SUPPLEMENTAL INFORMATION

for

Assessment of chronic low-dose elemental and radiological exposures to biota at the Kanab North uranium mine site in the Grand Canyon watershed

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This supplemental information describes sample processing (Section 1), microwave digestion (Section 1), and quality control results (Section 2) for this study. Metadata and digital datasets are available, per USGS policy, at DOI:10.5066/F7X0660R (chemistry and radiochemistry for vegetation/soils; chemistry for rodents) and DOI:10.5066/P99GDFWB (radiochemistry for rodents). Estimated method quantification limits (MQLs) are presented in Table S1. Literature-based comparison values, used to provide context for our results, are provided in Section 3, Table S2. A small survey of effects literature-based thresholds (multiple types; e.g., EC_{20}, LOAEL, etc.) that were not exceeded in Kanab North biota is provided in Table S3, while Section 4 describes some histopathological findings that could not be linked definitively to mining. It should be noted that one cliff chipmunk was collected at Kanab North;

those results are not discussed in the main text due to n=1, but are available in the digital datasets described above.

Section 1. Sample processing

Sample homogenization and lyophilization.

Rodent whole bodies were pre-homogenized by manual chopping with a titanium blade, then lyophilized and cryoground. Rodent kidneys were lyophilized whole to prevent loss of material to the container and for ease of transfer of the kidney tissues to microwave digestion tubes; lyophilized livers were homogenized using a cut-glass rod. The AGV and roots were similarly pre-homogenized by chopping prior to lyophilization. Lyophilized vegetation was then processed further using a hand-held immersion blender or by hand-crushing with cut glass rods. Soil samples were hand-mixed and then lyophilized. The lyophilized samples were stored at room temperature in a desiccator under a nitrogen atmosphere until digestion and instrumental analysis.

Microwave digestion.

All sample types were digested in closed Teflon vessels using a ramp to temperature (195 °C) program. The AGV and roots were digested in a 6:1 mixture of house-distilled concentrated nitric acid:high purity 30 % hydrogen peroxide. Soils were digested in an 11:1 mixture of house-distilled concentrated nitric:hydrochloric acids. Rodent kidneys and whole bodies were digested in house-distilled concentrated nitric acid.

Section 2. Quality Control

Method quantification limits for elemental analyses are summarized in Table S1. Quality control for elemental analyses included the use of NIST-traceable calibration standards and second-source calibration verification standards, certified reference materials, laboratory control samples, method replicates, analysis duplicates, method and analysis spikes, method blanks, and interference check standards, as appropriate for each analytical method. Laboratory control solutions indicated that the instruments were in-calibration, and analyte recoveries were generally 80-110 %. Duplicate and method replicate analyses had 0-10 % relative percent differences and 0-30 % relative standard deviations, respectively. Analysis and method spikes recoveries were 70-120 %. Method replicate and spike performance were likely affected by differing soil content in each sub-sampled portion, sample homogeneity, and small kidney sample masses. Analyte recoveries from certified reference materials were typically 80-120 %, although there were some instances of lower recoveries for Al (most likely due to silica-binding) and higher recoveries for Fe (most likely due to salt interferences). Accordingly, Al and Fe results are reported to fewer significant figures and have greater uncertainty than other analytes. Recoveries of ICP-MS targets in an interference check solution were 67-120 %, and dilution checks had < 30 % difference from undiluted samples. Analytical results were not adjusted for element recovery in control materials.

Reporting limits were 150 Bq/kg (dw) for gross alpha activity, 370 Bq/kg for gross beta activities, 37 Bq/kg for each U and Th isotope, and 18.5 Bq/kg for Ra-226. Quality control measures for radiochemical analyses included duplicate analyses, laboratory control samples, and method blanks. All method blanks were below the reporting limits. Control sample recoveries were 78-120 %, and duplicate relative percent differences were 0.3-120 %. Method spike recoveries ranged from 97 % to 119 %.

Section 3. Literature-based comparison values

As mentioned in the main text, a significant number of elements of interest for our study were lacking in critical threshold values. A number of tissue concentrations for samples from impacted and reference areas for comparison are provided in Table S2; threshold values that were not exceeded have been listed in Table S3. Neither Table S2 nor Table S3 is meant to be all-inclusive.

