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Tissue metal concentrations and histopathology of rats gavaged with vitrified soil obtained from the former Charleston Naval Shipyard (SC, USA)

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Abstract

Male Sprague Dawley® rats were administered a vitrified material obtained from the former Charleston Naval Shipyard (Charleston, SC, USA) by gavage once daily for 32 days. Group mean body weight of treated animals was within ±5.4% of controls. No gross or histopathological changes were observed when animals were treated with 67, 174, or 370 mg/kg per day. Analysis of heavy metals revealed a statistically significant increase only in the concentration of arsenic in the livers of animals treated with 174 or 370 mg/kg per day versus controls. Although there was a statistically significant increase in liver arsenic levels, the concentrations were far below mean soil concentrations for western and eastern United States. If the standard assumption of 100% absorption is used, the concentrations observed in the present study are about 20 times less than the average background soil levels in these regions. Based on this, it is concluded that the vitrified material would not pose a public health risk for its intended use as an additive for asphalt and glass beams.

Keywords: Waste disposal; Ex situ vitrification; Metal concentrations; Glasses; Risk assessment

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1. Introduction

Environmental decontamination is a critical step in the effort to clean up the USA's hazardous waste sites. Vitrification development began in 1980 as a method to treat soil contaminated with radioactive materials in place (in situ vitrification) to avoid the problems associated with excavation and transportation [1]. Over the years, this technique was applied to non-radioactive material. Vitrification of hazardous wastes has been determined to be environmentally compatible because of the high chemical resistance of the glass product [2]. In October 1996, the United States (US) Department of Energy (DOE) enacted Project Number 106 to coordinate the performance of research related to the clean up and recycling of contaminated soils from the Charleston Naval Complex (Charleston, SC, USA) and local area harbor dredge spoils. Tests conducted by the Westinghouse Savannah River Company (Aiken, SC, USA) at Clemson University (Clemson, SC, USA) have resulted in a new ex situ vitrification technology that can be used to treat the DOE's contaminated soils [2].

Previously, in situ vitrification systems have been used to treat toxic waste that is present in soil by the insertion of electrodes into the ground to melt the contaminated soil into a stable glass-like material [3]. The hazardous substances are destroyed or rendered non-accessible or non-leachable to the environment, but the vitrified material remains in the ground [2,4]. In contrast, ex situ vitrification is a highly efficient process that allows for the large-scale removal of hazardous materials [2]. This process provides high temperatures by creating current flow between electrodes. The high temperature of the melt zone restructures the

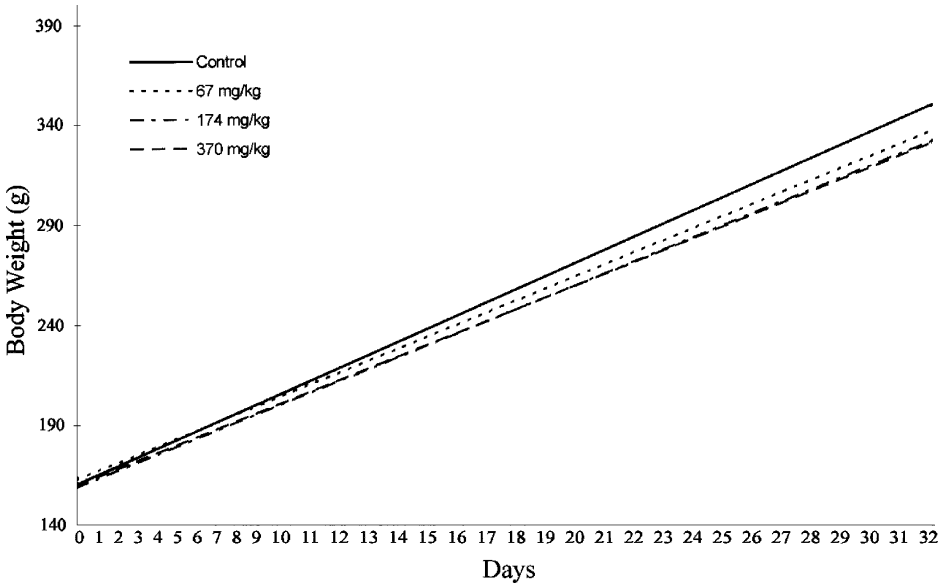

Fig. 1. Body weight gain of male Sprague Dawley® rats treated with vitrified material for 32 days. There was no statistically significant difference between treated animals and controls. Symbols represent the mean value for each group, $n = 7$.

molecular properties of the waste, creating a recoverable glass-like material. Organic substances are fully oxidized and metals are substantially removed and then recycled. Thus, instead of locking metals into the glass structure, this process physically separates most regulated metals from the glass [2]. In this respect, the thermal cleansing method of this process is unique and represents a step forward in technology. This vitrification process forms glasses of similar chemical compositions to those of basalt rocks, which are known to be among the oldest and most durable rocks in the earth's surface [2].

