 5 μm, stained (hematoxylin and eosin), and examined with a light microscope (Luna, 1968) for hepatocellular vacuolation by lipid (V-L), hepatic inflammatory cell infiltration (PPI), renal inflammatory cell infiltration (INF), hepatic and renal mineralization (LMIN and KMIN, respectively), hepatocellular degeneration or necrosis (DEG), hepatic granulomas (GR), liver parasites (metazoan and protozoan; PS), renal tubular regeneration (KREG), and renal tubular epithelial karyomegaly (KARY). These lesions are potentially associated with toxicosis and inflammatory responses (e.g., Thoolen et al., 2010). Liver lesions PPI, DEG, and GR, and kidney lesions INF, KREG, and KARY were qualitatively scored as absent, mild, moderate, or severe within the tissues. Liver lesions V-L, LMIN, and PS, and kidney lesion KMIN, were qualitatively scored as present or absent.

2.5. Statistical analyses

All computations and statistical analyses were performed with Version 9.4 of the Statistical Analysis System (SAS Institute, Cary, North Carolina). Elemental and radiochemical concentrations were statistically analyzed as dw values and were log10 transformed to mitigate the effect of a few large values and make the comparisons very conservative. Differences in concentrations were evaluated with analysis of variance (ANOVA) with SAS PROC GLM. Least-squares means (LSMs), which are adjusted for all factors in the ANOVA models (i.e., study site; species; tissue type, whole body or kidney; rodent sex), were evaluated as Fisher's unrestricted least significant difference (Saville, 1990). A conservative α-level of 0.01 was used for these comparisons to protect against experiment-wise error (as suggested by Saville, 1990). A value of one-half the MQL or RL was substituted for censored values in all statistical analyses. If all samples were <MQL or < RL for a given matrix, that matrix was excluded from the statistical analyses. Statistical comparisons among the rodent species were not made due to differences in life strategies (e.g., foraging and caching).

Elemental concentrations (mg kg^{-1} dw) and radioactivities (Bq kg^{-1} dw) are reported as arithmetic means and standard errors (SE) of all samples; pre-production site results are included for comparison (Hinck et al., 2017). Results for the pocket gopher from LRT (n = 1) were not included in the analyses; only gophers from Wild Band Reservoir (n = 5) were used to calculate the non-mineralized reference mean and SE. Comparisons of elemental concentrations between male and female rodents were performed within each site. As previously mentioned, isotopic Th and U data were not collected for the pre-production site with the exception of U-238 by gamma spectrometry; therefore, the pre-production site was excluded from inter-site comparisons for U-238. Concentrations were compared to literature-based thresholds (multiple types; e.g., lowest observable adverse effects levels, LOAELs; reproductive effects; inhibition of chlorophyll) to assess rodent, invertebrate, and vegetation exposures and the potential for dietary uptake. Vegetation and invertebrate concentrations were not explicitly used to evaluate dietary uptake (i.e., biomagnification factors) in rodents because rodent dietary preferences and seasonal shifts are not well-defined for these sites, but comparisons with literature-based dietary thresholds have been included when available. The histopathology results were qualitative; no statistical comparisons were made. The results tables in the main text have been focused on deer mice and pocket gophers since all four site types (i.e., non-mineralized reference, pre-production, active-production, and post-production) are available for these species; result tables for brush mice, cliff chipmunks, and kangaroo rats are given in the SI.

3. Results

3.1. Radiochemistry

3.1.1. Vegetation

Gross alpha activities (Table 1) were less than the RL (150 Bq kg^{-1} dw) for all vegetation types at the non-mineralized reference and pre-production sites; consequently, no isotopic U and Th analyses for the non-mineralized reference site were performed. Gross alpha activities were not statistically different at the active and post-production sites within each vegetation type (Table 1); likewise, there were no statistical differences for isotopic U or Th. However, gross beta activities were greater at the active and post-production sites compared to the pre-production or non-mineralized reference site.

3.1.2. Small rodents

Gross alpha and beta activities in small rodent whole bodies (Table 1, SI Table S3) were generally near or less than the RL (150 Bq kg^{-1} dw and 370 Bq kg^{-1} dw, respectively) for all small rodent species across the site types, and there were no statistical differences between site types when there were positive detections.

Isotopic U, Th, and Ra-226 results were not statistically different in deer mice at the active and post-production sites. However, the mean Ra-226 activity in deer mice and pocket gophers was 1.5–4 times greater than the U isotopes (U-234 and U-238) at the active

Table 1
Mean (±standard error) radioactivity results (Bq kg^{-1} dry weight)[1] for above-ground mixed species vegetation and small rodent whole body samples from uranium mine and reference locations. Pre-production site values (Hinck et al., 2017) are included for comparison.

Matrix, Site	Gross Activities			Isotopes							
	n	Gross α	Gross β	n	Th-228	Th-230	Th-232	U-234	U-235	U-238[2]	Ra-226
Vegetation											
Forbs[3]											
Non-mineralized reference	17	<150 nd	550 ± 42 a	0	nm	nm	nm	nm	nm	nm	nm
Active production	33	240 ± 38 a	780 ± 41 b	0	nm	nm	nm	nm	nm	nm	nm
Post-production	30	180 ± 26 a	750 ± 48 b	6	<37 nd	72 ± 13 nd	<37 nd	50 ± 16 nd	<37 nd	38 ± 15 nd	nm
Grasses											
Non-mineralized reference	12	<150 nd	310 ± 39 a	0	nm	nm	nm	nm	nm	nm	nm
Pre-production	8	<150 nd	360 ± 41 a	8	nm	nm	nm	nm	nm	<1700 nd	nm
Active production	33	640 ± 110 a	810 ± 80 b	10	<37 nd	130 ± 23 a	<37 nd	130 ± 29 a	<37 nd	120 ± 30 a	nm
Post-production	30	370 ± 53 a	700 ± 64 b	9	<37 nd	79 ± 18 a	<37 nd	64 ± 26 a	<37 nd	62 ± 30 a	nm
Shrubs											
Non-mineralized reference	17	<150 nd	520 ± 57 a	0	nm	nm	nm	nm	nm	nm	nm
Pre-production	8	<150 nd	530 ± 55 a	8	nm	nm	nm	nm	nm	<1700 nd	nm
Active production	33	750 ± 110 a	1100 ± 74 b	10	<37 nd	180 ± 46 a	<37 nd	180 ± 42 a	<37 nd	190 ± 47 a	nm
Post-production	30	460 ± 78 a	1500 ± 480 b	8	<37 nd	94 ± 27 a	<37 nd	79 ± 29 a	<37 nd	74 ± 28 a	nm
Rodent whole bodies											
Pocket gophers											
Non-mineralized reference	2	<150 nd	320 ± 140 a	0	nm	nm	nm	nm	nm	nm	nm
Pre-production	6	<150 nd	320 ± 63 a	6	nm	nm	nm	nm	nm	<2600 nd	nm
Active production	2	130 ± 57 nd	<370 nd	1	<37 nd	<37 nd	<37 nd	<37 nd	<37 nd	41 nd	63 nd
Post-production	1	<150 nd	380 nd	0	nm	nm	nm	nm	nm	nm	nm
Deer mice											
Non-mineralized reference	2	<150 nd	<370 nd	0	nm	nm	nm	nm	nm	nm	nm
Pre-production	10	<150 nd	440 ± 130 a	10	nm	nm	nm	nm	nm	<2600 nd	nm
Active production	7	130 ± 26 a	260 ± 46 a	3	<37 nd	26 ± 7 a	<37 nd	28 ± 9 a	<37 nd	41 ± 1 a	180 ± 33 a
Post-production	6	220 ± 53 a	370 ± 62 a	4	<37 nd	38 ± 7 a	<37 nd	40 ± 13 a	<37 nd	38 ± 13 a	110 ± 10 a