Section 4. Histopathological changes not likely associated with mining

Generally, we could not definitively attribute lesions to U mining. One deer mouse from Canyon Mine and two brush mice from Kanab North had mild discrete hepatocellular vacuolation consistent with lipid accumulation. Lipid accumulation can indicate hepatocellular injury, but can also have physiological causes, such as mobilization of fat to the liver after a period of fasting. Because it was mild in these samples, it is unlikely to represent a toxic lesion or a mining-related issue. Discrete hepatocellular vacuolation consistent with lipid accumulation can indicate hepatocellular injury, but can also have physiological causes, such as mobilization of fat to the liver after a period of fasting. Because it was mild in the samples and occurred infrequently, it is also unlikely to represent a toxic lesion or a mining-related issue.

Inflammatory cell infiltrates in the liver were more common, and in some cases more severe, in mice from Kanab North compared to Canyon Mine. Hepatic EMH, or the presence of leukocyte and erythrocyte precursor cells in sinusoids, was also noted in similar numbers in mice at Kanab North (37 %) and Canyon Mine (42 %) and is considered normal when mild. Likewise, hepatic mineralization (5 of 19 Kanab North mice, 26 %; absent in Canyon Mine mice, 0 %) may be a consequence of necrosis of the liver cell or reflect a generalized metabolic imbalance. However, metabolic imbalance is not suspected in these samples because of the absence of corresponding kidney mineralization in most of the animals. Tubular regeneration typically indicates previous non-lethal degeneration, the cause of which could not be determined. Three rodents at Kanab North had liver parasites, compared to 0 of 12 at Canyon Mine. Certain parasitic infections have been shown to affect concentrations of metals in rodent tissues (e.g., Jankovská et al 2008); however, the infection rate was too low to draw any definitive conclusions.

Figure S1. An overview map of the Kanab North site in 2013, showing the general orientation of the former detention pond, waste pile, and downwind locations for vegetation collection. Rodents were collected in 2015 within 200 m of the highlighted perimeter. See the main text for more information.

Table S1. Estimated method quantification limits (MQL, mg/kg dry weight) for elements in sample types from Kanab North Mine. Percent of samples having concentrations below the MQL is noted in parentheses.

Element	Vegetation			Small rodents	
	AGV (n=13)	Roots (n=11)	Root soil (n=11)	Whole-body (n=19)	Kidney (n=19)
Al	1.14 (0)	2.56 (0)	52 (0)	5.3-14 (0)	6 (89)
As	0.33 (0)	0.48 (0)	1.08 (0)	0.07-0.08 (0)	0.17 (53)
Bi	0.01 (38)	0.01 (0)	0.2 (100)	0.03-0.06 (95)	0.02 (95)
Cd	0.03 (15)	0.03 (0)	0.01 (18)	0.04-0.06 (58)	0.03 (5)
Co	0.01 (0)	0.01 (0)	0.06 (0)	0.02 (0)	0.20 (79)
Cu	0.04 (0)	0.42 (0)	5.31 (0)	0.12-0.43 (0)	0.17 (0)
Fe	6.5 (0)	5.7 (0)	9.4 (0)	9.1-16 (0)	102 (0)
Pb	0.02 (0)	0.02 (0)	0.03 (0)	0.06-0.08 (0)	0.02 (37)
Hg	0.06 (85)	0.06 (100)	0.06 (82)	0.15 (100)	na
Mo	0.06 (0)	0.01 (0)	0.05 (18)	0.07-0.08 (0)	0.05 (26)
Ni	0.03 (0)	0.05 (0)	0.16 (0)	0.07-0.12 (0)	0.14 (53)
Se	0.02 (0)	0.02 (0)	0.07 (0)	0.01-0.02 (0)	na
Ag	0.02 (53)	0.01 (9)	0.03 (18)	0.03 (100)	0.01 (100)
Tl	0.01 (0)	0.01 (0)	0.01 (0)	0.01 (68)	0.02 (47)
Th	0.003 (77)	0.013 (36)	0.06 (0)	0.02-0.06 (68)	0.01 (79)
U	0.01 (0)	0.01 (0)	0.02 (0)	0.03-0.05 (0)	0.01 (5)
Zn	2.2 (0)	1.9 (0)	1.7 (0)	0.29-0.63 (0)	3.33 (0)

n = number of samples
AGV = above-ground portion of the vegetation
na = not available; measurements of Hg in kidneys were not performed due to the small sample size, and Se concentration results were too low to be reliable by ICP-MS. See main text for details.

Table S2. Literature-based soil and rodent tissue concentrations (dry weight basis) from impacted and reference areas.