This study was designed to determine the toxicity of vitrified material obtained from the former Charleston Naval Shipyard, and subsequently to assess the risk associated with the materials proposed use, mostly in the Southeast USA, as an additive to asphalt or as replacement of steel beam with glass beam [4]. To our knowledge, this is the first study to evaluate the toxicity of hazardous waste treated by ex situ vitrification.

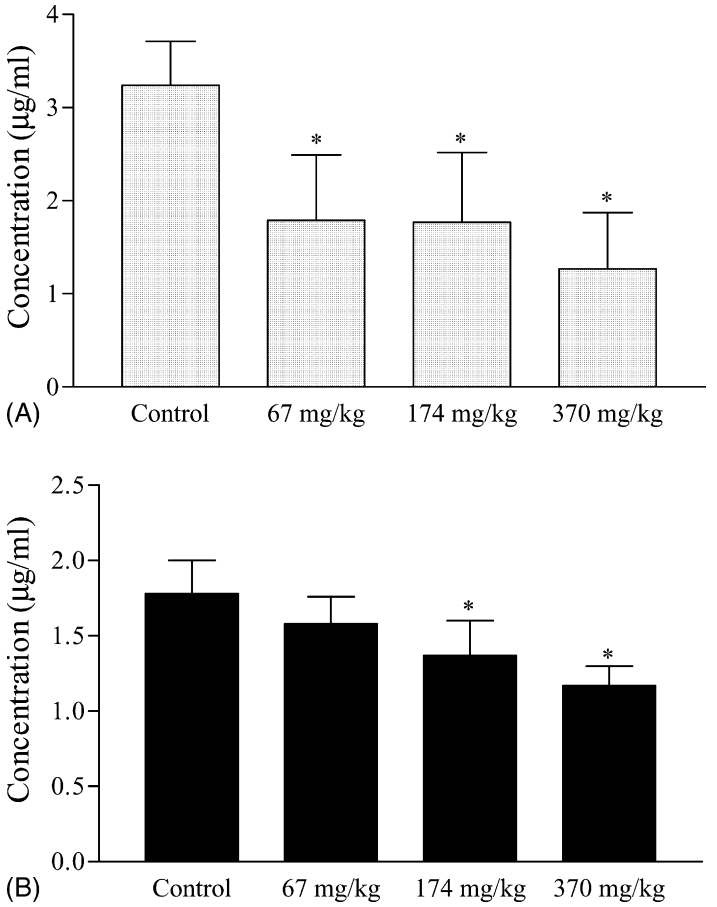

Fig. 2. Blood concentrations (mg/ml) for: (A) copper, and (B) selenium of male Sprague Dawley® rats treated with vitrified material for 32 days. Asterisk indicates a statistically significant difference from controls, $P < 0.05$. Bars represent the mean ± S.D., $n = 7$.