[1] Means followed by a different letter are significantly different at $p < 0.01$ (SAS PROC GLM/LSMs); comparisons were among mine site types within each rodent species or vegetation functional group, and n = number of samples. The reporting limits were 150 Bq kg^{-1} dw for gross alpha and 370 Bq kg^{-1} dw beta activities, 37 Bq kg^{-1} dw for isotopic U and Th, and 18.5 Bq kg^{-1} dw Ra-226. Means less than the reporting limits have at least one individual sample greater than the reporting limit. A "nd" notation indicates that statistical differences were not determined because all measured concentrations were below the reporting limit of the data set being compared, or n = 1. A "nm" notation indicates that the value was not measured. Rodent whole bodies include all tissues except livers and kidneys, which were removed for separate analyses. Lungs were also removed from deer mice and pocket gophers for a separate study. Vegetation and rodent furs were not washed prior to processing and may include elemental contributions from soil and dust. Animals were not depurated; digestive tracts may include vegetation (dietary) and soil contributions.
[2] U-238 in pre-production rodents (n = 10 deer mice and n = 6 pocket gophers), and vegetation samples (n = 8 grasses and 8 shrubs) was determined by gamma spectrometry; no samples had positive detections. Reporting limits for gamma spectrometry were not assigned; values above are the maximum value of the "minimum detectable concentration" for the matrix.
[3] Forbs from the pre-production site were not analyzed for radioactivities because the forbs at the pre-production site had limited sample size (n = 3); sampling efforts at that site were focused on shrubs and grasses.

and post-production sites (Table 1).

3.2. Elemental concentrations

3.2.1. Vegetation

Elemental concentrations differed among vegetation type and sampling location (Table 2, SI Table S4).

Mean concentrations of U in vegetation were generally greatest at the active production site followed by the post-production site; concentrations were lowest at the reference and pre-production sites. However, the limited forbs sample size at the pre-production site (n = 3) likely influenced the outcomes of the statistical comparisons for those samples. Mean U concentrations in forbs at the active (4.34 mg kg^{-1} dw) and post-production sites (1.26 mg kg^{-1} dw) were 4–15 times greater than the pre-production site (0.29 mg kg^{-1} dw); and mean U concentrations in grasses and shrubs were 36–244 times greater at the active and post-production sites (1.84–12.2 mg kg^{-1} dw) compared to the reference site (<0.03 mg kg^{-1} dw).

Similarly, mean concentrations of Cu and Mo in all 3 vegetation functional groups were up to 4-fold greater at the active production and post-production sites compared to the pre-production and non-mineralized reference sites (Table 2). Concentrations of As and Ni in grasses and shrubs were also greatest at the active and post-production sites (0.7–2.53 mg kg^{-1} dw), with mean concentrations up to 7 times greater than those at the non-mineralized reference and pre-production sites (0.32–0.68 mg kg^{-1} dw). Site differences for Cd, Pb, and Tl in grasses and Pb, Tl, and Th in shrubs were less-pronounced; and comparisons of Se were likely confounded by the measured concentrations having been at or near the MQL. Interestingly, concentrations of As, Cd, Pb, Ni, Tl, and Th were greater in the pre-production forbs than in the active and post-production forbs. Similarly, Th was greatest in pre-production grasses and shrubs, as was Cd in pre-production shrubs.

3.2.2. Invertebrates

Uranium concentrations were greatest in the aerial and terrestrial invertebrates at the active and post-production sites, and lowest at the pre-production site (Table 3, SI Table S5; mixed-order compositions of the aerial and terrestrial invertebrate samples by site are provided in Table S1). Indeed, mean U concentrations were 39–373 times greater in aerial and terrestrial invertebrates collected at the active and post-production sites (1.88–5.01 mg kg^{-1} dw U) compared to invertebrates from the pre-production site (0.01–0.09 mg kg^{-1} dw U).

There were few concentration differences among sites for the other elements in terrestrial and aerial invertebrates (Table 3). Mean concentrations of As in aerial and terrestrial invertebrates at the active-production site (3.46–5.70 mg kg^{-1} dw) were up to 5.9 times greater than As concentrations at the pre-production sites (0.96–2.11 mg kg^{-1} dw). Concentrations of Cd were greatest in terrestrial invertebrates at the pre-production site (1.86 mg kg^{-1}

Table 2
Mean (±standard error) concentrations (mg kg^{-1} dry weight)[1] of elements in above-ground mixed species forbs, grasses, and shrubs samples from non-mineralized reference, active production, and post-production uranium mine sites. Pre-production mine site results (Hinck et al., 2017) are provided for comparison. A summary of literature-based thresholds and background ranges for vegetation is provided in SI Table S13 for additional comparison.

Functional group, Element	Non-mineralized reference (n = 24)	Pre-production (n = 24)[2]	Active production (n = 33)	Post-production (n = 30)
Forbs				
As	0.57 ± 0.06 a	4.70 ± 1.26 c	1.58 ± 0.18 b	0.69 ± 0.07 a
Cd	0.06 ± 0.01 a	0.34 ± 0.03 b	0.07 ± 0.01 a	0.07 ± 0.01 a
Cu	7.52 ± 0.25 a	9.72 ± 1.29 ab	19.6 ± 1.42 c	10.9 ± 0.21 b
Pb	0.31 ± 0.02 a	4.68 ± 1.03 c	1.01 ± 0.19 b	0.66 ± 0.05 b
Mo	0.82 ± 0.05 a	1.00 ± 0.22 a	3.71 ± 0.36 b	3.39 ± 0.38 b
Ni	0.47 ± 0.06 a	8.23 ± 1.76 c	1.82 ± 0.12 b	1.46 ± 0.07 b
Se	0.14 ± 0.01 a	0.25 ± 0.04 abc	0.12 ± 0.01 ab	0.34 ± 0.07 c
Tl	<0.03 nd	0.20 ± 0.04 b	0.02 ± 0.01 a	0.02 ± 0.01 a
Th	0.16 ± 0.02 ab	1.87 ± 0.45 c	0.21 ± 0.02 b	0.13 ± 0.01 a
U	<0.03 nd	0.29 ± 0.05 a	4.34 ± 0.63 b	1.26 ± 0.23 ab
Grasses				
As	0.32 ± 0.04 a	0.58 ± 0.12 ab	1.34 ± 0.15 c	0.70 ± 0.07 b
Cd	<0.10 nd	0.08 ± 0.01 ab	0.15 ± 0.03 b	0.04 ± 0.01 a
Cu	3.68 ± 0.09 a	4.88 ± 0.19 a	13.2 ± 1.4 b	4.70 ± 0.21 a
Pb	0.17 ± 0.02 a	1.07 ± 0.14 c	0.73 ± 0.06 b	0.74 ± 0.07 bc
Mo	0.90 ± 0.03 a	1.56 ± 0.11 b	3.57 ± 0.39 c	3.87 ± 0.49 c
Ni	0.59 ± 0.02 a	1.98 ± 0.25 c	1.51 ± 0.13 bc	1.21 ± 0.06 b
Se	0.05 ± 0.01 a	0.17 ± 0.01 b	<0.20 nd	0.23 ± 0.04 b
Tl	<0.01 nd	0.04 ± 0.01 b	0.02 ± 0.01 a	0.04 ± 0.01 b
Th	0.12 ± 0.02 a	0.49 ± 0.07 c	0.15 ± 0.01 b	0.15 ± 0.01 ab
U	<0.03 nd	0.05 ± 0.01 a	5.95 ± 0.95 c	1.84 ± 0.36 b
Shrubs				
As	0.33 ± 0.03 a	0.68 ± 0.04 b	2.30 ± 0.30 c	1.70 ± 0.22 c
Cd	0.06 ± 0.01 a	0.28 ± 0.03 b	<0.1 nd	0.07 ± 0.01 a
Cu	8.84 ± 0.35 a	9.60 ± 0.50 a	31.1 ± 3.3 c	15.0 ± 0.6 b
Pb	0.32 ± 0.02 a	0.97 ± 0.14 b	1.01 ± 0.09 b	1.74 ± 0.20 c
Mo	0.49 ± 0.02 a	0.74 ± 0.08 a	1.60 ± 0.15 b	1.67 ± 0.13 b
Ni	1.21 ± 0.07 a	1.80 ± 0.16 b	2.53 ± 0.22 c	2.52 ± 0.19 c
Se	0.10 ± 0.01 a	0.17 ± 0.02 b	<0.2 nd	0.30 ± 0.05 c
Tl	<0.02 nd	0.03 ± 0.01 ab	0.02 ± 0.01 a	0.04 ± 0.01 b
Th	0.11 ± 0.01 b	0.21 ± 0.02 c	0.06 ± 0.01 a	0.14 ± 0.01 b
U	0.02 ± 0.01 a	0.05 ± 0.01 b	12.2 ± 1.9 d	5.88 ± 0.94 c