Matrix	Site type	Location	Literature value	Reference
Soil				
Gross α	Baseline	Bangladesh	1.13-5.66 Bq/kg	Biswas et al 2015
Gross α	Regional	USA	2.4-12.6 Bq/kg	Tyler and Wallace 2016
Gross α	U exploration/exploitation	India	175-2260 Bq/kg	Pathak and Pathak 2012
Gross β	Baseline	Bangladesh	30.7-132 Bq/kg	Biswas et al 2015
Gross β	Regional	USA	42-330 Bq/kg	Tyler and Wallace 2016
Ra-226	Agricultural; fertilized with U-containing phosphorus fertilizers	Syria	13-36 Bq/kg	Al-Masri et al 2008
Ra-226	U mine; adjacent agricultural soil	Portugal	11550 Bq/kg [1]	Abreu et al 2014
Th-228	Background		31-53 Bq/kg	Bhatti et al 2012
Th-230	Background		32-61 Bq/kg	Bhatti et al 2012
Th-232	Typical range for rocks and soils	USA	4-130 Bq/kg	Myrick et al 1983; National Research Council (US) Committee on Evaluation of EPA Guidelines for Exposure to Naturally Occurring Radioactive Materials 1999; NCRPM 1987
Th-232	Sand; monazite mining site	Brazil	21-18450 Bq/kg	Vasconcelos et al 2009
U-238	Agricultural; fertilized with U-containing phosphorus fertilizers	Syria	11-33 Bq/kg	Al-Masri et al 2008
U-238	Typical range for rocks and soils	USA	4-140 Bq/kg	Myrick et al 1983; National Research Council (US) Committee on Evaluation of EPA Guidelines for Exposure to Naturally Occurring Radioactive Materials 1999; NCRPM 1987
U-238	Sand; high background radiation area	Egypt	36-177 Bq/kg	Mubarek et al 2017
As	Partially remediated mine	France	75-18,900 mg/kg	Drouhot et al 2014
As	U mine overburden soil	Germany	185 mg/kg	Gramss and Voight 2014
Cd	U mine overburden soil	Germany	28.4 mg/kg	Gramss and Voight 2014
Co	U mine overburden soil	Germany	38.7 mg/kg	Gramss and Voight 2014
Cu	U mine overburden soil	Germany	356 mg/kg	Gramss and Voight 2014
Ni	U mine overburden soil	Germany	65.6 mg/kg	Gramss and Voight 2014
Pb	U mine overburden soil	Germany	136 mg/kg	Gramss and Voight 2014
Th	U mine overburden soil	Germany	10.7 mg/kg	Gramss and Voight 2014
Th	Reference; naturally occurring in soil		2-12 mg/kg	Bhatti et al 2012
Tl	Median US concentrations	USA	0.1-0.8 mg/kg	Smith and Carson 1977
Tl	Low-contaminated region	Germany	0.5 mg/kg median	Heim et al 2002
U	Reference		0.4 to 6 mg/kg	Shacklette and Boerngen 1984, United Nations 2010, Smith 1997
U	U mine overburden soil	Germany	73 mg/kg	Gramss and Voight 2014
U	U mine; adjacent agricultural soil	Portugal	660 mg/kg	Abreu et al 2014
Vegetation				
Gross α	Baseline	Bangladesh	0.23-1.81 Bq/kg	Biswas 2015
Gross α	U exploration/exploitation	India	48-477 Bq/kg	Pathak and Pathak 2012
Gross β	Baseline	Bangladesh	305-1676 Bq/kg	Biswas 2015
Ra-226	Crops; grown in soil fertilized with U-containing	Syria	< 3 Bq/kg	Al-Masri et al 2008