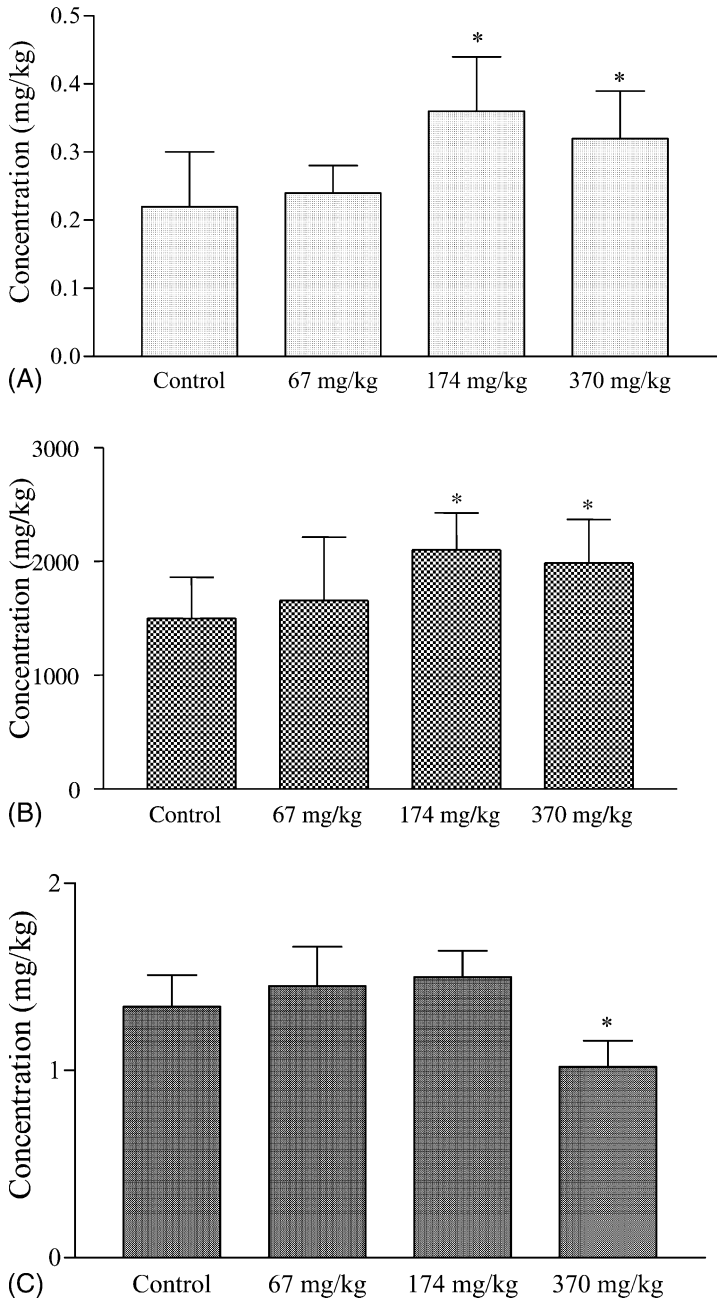

Fig. 3. Liver concentrations (mg/kg) for: (A) arsenic, (B) potassium, (C) selenium, and (D) titanium of male Sprague Dawley® rats treated with vitrified material for 32 days. Asterisk indicates a statistically significant difference from controls, $P < 0.05$. Bars represent the mean ± S.D., $n = 7$.

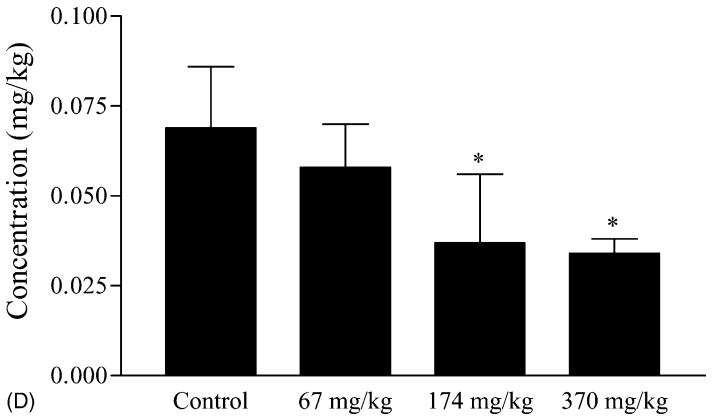

Fig. 3. (*Continued*).

2. Materials and methods

A sample of vitrified material was provided by the Center for Engineering Ceramic Manufacturing, Clemson University. The vitrified sample was prepared by the DOE and was a combined mixture of hazardous waste obtained from a processing foundry and an electroplating metal shop from the Charleston Naval Shipyard. Prior to receiving the sample, the substance was passed through a 35-mesh sieve to provide a sandy material less than 500 microns.