[1] Means followed by a different letter are significantly different at $p < 0.01$ (SAS PROC GLM/LSMs); comparisons were among mine site types within each vegetation functional group, and n = number of samples. Means less than the MQL have at least one individual sample greater than the reporting limit. Vegetation was not washed prior to processing for analyses and may include elemental contributions from soil and dust. A "nd" notation indicates that statistical differences were not determined because all measured concentrations were below the reporting limit of the data set being compared.
[2] n = 3 for forbs at the pre-production site because sampling was focused on shrubs and grasses

Table 3
Mean (±standard error) concentrations (mg kg^{-1} dry weight)[1] of elements in mixed-order aerial and terrestrial invertebrates from active and post-production uranium mine sites. Pre-production site results (Hinck et al., 2017) are provided for comparison. A summary of literature-based toxicity thresholds and background ranges for invertebrates is provided in SI Table S14 for additional comparison.

Element	Aerial Invertebrates			Terrestrial Invertebrates			
	Pre-production (n = 6)[2]	Active production (n = 5)[3]	Post-production (n = 9)	Non-mineralized reference (n = 1)	Pre-production (n = 12)[4]	Active production (n = 5)	Post-production (n = 7)[5]
As	0.96 ± 0.41 a	5.70 ± 1.75 b	3.46 ± 0.95 ab	3.47 nd	2.11 ± 0.29 a	5.38 ± 0.74 b	4.68 ± 1.49 ab
Cd	0.80 ± 0.22 a	0.74 ± 0.40 a	0.66 ± 0.28 a	0.32 nd	1.86 ± 0.69 b	0.17 ± 0.03 a	0.18 ± 0.06 a
Cu	23.9 ± 1.9 a	38.7 ± 7.3 a	35.8 ± 9.0 a	22.1 nd	41.5 ± 8.7 a	37.8 ± 6.4 a	24.2 ± 3.1 a
Pb	0.29 ± 0.04 a	0.49 ± 0.18 a	0.58 ± 0.14 a	1.53 nd	1.61 ± 0.16 a	1.15 ± 0.28 a	1.71 ± 0.48 a
Mo	1.09 ± 0.47 a	1.33 ± 0.22 a	1.11 ± 0.14 a	0.59 nd	0.83 ± 0.10 a	1.78 ± 0.41 a	1.49 ± 0.30 a
Ni	0.51 ± 0.08 a	1.17 ± 0.37 a	1.24 ± 0.24 a	2.06 nd	2.90 ± 0.27 a	2.81 ± 0.56 a	3.33 ± 0.84 a
Se	1.53 ± 0.18 a	0.63 ± 0.24 a	6.42 ± 3.26 a	0.40 nd	0.65 ± 0.10 a	0.56 ± 0.19 a	1.61 ± 0.91 a
Tl	<0.02 nd	<0.05 nd	<0.05 nd	<0.05 nd	0.06 ± 0.01 nd	<0.05 nd	<0.05 nd
Th	0.15 ± 0.06 b	0.03 ± 0.02 a	<0.02 nd	0.79 nd	0.67 ± 0.08 a	0.60 ± 0.18 a	0.73 ± 0.23 a
U	0.01 ± 0.01 a	3.73 ± 2.36 b	1.88 ± 0.64 b	0.10 nd	0.09 ± 0.01 a	5.01 ± 2.06 b	3.51 ± 0.86 b

[1]Means followed by a different letter are significantly different at $p < 0.01$ (SAS PROC GLM/LSMs); comparisons were among mine site types within each invertebrate type (aerial or terrestrial), and n = number of samples. Ground-dwelling terrestrial invertebrates were collected using unbaited pitfall traps; aerial invertebrates were collected with insect light traps and preserved in ethanol. Invertebrate samples were not depurated or rinsed prior to processing for analyses; samples may include elemental concentrations from surficial or ingested soil or dust. Means less than the MQL have at least one individual sample greater than the reporting limit. A "nd" notation indicates that statistical differences were not determined because all measured concentrations were below the reporting limit of the data set being compared. Terrestrial invertebrates collected at the non-mineralized reference site were not included in the comparison because n = 1 due to the need to composite multiple samples to provide sufficient weight for all analyses.
[2] n = 4 for Se due to low sample weights.
[3] n = 4 for Se due to low sample weights.
[4] n = 7 for Se due to low sample weights.
[5] n = 6 for Se due to low sample weights.

dw; approximately 11 times greater than the active and post-production sites), whereas concentrations of Th were greatest in aerial invertebrates at the pre-production site (0.15 mg kg^{-1} dw) and lowest at the active production site (0.03 mg kg^{-1} dw).

3.2.3. Small rodents

Mean U concentrations were greatest in deer mice at the active- and post-production sites (Table 4) and in brush mice at the active-production site (SI Table S7); further, deer mice whole body U concentrations at the post-production site were 1.9 times greater than those at the active production site, and 53 to 79 times greater than those at the reference and pre-production sites, respectively. Mean U in brush mice whole bodies was approximately 14 times greater at the active-production site compared to the post-production site. There were no statistical differences among site types for U in pocket gophers, cliff chipmunks, or kangaroo rat whole bodies or kidneys (Table 4; SI Table S7); however, the statistical comparisons may have been limited due to the relatively small sample sizes.

The whole body U concentrations for pocket gophers, cliff chipmunks, and kangaroo rats at the active production site were generally low (0.31–0.48 mg kg^{-1} dw; SI Table S7) compared to whole body U concentrations for *Peromyscus* spp. (0.82–1.54 mg kg^{-1} dw; Table 4 and SI Table S7). Deer mice whole bodies at the post-production site also had an elevated mean U concentration (1.59 mg kg^{-1} dw; Table 4) relative to the other rodent species (0.11–0.33 mg kg^{-1} dw; Table 4 and SI Table S7). Concentrations of U in deer mice kidneys (Table 4) were greatest at the post-production site and were below the MQL at the pre-production and non-mineralized reference sites. There were no other statistical differences among sites for U in rodent kidneys.

Concentrations of the co-occurring ore body elements in rodent whole bodies and kidneys had few differences among sites and were generally not indicative of mine status (Table 4; SI Tables S6 and S7). Most notably, brush mice kidneys at the active production site (0.26 mg kg^{-1} dw; SI Table S7) had a mean As concentration that was 3-fold greater than the As concentration at the post-production site (0.09 mg kg^{-1} dw; SI Table S7). Similarly, mean Pb were 6–13 times greater in whole bodies and kidneys of brush mice from the active production site compared to brush mice tissues from the post-production site. Whole body concentrations of Se were 1.4–7 times greater in deer mice at the post-production site (7.43 mg kg^{-1} dw; Table 4) compared to the other sites (0.97–1.51 mg kg^{-1} dw).