	phosphorus fertilizers			
U-238	Crops; grown in soil fertilized with U-containing phosphorus fertilizers	Syria	0.05-0.8 Bq/kg	Al-Masri et al 2008
Ag	Vascular plants; non-polluted		0-1.5 mg/kg	Bowen 1966, Cooper et al 1970, Vanselow 1966, Horovitz et al 1974
Ag	Agricultural crops grown on Ag-amended topsoil	USA	0.009-33.8 mg/kg	Hirsch 1998
As	Reference grasses and forbs	Canada	1-22 mg/kg [2]	Ollson et al 2009
Cd	“Normal” plant concentrations		0.05-0.4 mg/kg	Gramss and Voigt 2014 and references therein
Cu	“Normal” plant concentrations		2-20 mg/kg	Gramss and Voigt 2014 and references therein
Ni	“Normal” plant concentrations		0.1-3 mg/kg	Gramss and Voigt 2014 and references therein
Ni	Bushes and trees; near Au mine tailings, chronic exposure	Egypt	6-15 mg/kg	Rashed 2010
Ni	Kapok bush and twisted acacia; reference	Egypt	1-2 mg/kg	Rashed 2010
Th	Grasses grown on U tailings	Canada	< 0.001-0.047 mg/kg	Moffett and Tellier 1977
Tl	Most frequent Tl concentration in plant tissues	Worldwide	0.05 mg/kg	Kataba-Pendias and Pendias 1999
Tl	Crops; Tl-contaminated		< 0.004-321 mg/kg	Tremel et al 1997; Wang et al 2013; Wierzbicka et al 2004
Tl	Crops; grown in areas with natural Tl sources	China	1-500 mg/kg	Xiao et al. 2004
Tl	Schreber's big red stem moss, polytrichum moss; grown in low-contaminated region	Germany	0.01-0.13 mg/kg	Heim et al 2002
U	Grasses grown on U tailings	Canada	< 0.01-0.66 mg/kg	Moffett and Tellier 1977
U	“Normal” plant concentrations		0.002-0.015 mg/kg	Gramss and Voigt 2014 and references therein
Zn	“Normal” plant concentrations		1-100 mg/kg	Kataba-Pendias and Pendias 2001; Gramss and Voigt 2014 and references therein
Kidney				
As	Wood mice, bank voles; moderately contaminated As sites	Britain	0.27-1.7 mg/kg	Erry et al 2000
As	White-footed mice, rice rats; enriched U production site	USA	0.05-0.17 mg/kg	Smith et al 2002
As	Wood mice, Western Mediterranean mouse, common vole, white-toothed shrew; partially remediated Au mine	France	0.31-12.8 mg/kg	Drouhot et al 2014
As	Deer mice; reference areas	Canada	< 0.14-0.6 mg/kg [2]	Ollson et al 2009
Cd	White-toothed shrew; pyrite mine spill-impacted, chronic exposure	Spain	16.7 mg/kg mean	Sánchez-Chardi et al 2009
Cd	White-toothed shrew; reference	Spain	4.66 mg/kg mean	Sánchez-Chardi et al 2009
Cd	Deer mice; abandoned Cu mine	Canada	0.38 mg/kg	Laurinolli and Bendell-Young 1996
Cd	Bank voles, pygmy field mouse; Cu smelter	Urals	0.80 mg/kg mean	Kovalchuk et al 2017
Cd	Bank voles, pygmy field mouse; background	Urals	0.38-0.39 mg/kg mean	Kovalchuk et al 2017
Co	Deer mice; oil sands mining reference	Canada	0.138 mg/kg [2]	Rodriguez-Estival and Smits 2016
Cu	Deer mice ; oil sands mining reference	Canada	21.2 mg/kg [3]	Rodriguez-Estival and Smits 2016
Cu	Deer mice; pre-mining (oil shale region)	USA	22.0-22.7 mg/kg	Stelter 1980
Cu	Deer mice; abandoned Cu mine	Canada	3.96 mg/kg	Laurinolli and Bendell-Young 1996

Mo	Deer mice; oil sands mining reference	Canada	1.35 mg/kg [3]	Rodriguez-Estival and Smits 2016
Mo	Deer mice; pre-mining (oil shale region)	USA	5.7-7.8 mg/kg	Stelter 1980
Ni	Deer mice; serpentine area with naturally elevated Ni concentrations	USA	Not detected	Oswald 2004
Ni	White-footed mice, rice rats; enriched U production site	USA	0-4 mg/kg	Smith et al 2002
Tl	Bank voles, wood mice, yellow-necked mice, common shrew; reference areas	Poland	< 0.07 - 0.24 mg/kg	Dmowski et al 1998
Tl	Bank voles, wood mice, yellow-necked mice, common shrew; near Zn smelter	Poland	< 0.07 - 44.1 mg/kg	Dmowski et al 1998
Zn	Deer mice; abandoned Cu mine	Canada	15.9 mg/kg	Laurinolli and Bendell-Young 1996
Whole body				
Ra-226	Meadow voles; U mine tailings	Canada	1480 Bq/kg in bones; 370 Bq/kg in total body [4]	Cloutier et al 1985
As	Wood mice, bank voles; moderately contaminated As sites	Britain	0.22-9.4 mg/kg	Erry et al 2000
As	Deer mice carcass; reference areas	Canada	0.65-1.3 mg/kg [2]	Ollson et al 2009
As	Deer mice carcass; gold mining areas	Canada	12-43 mg/kg [2]	Ollson et al 2009
Se	Typical mammal background body burden	USA	< 1-4 mg/kg	US DOI 1998
U	Southern red-backed and meadow voles; U tailings and milling sites	Canada	0.03-0.65 mg/kg [2]	Thomas 2000
U	Deer mice, carcass; Los Alamos firing and surface disposal site	USA	< 0.5-30 mg/kg	Hanson and Miera 1978