Male Sprague Dawley® rats, weighing approximately 150 g, were obtained from Harlan Sprague Dawley®, Inc. (Indianapolis, IN, USA). Rats weighing in this range were chosen because they are rapidly growing, and a reduction in the rate of body weight gain can be used as an indicator of an effect of the test substance. Animals were kept in an environmentally controlled room (25 °C, 40–70% relative humidity, 12 h light/dark cycle). They were housed in cages (three per cage for phase I and two per cage for phase II) with corncob bedding (Bed-o'cobs®; The Andersons, Inc., Maumee, OH, USA) and acclimated for 1 day (phase I) or 5 days (phase II) prior to experimentation. The rats had free access to food (Rodent Lab Diet; PMI Feeds, Inc., Richmond, IN, USA) and water. Animals were observed twice daily for any clinical signs of morbidity. Individual body weight and water consumption were monitored daily. Metal levels were measured in food, water, and bedding (data not shown). All animals in this study were sacrificed via CO_2 asphyxiation. Gross autopsies and histopathological evaluations were performed on each animal.

The study was conducted in two phases. Phase I involved a 72 h pilot test in which 12 rats were studied. Controls received the vehicle once daily by gavage, pH buffered saline with no preservative (Fisher Scientific International, Inc., Pittsburgh, PA, USA), via a 5 ml Luer-Lok syringe (Becton Dickinson & Co., Franklin Lakes, NJ, USA) prefitted with a shortened surgical feeding tube (Sherwood Medical Co., St. Louis, MO, USA). Experimental animals received once daily by gavage: low dose 67 mg/kg, medium dose 174 mg/kg, or high dose 370 mg/kg of the vitrified material suspended in saline. After 72 h, all animals were sacrificed. The purpose of the phase I study was to determine the feasibility

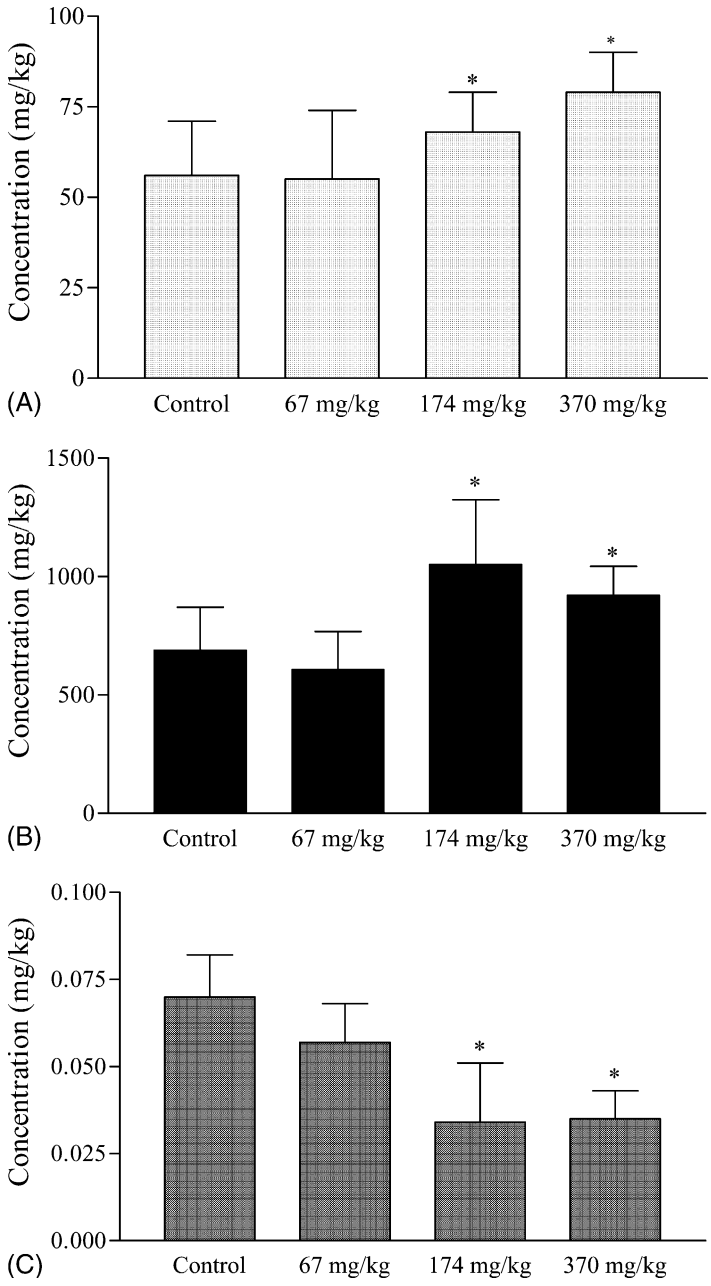

Fig. 4. Kidney concentrations (mg/kg) for: (A) calcium, (B) sodium, and (C) titanium of male Sprague Dawley® rats treated with vitrified material for 32 days. Asterisk indicates a statistically significant difference from controls, $P < 0.05$. Bars represent the mean ± S.D., $n = 7$.