Concentrations of Ni in deer mice kidneys (0.75 mg kg^{-1} dw; Table 4) were up to 15 times greater at the non-mineralized reference site compared to the other sites (0.05–0.14 mg kg^{-1} dw), whereas the Ni concentrations of kidneys in the other rodent

Table 4
Mean (±standard error) concentrations (mg kg^{-1} dry weight)[1] of elements in whole bodies and kidneys of deer mice (*Peromyscus maniculatus*) and pocket gophers (*Thomomys bottae*) from non-mineralized reference, active production, and post-production uranium mine sites. Pre-production site results (Hinck et al., 2017) are provided for comparison. A summary of literature-based thresholds and background ranges for small rodents is provided in SI Table S15 for additional comparison.

Element	Deer mice				Pocket gopher			
	Non-mineralized reference (n = 10)	Pre-production (n = 10)	Active production (n = 12)[2]	Post-production (n = 7)	Non-mineralized reference (n = 5)	Pre-production (n = 6)	Active production (n = 5)	Post-production (n = 4)
As								
Whole body	0.65 ± 0.09 ab	<1.2 nd	0.55 ± 0.07 a	0.99 ± 0.18 b	0.34 ± 0.04 a	<1.2 nd	0.59 ± 0.07 b	0.52 ± 0.03 b
Kidney	0.12 ± 0.04 a	0.11 ± 0.02 ab	0.24 ± 0.03 b	0.19 ± 0.08 ab	0.31 ± 0.02 a	0.34 ± 0.11 a	0.32 ± 0.03 a	0.39 ± 0.06 a
Cd								
Whole body	0.04 ± 0.01 a	0.08 ± 0.02 a	0.04 ± 0.01 a	0.03 ± 0.01 a	0.05 ± 0.01 b	0.17 ± 0.04 c	0.02 ± 0.01 a	0.04 ± 0.01 b
Kidney	1.50 ± 0.33 a	0.89 ± 0.45 a	0.95 ± 0.32 a	0.68 ± 0.31 a	1.80 ± 0.55 a	8.55 ± 1.41 b	2.15 ± 0.47 a	3.54 ± 0.69 ab
Cu								
Whole body	8.52 ± 0.37 a	10.4 ± 0.8 a	9.96 ± 0.78 a	10.1 ± 0.7 a	7.14 ± 0.42 a	7.14 ± 0.20 a	5.70 ± 0.47 a	6.34 ± 0.62 a
Kidney	19.4 ± 0.5 a	17.6 ± 0.8 a	17.5 ± 0.5 a	19.2 ± 0.8 a	12.4 ± 1.3 a	15.1 ± 0.7 a	11.3 ± 1.2 a	13.3 ± 0.5 a
Pb								
Whole body	0.65 ± 0.12 ab	0.37 ± 0.10 a	0.65 ± 0.19 ab	1.68 ± 0.49 b	0.74 ± 0.13 a	0.55 ± 25 a	0.74 ± 0.13 a	1.06 ± 0.27 a
Kidney	<0.04 nd	0.15 ± 0.04 a	0.70 ± 0.49 ab	0.74 ± 0.26 b	0.14 ± 0.05 a	0.08 ± 0.01 a	0.13 ± 0.02 a	0.09 ± 0.05 a
Mo								
Whole body	0.70 ± 0.07 b	0.70 ± 0.04 b	0.43 ± 0.11 a	1.08 ± 0.15 b	0.09 ± 0.06 ab	0.81 ± 0.09 ac	0.33 ± 0.18 b	0.86 ± 0.20 bc
Kidney	2.54 ± 0.13 a	2.41 ± 0.11 a	2.32 ± 0.09 a	2.45 ± 0.09 a	0.97 ± 0.14 a	1.93 ± 0.18 b	1.46 ± 0.18 ab	2.42 ± 0.24 b
Ni								
Whole body	1.63 ± 0.11 b	0.60 ± 0.10 a	1.77 ± 0.09 b	2.26 ± 0.17 b	1.66 ± 0.09 b	0.75 ± 0.10 a	2.46 ± 0.17 b	2.40 ± 0.23 b
Kidney	0.75 ± 0.23 b	0.05 ± 0.001 a	0.14 ± 0.06 a	0.13 ± 0.04 a	0.03 ± 0.01 a	0.08 ± 0.02 a	0.15 ± 0.08 a	<0.04 nd
Se								
Whole body	1.18 ± 0.06 a	1.51 ± 0.22 a	0.97 ± 0.06 a	7.43 ± 2.14 b	0.69 ± 0.03 ab	0.77 ± 0.10 ab	0.57 ± 0.04 a	1.10 ± 0.27 b
Tl								
Whole body	<0.05 nd	0.01 ± 0.01 a	<0.03 nd	0.09 ± 0.02 b	<0.02 nd	0.02 ± 0.01 nd	<0.03 nd	<0.02 nd
Kidney	<0.02 nd	0.03 ± 0.01 a	0.02 ± 0.01 a	0.55 ± 0.12 b	<0.1 nd	0.12 ± 0.08 a	0.03 ± 0.02 a	<0.1 nd
Th								
Whole body	0.11 ± 0.01 a	0.19 ± 0.05 a	<0.5 nd	0.19 ± 0.03 a	0.07 ± 0.06 a	0.25 ± 0.05 b	<0.04 nd	<0.06 nd
Kidney	0.12 ± 0.02 b	0.08 ± 0.03 a	<0.3 nd	<0.2 nd	<0.3 nd	0.05 ± 0.02 nd	<0.3 nd	<0.3 nd
U								
Whole body	0.03 ± 0.01 a	0.02 ± 0.01 a	0.82 ± 0.20 b	1.59 ± 0.48 b	<0.05 nd	0.01 ± 0.01 a	0.48 ± 0.31 a	0.33 ± 0.18 a
Kidney	<0.04 nd	<0.01 nd	0.12 ± 0.03 a	0.98 ± 0.38 b	<0.02 nd	<0.01 nd	0.03 ± 0.02 a	0.02 ± 0.01 a

[1] Means followed by a different letter are significantly different at $p < 0.01$ (SAS PROC GLM/LSMs); comparisons were among mine site types within each rodent species, and n = number of samples. Whole bodies include all tissues except livers, kidneys, and lungs, which were removed for separate analyses. Rodent furs were not washed prior to processing and may include elemental contributions from soil and dust. Animals were not depurated; digestive tracts may include vegetation (dietary) and soil contributions. Comparisons among species were not made due to differences in life strategies. Means less than the MQL have at least one individual sample greater than the reporting limit. An "nd" notation indicates that statistical differences were not determined because all measured concentrations were below the reporting limit of the data set being compared. Concentration results for Se measured by ICP-MS in kidney tissues were too low to be reliable and are therefore not reported.

[2] One whole body deer mouse sample from the active production site (n = 1 of 13) was lost during cryogrinding due to an equipment failure. The corresponding kidney was not removed from the dataset, and kidney n = 13.

species (Table 4; SI Table S7) had no statistical differences by site type. Whole bodies of cliff chipmunks from the post-production site had 5.5 times greater concentrations of Ni (1.90 mg kg^{-1} dw; SI Table S7) than whole bodies from the pre-production site (0.34 mg kg^{-1} dw).

There were few differences in elemental concentrations by rodent sex. Male deer mice at the active production site had greater whole body concentrations of Cd (0.06 mg kg^{-1} dw) than females (0.02 mg kg^{-1} dw; SI Table S8). Female pocket gophers at the non-mineralized reference site (0.34 mg kg^{-1} dw) had greater concentrations of Co in kidney tissues than males (0.11 mg kg^{-1} dw; SI Table S9). Brush mice (SI Table S10), and kangaroo rats and cliff chipmunks (SI Table S11) tissues had no statistical differences in elemental concentrations by sex, although the comparisons were likely limited due to low sample numbers.