Bank vole = *Clethrionomys glareolus*; Common shrew = *Sorex araneus*; Common vole = *Microtus arvalis*; Deer mice = *Peromyscus maniculatus*; Kapok bush = *Aerva javanica*; Meadow vole = *Microtus pennsylvanicus*; Polytrichum moss = *Polytrichum formosum*; Pygmy field mouse = *Apodemus uralensis*; Rice rat = *Oryzomys palustris*; Schreber's big red stem moss = *Pleurozium schreberi*; Southern red-backed vole = *Clethrionomys gapperi*; Twisted acacia = *Acacia raddiana*; Western Mediterranean mouse = *Mus spretus*; White-footed mouse = *Peromyscus leucopus*; White-toothed shrew = *Crocidura russula*; Wood mice = *Apodemus sylvaticus*; Yellow-necked mice = *Apodemus flavicollis*

[1] Adjusted from 2310 Bq/kg, representing 20 % of concentration
[2] Assumed 80 % moisture
[3] Geometric mean using the stated conversion factor of 4.10
[4] Estimated from Figure 14

Table S3. Literature-based thresholds that were not exceeded at Kanab North.

Analyte	Threshold (dry weight basis)	Reference
Soil		
Ag	< 10 mg/kg; proposed for sensitive crop species (germination, emergence, biomass)	Hirsch 1993
As	200 mg/kg; phytotoxic soil concentration for growth of Scots pine seedlings	Sheppard et al 1985
Co	800 mg/kg; phytotoxic soil concentration for Scots pine seedlings (*Pinus sylvestris*)	Sheppard et al 1985
Mo	> 525 mg/kg; inhibition of shoot yields, stem discoloration, and chlorosis	McGrath et al. 2010
Zn	100-5400 mg/kg; soil toxicity limit for growth and tillering, sensitive crops	Kataba-Pendias and Pendias 2001
Vegetation		
Ag	5.1 mg/kg (AGV), 1760 mg/kg (roots); reduced yields of bush beans (*Phaseolus vulgaris*)	Wallace et al. 1977
As	62 mg/kg; toxicity threshold (survival), Scots pine shoots	Sheppard et al 1985
As	1-20 mg/kg; phytotoxicity, 10 % yield depression, agricultural crops and fruit tree leaves	Kataba-Pendias and Pendias 2001 and references therein
As	28 mg/kg dietary threshold; tissue damage in bank vole (*Clethrionomys glareolus*)	Griffin et al 2001
Cd	5-30 mg/kg; phytotoxic, upper critical level based on chlorophyll inhibition, sensitive crops	Kataba-Pendias and Pendias 2001 and references therein
Cd	0.05-0.4 mg/kg Cd; tolerable limits in forage plants	Gramss and Voigt 2014
Ni	> 10 mg/kg; plant leaf toxicity limit, agricultural crops	Kataba-Pendias and Pendias 2001
Pb	30-100 mg/kg; plant leaf toxicity limit, agricultural crops	Kataba-Pendias and Pendias 2001
Se	1.4 mg/kg dietary Se exposure; sublethal effects threshold, lifetime exposure of rats 3 mg/kg dietary Se exposure; reduced longevity threshold and LOAEL for reproductive selenosis in rats, lifetime exposure of rats	US DOI 1998
Se	6.4 mg/kg dietary Se exposure; enlarged spleens and reduced growth rats	Halverson et al 1966
Se	4-250 mg/kg Se; experimental sublethal LOAEL in plant tissues for growth (800 mg/kg lethal experimental LOAEL)	US DOI 1998
Tl	2 mg/kg (roots); inhibits germination, plant growth, and chlorophyll content	Kataba-Pendias and Pendias 2001
Kidneys		
Cd	100-120 mg/kg; critical threshold; rat proteinuria, cell necrosis, tubular degeneration, other effects	Cooke 2011
Cd	33-190 mg/kg; reduced fetal and pup development	Shore and Douben 1994
Cd	105 mg/kg; LOAEL based on necrotic changes in adult animal kidneys	Chmielnicka et al 1989
Pb	> 16 mg/kg; kidney damage and weight loss in mammals	Ma 2011
U	1-3.5 mg/kg; LOEC for renal damage in rats	Gilman et al 1998
Whole body		
Se	> 10 mg/kg; mammal reproductive depression threshold	US DOI 1998

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