of gavaging rats with vitrified material through the above described device and the physical limitations of the test animals to tolerate the test volume. Phase II involved a subchronic treatment (14 and 32 days) in which 40 rats were randomized into four groups receiving the following: group I, saline control; group II, 67 mg/kg; group III, 174 mg/kg; group IV, 370 mg/kg of vitrified material, once daily by gavage. On day 14, 3 rats from each group were sacrificed; the remaining 28 rats were sacrificed on day 32.

For each phase of the study, samples of liver, heart, lungs, small bowel, colon, iliac peripheral nerve, kidney, para-aortic lymph nodes, thymus, spleen, vertebrae and rib bone, psoas muscle, adrenal gland, para-ganglia, inter-vertebral and costosternal cartilage, pancreas, trachea, esophagus, and stomach were removed for histological examination. All tissues with the exception of blood were immediately fixed in 10% neutral buffered formalin (Sigma Chemical Company, St. Louis, MO, USA) and embedded in paraffin. Histopathological parameters were evaluated independently and assigned an intensity score as described previously [5]. Additional samples of liver (phase II, 14 and 32 days) and kidney (phase II, 32 days only) were stored unfixed at −80 °C for metal analysis. Cardiac blood samples were immediately collected after the animals were sacrificed and were kept refrigerated for metal analysis (phase II, 14 and 32 days).

All samples (food, water, corncob bedding, and tissues (blood, liver, and kidney)) were sent to a US Environmental Protection Agency (EPA) certified laboratory (Ecology and Environment, Inc., Lancaster, NY, USA) for metal analyses. All metals were analyzed using Optima 3000XL inductively coupled plasma–atomic emission spectrophotometer

Table 1
Phase II (32-day) metal concentrations for blood (μg/ml)

Element	Control	67 mg/kg	174 mg/kg	370 mg/kg
Silver (Ag)	0.008 ± 0.022	0	0	0
Aluminum (Al)	1.63 ± 1.98	0.05 ± 0.10	0.09 ± 0.18	0.21 ± 0.21
Arsenic (As)	6.0 ± 0.6	5.6 ± 0.2	5.0 ± 0.7	5.4 ± 0.9
Barium (Ba)	0.49 ± 0.37	0.23 ± 0.08	1.34 ± 3.30	0.06 ± 0.01
Calcium (Ca)	83 ± 24	80 ± 16	75 ± 17	61 ± 12
Cadmium (Cd)	0	0	0	0
Chromium (Cr)	0.004 ± 0.010	0	0	0
Copper (Cu)	3.24 ± 0.472	1.79[a] ± 0.70	1.77[a] ±0.75	1.27[a] ± 0.60
Iron (Fe)	491 ± 19	475 ± 18	460 ± 59	487 ± 69
Potassium (K)	2038 ± 48	1985 ± 89	1928 ± 170	1971 ± 213
Magnesium (Mg)	16 ± 10	21 ± 3	23 ± 3	22 ± 2
Manganese (Mn)	0.026 ± 0.039	0.035 ± 0.035	0.008 ±0.012	0.009 ± 0.024
Sodium (Na)	1791 ± 111	1757 ± 113	1842 ± 171	1828 ± 236
Nickel (Ni)	0.277 ± 0.127	0.365 ± 0.205	0.239 ± 0.073	0.201 ± 0.254
Lead (Pb)	0.34 ± 0.48	0.08 ± 0.02	0.069 ± 0.031	0.058 ± 0.009
Selenium (Se)	1.78 ± 0.22	1.58 ± 0.18	1.37[a] ± 0.23	1.17[a] ± 0.13
Silicon (Si)	5.22 ± 1.30	5.21 ± 2.29	4.53 ± 2.59	5.02 ± 1.62
Tin (Sn)	0.961 ± 0.382	1.432 ± 0.973	0.970 ± 0.328	0.397 ± 0.070
Titanium (Ti)	0.093 ± 0.020	0.105 ± 0.008	0.097 ± 0.035	0.072 ± 0.006
Zinc (Zn)	7.4 ± 1.2	7.6 ± 1.0	6.1 ± 0.7	6.5 ± 1.4
Zirconium (Zr)	0.022 ± 0.023	0	0.006 ± 0.008	0.005 ± 0.012

[a] Indicates a statistically significant difference from a control. Values represent the mean ± S.D., $n = 7$.