3.3. Histopathology

Lesions having positive detections in the rodent liver and kidney tissues (Table 5; SI Table S12) were V-L, PPI, LMIN, DEG, GR, PS, INF, KMIN, KREG, and KARY. Lesions V-L (n = 4), LMIN (n = 4), GR (n = 17), PS (n = 2), INF (n = 18), KMIN (n = 12), KREG (n = 13), and KARY (n = 5) had relatively low prevalence (present in <20% of all rodents, total n = 95 livers, n = 93 kidneys). The GR lesion had 21% prevalence, and the PPI lesion prevalence was 42%. All scored lesions were mild with the following exceptions: one moderate PPI lesion in a deer mouse at the post-production site (Table 5); one moderate KARY lesion in a deer mouse at the non-mineralized reference; two moderate DEG lesions in non-mineralized reference pocket gophers (Table 5); one moderate GR lesion in a pre-production pocket gopher; one severe PPI lesion in a brush mouse at the post-production site (SI Table S12); one moderate DEG lesion, one moderate GR lesion, and one moderate KARY lesion in post-production brush mice; one moderate PPI lesion in a post-production cliff chipmunk; and one moderate and one severe DEG lesion in cliff chipmunks at the post-production site.

The prevalence or severity of the lesions had no consistent pattern by site mineralization or mine life stage. For example, inflammatory cell infiltrates in the liver (PPI), which is typically characterized by portal infiltrates of mononuclear cells, was the most prevalent lesion; yet deer mice livers tended to have the PPI lesion at varying degrees of severity regardless of mining stage. The next most-common lesion, GR, was present in both non-mineralized reference site rodents and rodents from the pre-production, active production, and post-production sites.

4. Discussion

4.1. Radiochemistry

4.1.1. Vegetation

Greater gross alpha and beta activities in grasses, forbs, and shrubs at active and post-production sites compared to pre-production and reference sites indicates that U mining activities have increased radioactivity on the surface of the mine sites and their surrounds.

Similar elevated radioactivity was observed in grasses and shrubs at a chronic exposure breccia pipe U mine site (Cleveland et al., 2019). The gross alpha activities at the active and post-production sites were within a range for vegetation from a U exploration and exploitation area (48–477 Bq kg^{-1} dw; Pathak and Pathak, 2012). Gross beta activities were generally within background range at all sites (Hinck et al., 2017), but the greater beta activities at the active and post-production sites relative to the pre-production and reference sites may be due to the presence of U decay chain beta emitters in the ore. For example, Pb-210 is a naturally-occurring beta emitter and radon (Rn) progeny. Elevated Rn has been detected near the ore piles at the active production site (Naftz et al., 2020), and aeolian transport may have moved radionuclide-laden dust onto the mine surrounds (Bidar et al., 2009; Pozolotina et al., 2000). We previously demonstrated that surficial dust was the source of elevated concentrations of Ni, Pb, Tl, and U on grasses downwind of the pre-production site (Hinck et al., 2017).

The U-238 activities in vegetation at the active and post-production sites were greater than a range for crops grown in soil treated with U-containing fertilizers (0.05–0.8 Bq kg^{-1} dw, Al-Masri et al., 2008). Mean U-238 in vegetation at the active and post-production sites was generally greater than U-238 found in grasses and forbs growing at a former U mine site (3.5–11.5 Bq kg^{-1} dw assuming 80% moisture; Skipperud et al., 2013). Notably, the U-238 activities were within or above the typical range for rocks and soils in the US (4–140 Bq kg^{-1} dw, NCRPM, 1987). This may indicate that the U-238 results reflect uptake of U by the vegetation and/or dust-loading on the surfaces of the unwashed vegetation. The hypothesis that our results reflect surficial dust-loading is supported

Table 5
Summary of histopathological findings[1] in deer mouse and pocket gopher livers and kidneys from reference, pre-production, active production, and post-production uranium mine sites. Data indicate the number of samples that contained lesions that were scored absent-mild-moderate-severe, or the number of samples having the lesion absent/present.

Species, Site		Liver lesions						Kidney lesions			
	n[2]	V-L	PPI	LMIN	DEG	GR	PS	INF	KMIN	KREG	KARY
Deer mice											
Non-mineralized reference	10	10/0	3–7–0–0	10/0	10–0–0–0	8–2–0–0	10/0	6–4–0–0	9/1	6–3–1–0	10–0–0–0
Pre-production	12[3]	11/1	11–1–0–0	12/0	9–3–0–0	12–0–0–0	12/0	10–0–0–0	10/0	9–1–0–0	9–1–0–0
Active production	13[4]	13/0	2–11–0–0	13/0	13–0–0–0	12–1–0–0	13/0	9–4–0–0	11/2	13–0–0–0	12–0–0–0
Post-production	6	6/0	2–3–1–0	5/1	5–1–0–0	6–0–0–0	6/0	5–1–0–0	4/2	5–1–0–0	6–0–0–0
Pocket gopher											
Non-mineralized reference	5	5/0	5–0–0–0	3/2	2–1–2–0	4–1–0–0	5/0	4–1–0–0	3/2	5–0–0–0	5–0–0–0
Pre-production	5	5/0	5–0–0–0	4/1	3–2–0–0	4–0–1–0	5/0	5–0–0–0	5/0	4–1–0–0	5–0–0–0
Active production	5	5/0	4–1–0–0	5/0	4–1–0–0	2–3–0–0	5/0	5–0–0–0	3/2	3–2–0–0	5–0–0–0
Post-production	4	4/0	3–1–0–0	4/0	4–0–0–0	4–0–0–0	4/0	4–0–0–0	3/1	4–0–0–0	4–0–0–0

[1] V-L = hepatocellular vacuolation by lipid; PPI = hepatic inflammatory cell infiltration; LMIN = hepatic mineralization; DEG = hepatocellular degeneration or necrosis; GR = hepatic granulomas; PS = liver parasites (metazoan and protozoan); INF = renal inflammatory cell infiltration; KMIN = renal mineralization; KREG = renal tubular regeneration; and KARY = renal tubular epithelial karyomegaly.
[2] n = number of samples.
[3] n = 12 for deer mouse liver and n = 10 for deer mouse kidney.
[4] One deer mouse kidney at the active production site could not be scored for the KARY lesion because no medulla was present (n = 12).

by Pulhani et al. (2005), who found that a major percentage (>50%) of total U-238, Th-232, and Ra-226 activity in wheat grain is sequestered in the roots. In other words, translocation was likely low, indicating an external source of the radionuclides. However, differences in plant physiology between wheat grain and our sample species (e.g., water and nutrient uptake rates, external structures, growth rate, transpiration) may also have contributed.

Interestingly, a trend toward radioactive disequilibrium and enrichment of mean Th-230 relative to U-238, by a factor of 2, was evident in forbs at the post-production site. This could indicate that surface weathering and fractional crystallization processes (e.g., Scott, 1968; Santos and Marques, 2007) occurred while the post-production site was on standby; in this way, Th-230 would have become more bioavailable for translocation into the above-ground vegetation. However, plant Th concentrations are generally thought to be not dependent on soil Th (Sheppard et al., 1989), so the forbs may have unique physiological processes and external structures (e.g., stellate hairs, petals) that permitted them to surficially trap and retain weathered dust. Alternately or in addition, the different mixtures of species collected at each site (Hinck et al., 2014; Mann and Duniway, 2020) may have also contributed.

4.1.2. Small rodents

The results for gross alpha and beta activities in rodent whole bodies indicate that life history strategies affect rodent exposure to radioactivity and radioisotopes.

For example, there were no statistical differences in gross alpha activities among site types for pocket gophers (Table 1), whereas the mean alpha activity in deer mice at the post-production site (220 Bq kg^{-1} dw, Table 1) was approximately 2 times greater than the means at the other sites. This may reflect differences in dietary preferences or grooming habits for pocket gophers and deer mice (e.g., Hanson and Miera, 1978). Gophers are strict herbivores that spend much time underground eating the roots and fleshy parts of vegetation, whereas deer mice forage above ground, and have an omnivorous diet that changes seasonally. Although gophers might be exposed to U sequestered in the roots of vegetation, deer mice and their food caches would likely be subject to more blowing dust from ore and waste piles, thereby enhancing the opportunity for incidental ingestion of soils with elevated concentrations of mining-related elements. However, the small samples sizes may have confounded site comparisons.