(Perkin-Elmer Corp., Norwalk, CT, USA) with argon as the inert gas. The analysis used is suitable for quality control according to EPA SW846 Method 6010A.

One-way analysis of variance (ANOVA) and Tukey's honestly significant difference test were used to determine differences in weight gain and metal concentrations of treated and control groups. Differences in histopathology of treated and control groups were determined by the Kruskal–Wallis test followed by Dunn's post-test. Alpha less than 0.05 was considered statistically significant.

3. Results and discussion

Body weight gain was used as an indicator for the effect of the vitrified material on body development [6]. No statistically significant differences were observed in body weight gains between treated animals and controls (Fig. 1). Histopathological examinations of the tissues from treated animals were consistent with findings from controls. There was no treatment-induced pathology in any of the 52-test animals (data not shown).

Arsenic, cadmium, chromium, and lead were the primary metals of toxicological concern in this study. In phase II (14 days), there was no statistically significant increase in levels of these metals in the blood or liver of animals treated with up to 370 mg/kg per day of vitrified material (data not shown). Blood samples revealed a statistically significant increase in sodium between the experimental group receiving 67 mg/kg and controls. Liver analyses

Table 2
Phase II (32-day) metal concentrations for liver (mg/kg)

Element	Control	67 mg/kg	174 mg/kg	370 mg/kg
Silver (Ag)	0	0	0	0
Aluminum (Al)	0.02 ± 0.06	0	0.12 ± 0.22	0
Arsenic (As)	0.22 ± 0.08	0.24 ± 0.04	0.36[a] ± 0.08	0.32[a] ± 0.07
Barium (Ba)	0.17 ± 0.04	0.12 ± 0.02	1.92 ± 4.46	0
Calcium (Ca)	35 ± 16	40 ± 10	39 ± 22	36 ± 19
Cadmium (Cd)	7.4 ± 19	0	0.01 ± 0.04	0.001 ± 0.002
Chromium (Cr)	0	0.004 ± 0.010	0	0
Copper (Cu)	2.90 ± 0.42	3.68 ± 0.59	3.94 ± 1.29	3.47 ± 0.48
Iron (Fe)	60 ± 16	62 ± 25	89 ± 12	83 ± 11
Potassium (K)	1498 ± 363	1657 ± 556	2100[a] ± 326	1985[a] ± 384
Magnesium (Mg)	157 ± 28	254 ± 34	170 ± 15	175 ± 16
Manganese (Mn)	1.670 ± 0.637	1.540 ± 0.632	2.028 ± 0.179	1.857 ± 0.207
Sodium (Na)	455 ± 80	560 ± 174	510 ± 47	472 ± 63
Nickel (Ni)	0.180 ±0.113	0.251 ± 0.126	0.172 ± 0.072	0.234 ± 0.106
Lead (Pb)	0.241 ± 0.283	0.100 ± 0.077	0.580 ± 0.687	0.139 ± 0.087
Selenium (Se)	1.34 ± 0.17	1.45 ± 0.21	1.50 ± 0.14	1.02[a] ± 0.14
Silicon (Si)	4.49 ± 0.91	4.51 ± 1.54	3.82 ± 1.39	3.74 ± 1.62
Tin (Sn)	0.407 ± 0.068	0.408 ± 0.078	0.388 ± 0.081	0.437 ± 0.062
Titanium (Ti)	0.069 ± 0.017	0.058 ± 0.012	0.037[a] ± 0.019	0.034[a] ± 0.004
Zinc (Zn)	18.9 ± 4.3	19.7 ± 5.3	43.2 ± 51.4	20.8 ± 2.4
Zirconium (Zr)	0.009 ± 0.012	0.016 ± 0.032	0.141 ± 0.374	0

[a] Indicates a statistically significant difference from a control. Values represent the mean ± S.D., $n = 7$.

Table 3
Phase II (32-day) metal concentrations for kidney (mg/kg)

Element	Control	67 mg/kg	174 mg/kg	370 mg/kg
Silver (Ag)	0	0	0	0
Aluminum (Al)	0.16 ± 0.44	0.07 ± 0.14	0	0
Arsenic (As)	0.18 ± 0.06	0.15 ± 0.08	0.15 ± 0.04	0.14 ± 0.07
Barium (Ba)	0.13 ± 0.04	0.11 ± 0.03	0.39 ± 0.97	0
Calcium (Ca)	56 ± 15	55 ± 19	68[a] ± 11	79[a] ± 11
Cadmium (Cd)	0.001 ± 0.001	0	0.006 ± 0.006	0.007 ± 0.011
Chromium (Cr)	0.008 ± 0.01	0	0	0
Copper (Cu)	3.77 ± 0.56	3.95 ± 0.82	4.34 ± 0.75	5.14 ± 0.79
Iron (Fe)	39 ± 16	40 ± 16	34 ± 7	35 ± 7
Potassium (K)	1152 ± 262	1241 ± 332	1500 ± 365	1414 ± 291
Magnesium (Mg)	128 ± 21	131 ± 24	128 ± 19	140 ± 17
Manganese (Mn)	0.629 ± 0.562	0.751 ± 0.717	0.414 ± 0.064	0.414 ± 0.190
Sodium (Na)	688 ± 182	607 ± 161	1051[a] ± 273	920[a] ± 123
Nickel (Ni)	0.264 ± 0.159	0.157 ± 0.093	0.241 ± 0.234	0.153 ± 0.089
Lead (Pb)	0.111 ± 0.034	0.104 ± 0.068	0.333 ± 0.323	0.274 ± 0.497
Selenium (Se)	1.30 ± 0.60	1.47 ± 0.13	1.38 ± 0.15	1.02 ± 0.20
Silicon (Si)	3.99 ± 1.11	3.94 ± 2.19	2.66 ± 1.16	3.81 ± 2.03
Tin (Sn)	0.320 ± 0.089	0.441 ± 0.385	0.289 ± 0.127	0.316 ± 0.126
Titanium (Ti)	0.070 ± 0.012	0.057 ± 0.011	0.034[a] ± 0.017	0.035[a] ± 0.008
Zinc (Zn)	15.0 ± 3.0	15.2 ± 4.5	15.4 ± 3.1	13.5 ± 1.5
Zirconium (Zr)	0.014 ± 0.014	0.005 ± 0.009	0.008 ± 0.011	0

[a] Indicates a statistically significant difference from a control. Values represent the mean ± S.D., $n = 7$.

of 21 metals revealed no statistically significant differences between treated and control groups (data not shown).

Phase II (32 days) metal analyses for blood revealed a statistically significant decrease in copper and selenium (Fig. 2). Liver metal analyses showed a statistically significant increase in arsenic and potassium and a decrease in selenium and titanium (Fig. 3). Kidney metal analyses revealed a statistically significant increase in calcium and sodium and a decrease in titanium (Fig. 4). The remaining metals analyzed were not significantly different in treated versus control animals. A complete listing of the 21 elements analyzed in blood, liver, and kidney are presented in Tables 1–3, respectively.

Although there was a statistically significant increase in liver arsenic levels (from 0.22 to 0.36 and 0.32 mg/kg), this increase is not clinically significant considering the concentration was far below the mean background soil levels for the western (7.0 mg/kg) and eastern (7.4 mg/kg) part of USA (Table 4). If the standard assumption of 100% absorption is used, the concentrations observed in the present study are about 20 times less than the average soil levels in these regions.

Concentrations of metals in the vitrified material used in this study were determined by the Albany Research Center, DOE (Albany, OR, USA). Metals in the samples, with the exception of arsenic and chromium, were below the EPA Region III (mid-Atlantic region) risk-based concentrations (RBCs) for soil (Table 4) [7]. EPA Region III RBCs are used to screen environmental hazards for baseline risk assessments. For a single contaminant in a single medium, the RBCs use standard default assumptions for assessing exposure (i.e.