As previously mentioned, Ra-226 activity in deer mice was approximately 3–4 times greater than the U-234 and U-238 activities (Table 1). Cloutier et al. (1985) demonstrated transfer of Ra-226 from soil and vegetation into meadow vole gut, skin, and bone; this indicates that, as a carcinogen, Ra-226 could have greater health effects than U in small rodents. However, Ra-226 concentrations up to 4 times those of U do not intrinsically equate to radiotoxicity; and dose calculations (RESRAD-BIOTA; Minter et al., 2019) indicated that the total radioactivity doses to our rodents did not exceed the U.S. Department of Energy's average dose criteria rate of 1 mGy d^{-1}.

4.2. Elemental concentrations

4.2.1. Vegetation

Elemental concentrations were nearly always greater at the mineralized breccia pipe sites, regardless of mine production stage, compared to the non-mineralized reference site (Table 2). Mining activities at the active and post-production sites appear to have resulted in elevated (relative to the pre-production site) concentrations of U (forbs, grasses, shrubs), As (grasses, shrubs), Cd (grass), Cu (forbs, grasses, shrubs), Mo (forbs, grasses, shrubs), Ni (shrubs), Pb (shrubs), and Se (shrubs). This suggests that these elements, particularly U, Cu, and Mo, represent an active or recent U mining signature; a similar signature for U, Co, and Pb was observed at a chronic U exposure site (Cleveland et al., 2019).

However, there were also instances where the elemental concentrations were greatest at the pre-production site compared to the active or post-production sites. While these greater elemental concentrations cannot be directly tied to ore production, differences may reflect anthropogenic activities at the site (i.e., surface clearing, scraping, bulldozing) related to mine site preparation and subsequent increased dust loads of mineralized soils on the vegetation (e.g., Bidar et al., 2009; Hinck et al., 2017). In addition, some elemental differences may represent differences in natural mineralization (Van Gosen, 2016) or differences in soil chemistry (e.g., pH, presence of ligands, elemental speciation; Violante et al., 2010) among the pre-, active, and post-production sites.

Moreover, different dominant plant species were collected at each site (Mann and Duniway, 2020), which may have influenced the measured elemental concentrations. For example, shrub species like sagebrush and rubber rabbitbrush (the latter of which was found to be more prevalent at the pre-production site) may have retained greater loads of depositional soil and dust via trichomes and flower structures, respectively, which might have increased concentrations of soil-associated elements (e.g., As, Pb, Ni, Th) in our unwashed vegetation samples relative to grasses or other species lacking these structures (Hinck et al., 2017). In addition, atmospheric deposition of elements in dust may have permitted foliar uptake (Kataba-Pendias and Pendias, 2001).

Additional factors affecting elemental differences among vegetation species include elemental bioavailability, binding of dissolved or complexed elements by absorption, species selectivity for specific ions, nutrient cycling and root uptake, and translocation and sequestration (Kataba-Pendias and Pendias, 2001). One example of differential uptake of elements in vegetation is Mo and Cu in sagebrush (*Artemisia tridentata*; Rickard and Van Scoyoc, 1984); Mo applied to the soil was shown to be readily translocated sagebrush leaves whereas Cu was not translocated. The exact mechanism for the observed differences is not known, but the Mo concentrations remained elevated in the sagebrush leaves for 2-years post application to the soil. In this way, ingested vegetation that contains translocated Mo (or other elements) could represent a dietary exposure pathway for elements in herbivores, omnivores, and grazers.

Mean U concentrations in vegetation at the non-mineralized reference site were generally near a literature background range (0.002–0.015 mg kg^{-1} dw; Gramss and Voigt, 2014). However, U was greater than literature-based background concentrations at the pre-production, active, and post-production sites, ranging from 3x background at the pre-production site to 800x background at the active production site. Further, mean U concentrations in grasses from active production site and shrubs from active and post-production sites exceeded a soil-screening benchmark of 5 mg kg^{-1} dw (Efroymson et al., 1997). By plant functional group, $n = 16$ of 33 grasses, $n = 20$ of 33 shrubs, and $n = 13$ of 33 forbs samples at the active production site, and $n = 3$ of 30 grasses, $n = 13$ of 30 shrubs, and $n = 1$ of 30 forbs at the post-production site exceeded this benchmark (https://doi.org/10.5066/P94OVQO9); shrubs at both sites had the highest rates of exceedance.

Concentrations of As, Cd, Cu, Pb, Ni, Se, and Tl in vegetation were generally within literature background ranges and did not exceed literature-based thresholds (SI Table S13), with a few exceptions for As, Cu, and Ni. For example, we noted that the literature-based background range for As (1–22 mg kg^{-1} dw; Kataba-Pendias and Pendias, 2001; Ollson et al., 2009) overlaps a phytotoxicity range (1–20 mg kg^{-1} dw) that results in 10% yield depression of sensitive agricultural crops and fruit tree leaves (Kataba-Pendias and

Pendias, 2001). Mean As concentrations in forbs (pre- and active production), grasses (active production), and shrubs (active and post-production) were 1–5 mg kg^{-1} dw, exceeding the lower end of that phytotoxicity range. No As concentrations in vegetation exceeded a dietary threshold (28 mg kg^{-1} dw) that results in tissue damage in bank voles (*Clethrionomys glareolus*; Griffin et al., 2001).

Similarly, mean Cu concentrations in shrubs at the active production site (31.1 mg kg^{-1} dw; Table 2) and Ni in forbs at the pre-production site (8.23 mg kg^{-1} dw) exceeded literature background ranges (SI Table S13; Cu: 2–20 mg kg^{-1} dw, Gramss and Voigt, 2014 and references therein; Ni: 0.1–3 mg kg^{-1} dw, Gramss and Voigt, 2014 and references therein; Rashed, 2010). The Cu concentrations in shrubs at the active production site also exceeded a dietary threshold that reduced moth (*Spodoptera litura*) pupation and emergence rates (25 mg kg^{-1}; Huang et al., 2012) and a threshold that reduced yields of spring barley (20 mg kg^{-1} dw; *Hordeum vulgare*; Davis et al., 1978). Mean Mo concentrations in forbs and grasses at the active and post-production sites exceeded a soil-screening benchmark of 2 mg kg^{-1} dw (Efroymson et al., 1997); however, this benchmark is within a range for Mo in vegetation that is considered to be nontoxic to grazing animals (0.01–21 mg kg^{-1} dw, Kataba-Pendias and Pendias, 2001).

4.2.2. Invertebrates

Our results indicate that anthropogenic activities associated with active and recent U ore production can result in greater concentrations of As and U in aerial and terrestrial invertebrates (Table 3).

Notably, mean U concentrations in both aerial and terrestrial invertebrates at the active and post-production sites were similar to those in Coleoptera collected at a U production site (0.68–3.54 mg kg^{-1}; Gongalsky, 2006), whereas U concentrations in invertebrates at the non-mineralized reference and pre-production sites were within a background range for U in weaver ants (*Oecophylla smaragdina*; <0.01–0.17 mg kg^{-1} dw; Doering and Bollhöfer, 2016). Aerial and terrestrial invertebrate elemental uptake and exposure are generally accepted to be closely linked to trophic transfer, depuration, sub-cellular sequestration, elemental speciation and bioavailability, and other physiological and species-specific biochemical processes (Morgan et al., 2007; Skip et al., 2014). In this way, the different invertebrate orders and resultant composite samples collected at each site (SI Table S1) may have influenced the results. Our terrestrial invertebrates were analyzed unwashed; therefore, the samples may have included surficial soil and dust as a source of the measured elements. In contrast, the external surfaces of the aerial invertebrates were likely rinsed by the ethanol in the light traps, thereby reducing or eliminating the external soil and dust loads in the aerial tissues. The elevated U and As concentrations in aerial invertebrates may indicate that external soils and dust were not the sole source of elements in our invertebrate samples; aerial and terrestrial results likely included dietary exposure and bioaccumulation.

There are few available protective thresholds for elements that are based on body burdens for invertebrates (SI Table S14); few were exceeded in our study. At the pre-production site, n = 3 of 4 Se concentrations in aerial invertebrates (https://doi.org/10.5066/P94OVQO9) exceeded the lower end of a dietary threshold range (1.4–6.6 mg kg^{-1} dw Se) causing sublethal effects in rats (e.g., reduced longevity, reproductive selenosis, reduced growth, renal damage; Halverson et al., 1966; USDOI, 1998). In terrestrial invertebrate samples, n = 2 of 7 concentrations (2.28 mg kg^{-1} dw and 6.84 mg kg^{-1} dw; https://doi.org/10.5066/P94OVQO9) exceeded Se thresholds at the post-production site; and n = 4 of 8 aerial mixed-order samples had Se concentrations (3.69, 3.76, 15.1, and 25.8 mg kg^{-1} dw) that exceeded one or more Se toxicity thresholds (i.e., 1.4–6.6 mg kg^{-1} dw Se for sublethal effects in rats, Halverson et al., 1966, USDOI, 1998; 2.5–15 mg kg^{-1} dw Se for growth effects in invertebrates, USDOI, 1998; 3.75 mg kg^{-1} dw assuming 80% moisture as a risk threshold for the entire food web in the Colorado River, Walters et al., 2015). However, the typical background range for Se in terrestrial invertebrates (0.1–2.5 mg kg^{-1} dw; USDOI, 1998) overlaps these thresholds. The greatest Se concentrations (>6 mg kg^{-1} dw Se) were in ants (Hymenoptera) and Dipterans at the post-production site (https://doi.org/10.5066/P94OVQO9). As previously mentioned, the differences in species collected at each site and the resultant composites may have influenced the results. Alternately or in addition, Se (and As) concentrations were previously linked to the aquatic exposure pathway at the pre-production site (Hinck et al., 2017); therefore, the containment ponds at the active and post-production sites may have been a source of dissolved elements for uptake by aerial invertebrates.

Studies have indicated that local invertebrate populations may be element-tolerant, with tissue accumulation having no effect on the invertebrate development into adulthood (Greville and Morgan, 1991; Jaffe et al., 2019; Tollett et al., 2009). Further, Cu and other nutrients are likely regulated by physiological processes (Grześ, 2012). While mining-related elements may not directly impact invertebrate populations, Se, Cu, and other elements can be bioaccumulated and biomagnified (Cheruiyot et al., 2013; Hopkins et al., 2005), which may increase uptake and exposure of omnivorous and insectivorous predators. In this way, it may be prudent for land managers to use invertebrates as biomonitors of adverse conditions, particularly for assessing risk to invertivores or omnivores.

4.2.3. Small rodents

The statistically greater concentrations of U in whole bodies and kidney tissues of deer mice and brush mice at the active and post-production sites compared to the pre-production and non-mineralized reference sites indicate that these species have been exposed to U associated with mining.

Pocket gophers, cliff chipmunks, and kangaroo rats appear to have received less exposure. Moreover, the exposure and uptake of U in brush mice appears to have attenuated once the post-production site was placed on standby (i.e., U concentrations in whole body brush mice were lower at the post-production site compared to the active site).

The kidney and bones are generally thought to be primary targets for U toxicity and accumulation; the mean U concentrations in our rodent kidneys were generally less than U concentrations in whole bodies. This may suggest that the primary source of U in the whole body samples was (1) the bones, or (2) fur-borne soils and dust; alternately, ingested soils may have contained U species that are not bioavailable for kidney uptake (e.g., U bound in siliceous minerals would not be expected to be accessible to gastric juices; soil particle size effects; Jovanovic et al., 2012 and references therein). Although some studies indicate that age or sex of our sampled rodents may have influenced the statistical differences observed for the elemental concentrations (e.g., Blagojević et al., 2012; Zarrintab and Mirzaei, 2017), we found few statistical differences by sex (SI Tables S8-11). This may be due to relatively low sample numbers; we did not consider the ages of our sampled rodents when determining mean concentrations for the same reason.

Tissue-based (whole body or kidney) concentration thresholds and literature-based ranges for the small rodent species that we studied are relatively limited. Most thresholds are based on a daily uptake rate; dose response and risk assessment are beyond the scope of this study. The mean U concentration in kidneys of deer mice at the post-production site (0.98 mg kg^{-1} dw) was just below

a lowest-observed effect concentration (LOEC) range for renal damage in rats (1–3.5 mg kg^{-1}; Diamond et al., 1989; Gilman et al., 1998a); individually, n = 3 of 7 deer mice kidneys had a U concentration >1 mg kg^{-1} dw (https://doi.org/10.5066/P94OVQO9). A kidney concentration of 0.2 mg kg^{-1} dw U (assuming 80% moisture) is a LOEC for histopathological changes in the renal tubular nuclei of rabbits (Gilman et al., 1998b); n = 2 of 12 deer mice kidneys at the active production site, n = 5 of 7 deer mice kidneys at the post-production site, and n = 1 of 5 cliff chipmunk kidneys at the post-production site exceeded this 0.2 mg kg^{-1} dw U threshold (https://doi.org/10.5066/P94OVQO9). Concentrations of 0.16 mg kg^{-1} dw U in wood mice (*Apodemus sylvaticus*) exposed to mine wastes and contaminated waters from an abandoned U mine had loss of DNA integrity in blood cells, up-regulation of the tumor suppressor gene P53 (Lourenço et al., 2013); our mean kidney concentrations of U in deer mice (post-production site), brush mice (active-production site), and kangaroo rats (active and post-production) all exceeded this sublethal threshold.

Concentrations of Cd, Pb, and Se in rodent whole body and kidney tissues (Table 4; SI Table S7) did not exceed literature-based toxicity thresholds (SI Table S15) at any of the sites. Moreover, mean concentrations of As, Cd, Mo, Se, and Tl were generally within literature-based background ranges (i.e., concentrations in animal tissues collected from areas not associated with U mining or having no mineralization; SI Table S15).

In all rodent species, Cd, Cu, and Mo were concentrated in the kidney relative to the whole bodies; the kidneys are a known target organ for Mo- and Cd-induced toxicity, and exposure to excessive Cu can cause kidney damage and other adverse health effects (e.g., RAIS, 2020). However, while the kidney is also a known target of As, Pb, and Ni, the mean concentrations of these elements in the rodent whole bodies were similar to or greater than the concentrations in the kidneys. This may suggest fur-borne soils and dust are the primary source of As, Pb, and Ni in our rodent samples; Pb may also be associated with bones and teeth in the whole body samples. Alternately, or in addition, the speciation of these elements may be less nephrotoxic than dissolved or other more bioavailable species, or the elemental dosages were sufficiently low for renal biotransformation and elimination.

As previously mentioned for U, co-occurring ore body element uptake mechanisms in rodents likely included dietary exposure to translocated or surficial elements in or on vegetation, direct soil exposure by dermal contact and accumulation in the fur, and incidental ingestion of soil on external surfaces of foodstuffs or via grooming. In this way, the observed elemental variations among rodent species likely reflect differences in dietary preferences, life history strategies (e.g., foraging, caching), and habitat preferences (e.g., vegetation cover) (Gottesman et al., 2004; Hanson and Miera, 1978; Vander Wall et al., 2001). Similarly, differences in availability of dietary items (e.g., vegetation species differences) at each site may have contributed to differences in element burdens in rodent whole bodies and kidneys (Everett et al., 1978; Heikens et al., 2001).