Table 4
Risk comparison: vitrified material vs. EPA Region III RBCs and western/eastern USA soil metal contents (mg/kg)

Elements	Sample #1[a]	Sample #2[a]	Industrial[b]	Residential[b]	Western USA[c]	Eastern USA[d]
Silver (Ag)	<2	<2	10 000 N	390 N	ND	ND
Aluminum (Al)	27 100	22 800	2 000 000 N	78 000 N	5000–>100 000 (74 000)	7000–>100 000 (57 000)
Arsenic (As)	60	70	3.8 C	0.43 C	<0.1–97 (7)	<0.1–73 (7.4)
Barium (Ba)	130	130	140 000 N	5500 N	70–5000 (670)	10–1500 (420)
Calcium (Ca)	142 000	130 000	ND	ND	600–320 000 (33 000)	100–280 000 (6300)
Cadmium (Cd)	<10	<10	1000 N	39 N	ND	ND
Chromium (Cr)	1960	1170	Cr III: 3 100 000 N	Cr III: 120 000 N	3–2000 (56)	1–1000 (52)
	ND	ND	Cr VI: 6100 N	Cr VI: 230 N	ND	ND
Copper (Cu)	30	<10	82 000 N	3100 N	2–300 (27)	<1–700 (22)
Metallic Iron (Fe)	1200	1400	Iron: 610 000 N	Iron: 23 000 N	ND	ND
Fe^{2+}	21 000	16 000	ND	ND	1000–>100 000 (26 000)	100–>100 000 (25 000)
Fe^{3+}	<100	<100	ND	ND	ND	ND
Potassium (K)	6260	6490	ND	ND	1900–63 000	50–37 000
Magnesium (Mg)	69 700	66 100	ND	ND	300–>100 000 (10 000)	50–50 000 (4600)
Manganese (Mn)	150	170	41 000 N	1600 N	30–5000 (480)	<2–7000 (640)
Sodium (Na)	1270	1460	ND	ND	500–100 000 (12 000)	<500–50 000 (7800)
Nickel (Ni)	<20	<20	41 000 N	1600 N	<5–700 (19)	<5–700 (18)
Lead (Pb)	40	40	ND	ND	<10–700 (20)	<10–300 (17)
Selenium (Se)	60	70	10 000 N	390 N	<0.1–4.3 (0.34)	<0.1–3.9 (0.45)
Silicon (Si)	275 000	284 000	ND	ND	150 000–440 000	17 000–450 000 (15 000)
Tin (Sn)	<50	<50	1 200 000 N	47 000 N	<0.1–7.4 (1.2)	<0.1–10 (1.5)
Titanium (Ti)	970	1030	8 200 000 N	310 000 N	500–20 000 (2600)	70–15 000 (3500)
Zinc (Zn)	10	<10	610 000 N	23 000 N	10–2100 (65)	<5–2900 (52)
Zirconium (Zr)	60	50	ND	ND	<20–1500 (190)	<20–2000 (290)

ND: not determined; N: non-carcinogenic effect; C: carcinogenic effect.

[a] Albany Research Center, US DOE, Charleston Naval Shipyard samples (sample #1: CNC-GP21b; sample #2: CNC-GP23b).

[b] US EPA Region III RBCs for soils (October 2002) [7].

[c] Soils, west of 96th meridian. Observed range (mean) [9].

[d] Soils, east of 96th meridian. Observed range (mean) [9].

adult body weight is 70 kg, adults ingest 100 mg of soil per day, children (15 kg) ingest 200 mg of soil per day) [8]. Target risks coupled with these assumptions produce concentrations, which are expected to be protective. When comparing the vitrified material to normal soils found in the western and eastern US, metals were within the background concentration ranges, with the exception of chromium (eastern US), magnesium (eastern US), selenium, and tin (Table 4) [9]. However, none of the metals in the vitrified test substance were leachable in concentrations sufficient to fail the EPA Toxicity Characteristic Leaching Procedure (data not shown). In addition, even the maximum dose used in the current study did not result in a statistically significant increase in any of the latter elements.

4. Conclusions

All metal levels in tissues of treated animals were below EPA Region III RBCs, and all but four metal concentrations in the vitrified material were similar to the background levels found in soils from the western and eastern USA. Test animals treated with 370 mg/kg per day of the vitrified material showed no evidence of adverse health effects, as determined by body weight gain and histopathological analyses. The 370 mg/kg per day dose provided to the test animal is analogous to 25.9 g/70 kg per day (adult) or 5.6 g/15 kg per day (child) of the vitrified material, a value which by far exceeds the estimated average daily ingestion of soil [10]. Thus, there is a large margin of safety associated with its use. Based on this, it is concluded that the vitrified material, obtained from the former Charleston Naval Shipyard, would not pose a public health risk for its intended purpose as a constituent of asphalt or glass beams.

Acknowledgements

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