4.3. Histopathology

Pathological effects of U and co-occurring metals and radiation on the liver and kidney have been reported in various species (e.g., Cooke, 2011; Morley, 2012) and include many of the changes assessed in the current study (e.g., tubular degeneration, kidney inflammation). For example, rats exposed to dietary Cu (>100 mg kg^{-1} day^{-1}) have exhibited liver degeneration, kidney necrosis, and associated inflammatory responses (Haywood, 1985; Rana and Kumar 1978). Lourenço et al. (2013) found that wood mice exposed to mine wastes and contaminated waters from an abandoned U mine had loss of DNA integrity in blood cells, up-regulation of the tumor suppressor gene P53. Indirect effects, such as immunosuppression leading to an increased rate and severity of parasitism (Maslov et al., 1967; Silverman et al., 1969), are also possible. These effects are not necessarily specific to elemental exposure and could also result from various other causes, including other toxicoses and infectious organisms. These microscopic changes, particularly when mild, can also be present at background levels in healthy or reference populations (e.g., Tête et al., 2014).

In this study, the majority of microscopic lesions were mild (Table 5; SI Table S12). Furthermore, some lesions were more or equally prevalent in rodents from reference or pre-production sites compared to active or post-production sites. For example, the DEG lesion occurred in 20–23% of rodents from all site types. The PPI lesion occurred in 47% of non-mineralized reference animals, 62% of active production animals, and 54% of post-production animals compared to 4% of pre-production animals. These findings indicate that in most cases, we were observing normal fluctuations of microscopic lesions in wild rodent populations (i.e., background; Thoolen et al., 2010) rather than lesions occurring due to exposure to elements or radiation. The animals appeared healthy and abundant at our study sites; however, the relatively short lifespans (1–2 years) of these species may have reduced the opportunity for the lesions to increase in severity; or the animals may have adapted to the natural radiation and mineralization at the sites over generations (e.g., Kudyasheva et al., 2007).

A similar observation showing increased prevalence of hepatic inflammation and granulomas in mice from a chronic exposure site versus a pre-production site was reported by Cleveland et al. (2019). In that work, concentrations of U, Cd, Ni were significantly greater in rodent whole bodies collected at a low-dose chronic U exposure site compared to a pre-production site; U was also greater in kidney tissues from the chronic exposure site. However, Cleveland et al. (2019) did not include the non-mineralized reference site for comparison. Animals having close contact with radionuclide-contaminated soil (e.g., voles, shews) had greater prevalence of ectoparasite infection than animals from non-contaminated areas (Maslov et al., 1967); therefore, the prevalence of PPI in the livers may indicate a general immune response (Cattley and Cullen, 2013). In addition, liver gene expression effects related to lipid metabolic processes and suppressed immune responses have been observed in bank voles (*Myodes glareolus*) exposed to low-dose radionuclides (Kesäniemi et al., 2019). These effects could also be due to parasitic host and environmental factors, such as increased susceptibility to infections or increased exposure to infectious organisms due to ecological disturbance at developed (i.e., pre-, active, and post-production) sites.

Overall, the mild and inconsistent prevalence (by site) of most lesions indicates a lack of direct biological effects of mining exposure in the examined animals. There is evidence that metallothioneins mitigate pathological injuries (e.g., nephrotoxicity) in mice exposed to depleted U; biological detoxification and clearance mechanisms may account for the lack of direct effects (Hao et al., 2015). Similarly, gut microbiota may act as a barrier to limit the uptake of elements by rodents chronically exposed to dietary sources of Pb and Cd (Breton et al., 2013). The histopathological effects observed were mild or indirect; therefore, examination of a larger number of animals may be necessary to detect significant differences among sites.

5. Conclusions

The results presented here indicate that small rodents, invertebrates, and vegetation have been exposed to and taken up mining-related elements and radioisotopes at active and post-

production U mine sites (most notably As, Co, Pb, Ni, U, and gross beta activity) relative to non-mineralized reference sites, and in some cases, to pre-production sites (e.g., Cu, Mo, U). However, few protective thresholds were exceeded, and microscopic examinations of liver and kidney tissues revealed no clear, direct biological effects of U mining on small rodents. Our results, along with the relatively small footprint of breccia pipe uranium mines and the ease at which species were collected, indicate that population-level impacts are unlikely for rodents.

Nevertheless, these results may assist land managers in making decisions about relative U mining risks, approaches to site remediation, and resource protection. Our data indicate that land managers might consider a number of factors when determining breccia pipe site permitting, remediation, and restoration activities in the Grand Canyon watershed. For example, the interspecies differences in both small rodent whole body and kidney burdens are likely due, in part, to differences in physiology, foraging behaviors, dietary preferences, and the site-specific bioavailability of elements in the preferred habitat (Hickey et al., 2001; Pereira et al., 2006; Sample et al., 2014). Seasonal changes in element and radioisotope bioavailability, effects of precipitation and wind, soil conditions and leaching, plant uptake and bioconcentration, and animal foraging and caching behaviors may be worth further consideration (e.g., Alfani et al., 1996; Bidar et al., 2009; Erry et al., 1999).

This study focused primarily on terrestrial exposure, but aquatic pathways provide an important route for biomagnification of mining-related elements at these sites (Hinck et al., 2017). We were unable to directly study the containment ponds at these sites beyond locating aerial invertebrate traps as close as possible to the ponds from outside the fenced perimeters. In this way, the effects of active and post-production U mining on the aquatic food web at breccia pipe sites in the Grand Canyon watershed remains largely unknown in terms of elemental and radiochemical characterization, exposure, and uptake.

Further, ruminants and humans may have different toxicological thresholds than the small rodents studied here (Gál et al., 2008); effects on grazers should be studied if reclaimed lands are to be reopened to public grazing. Toxic effects (e.g., survival, abundance) may also be greater in taxa with longer natal dispersal distances and in taxa with higher population densities (Møller and Mousseau, 2011). Moreover, our comparisons to literature-based thresholds only consider a single element at a time; there may be shifts toward enhanced toxicity in the presence of multiple elements (Wallace and Berry, 1983).

Our data indicates that dust-loading on vegetation surfaces was a likely source of elevated concentrations of As, Cd, Pb, Ni, Th, U, and gross beta activity. Breccia pipe sites tend to be highly disturbed, even while in the pre-production stage. Therefore, it may be important to establish practices that stabilize breccia pipe sites and reduce blowing dust, especially during active ore production and site reclamation. Strategies might include the use of chemical dust reduction agents (Petavratzi et al., 2005), biocrust inoculations (e.g., Belnap and Büdel, 2016), or phytostabilization (Mendez and Maier, 2008). However, it will be important to avoid or minimize the use of approaches that might solubilize the U and co-occurring elements or radionuclides (e.g., direct watering of surface operations for dust control) and subsequently increase bioavailability (e.g., efflorescent salts). Further, during site reclamation, it will be important for land managers to select vegetation species that sequester elements and radionuclides in the roots such that dietary uptake and bioaccumulation of elements by animals consuming vegetation grown atop former mine sites is minimized (e.g., Sorensen et al., 2009). Reclamation strategies that have the potential to improve reseeding success, such as the use of connectivity modifiers, may also be useful (Fick et al., 2016). Uptake and translocation in cultural resources, medicinal or ceremonial plants, and agricultural crops should also be considered, and elemental and radionuclide loads might usefully be extrapolated to human uptake and effects (e.g., inhalation, ingestion, and dermal exposures; Samuel-Nakamura et al., 2019).

Credit author statement

Danielle Cleveland: Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing; Jo Ellen Hinck: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing; Julia S. Lankton: Investigation; Resources; Writing - original draft.

Data availability

Metadata and digital datasets are available, per USGS Data Management Policy, at https://doi.org/10.5066/P94OVQO9. Premining site metadata and digital datasets are available at https://doi.org/10.5066/F7QF8R16.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.127908.

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