Development of a New Biomonitoring Technique Using Domestic Pets as Sentinel Species

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Abstract

The goal of this research project was to develop a new way of investigating residential exposure to environmental contaminants. Specific objectives were to 1) develop a new method of monitoring biological exposure, 2) test the method in the field, and 3) develop a technique for analyzing the data. Domestic pets were chosen as the sentinel species, and the protocol involved collection of hair samples with subsequent analysis using inductively coupled plasma–mass spectrometry (ICP-MS). The method was tested using ~ 100 pets residing in the Butte area, and a new technique was devised that defines hazard quotients and hazard indices commonly employed in the field of risk assessment to identify pets of concern (POCs) and elements of concern (EOCs). In the field campaign, 76 percent of hair samples had arsenic concentrations ≥ the reference concentration of 0.02 mg percent. Twenty-five pets were identified as POCs based on pet hazard indices (HI_j) ≥ 1.0, and only 10 of the 25 POCs resided within the Butte Priority Soils Operable Unit (BPSOU), a boundary set by the EPA to represent the bulk of residential contamination in Butte. We also identified the following elements as EOCs based on element hazard indices (HI_i) ≥ 1.0: sodium, copper, manganese, selenium, boron, molybdenum, arsenic, lead, aluminum, lithium, and zirconium. The new biomonitoring technique was designed as a screening-level tool for studying residential exposure to environmental contaminants, but pets are companion animals and results may have implications for human health risk assessment.

Key Words: biomonitoring, domestic pets, hair sampling, residential contamination

Introduction

Nearly a century of mining and smelting activities in the Butte/Anaconda area of Montana resulted in widespread contamination. Arsenic, cadmium, copper, lead, and zinc are pollutants commonly identified in tailings and mine waste. Some remediation has taken place over the past two decades as a direct outcome of the National Priorities List (NPL) designation of sites in the area. However, much contamination remains in the residential neighborhoods, and little is known about long-term health impact on populations exposed on a daily basis to residual contaminants.

We describe a new way of investigating residential exposure to environmental pollutants. The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as Superfund, employs simple methods for addressing human health risk based on concentrations of contaminants measured in the soil, sediments, water, and air at a site (U.S. Environmental Protection Agency 1989); general assumptions are made about intake to estimate potential cancer risks and health hazards for "hypothetical" receptors, such as residents, workers, adults, and children. These methods were not designed for communities such as Butte where the Superfund site is in the backyard, receptors are not hypothetical, and contaminated waste is in the yards, gardens, water supply, and house dust.

To improve our understanding of actual exposure to contamination in a residential area such as Butte, we propose using domestic pets as our sentinel species. Although levels of contact with contaminated soil and water might be much higher for pets than humans, they might act as good surrogate monitors for humans in contaminant exposure assessments.

This paper describes the first in a series of research campaigns for which the main objectives of this project were to 1) design a simple biomonitoring method, 2) test the method in the field, and 3) develop a technique for analyzing the data. Regarding objective 1, our novel protocol involved collection of hair samples with subsequent analysis using inductively coupled plasma mass spectrometry (ICP-MS) as an easy, non-invasive way to obtain biological data regarding toxin/contaminant environmental exposure. For objective 2, we tested the method in winter of 2004 by sampling ~ 100 pets, including a variety of breeds, ages, and residence locations. Finally, because a technique was not available for interpreting the data from samples of pet hair, a simple method was devised that utilized some of the concepts currently used in the field of risk analysis (objective 3).

Although use of animals as sentinel species is not a new idea, this project is the first of its kind to draw on domestic pets as biosamplers with subsequent hair sampling and analysis to study chronic exposure of environmental contaminants in a residential Superfund area. Although results from our campaign were site specific, the method developed and tested here has potential for applications elsewhere.

Background

Mining History in the Butte Area

Gold mining began in Butte in 1864 (U.S. Environmental Protection Agency 2005), and by 1884, more than 300 mines, nine stamp mills, and as many as seven copper smelters were present (MacMillan 2000). Heap roasting was a common form of smelting in the late 1800s, and the heaps in Butte consisted of large masses of sulfide ore intermixed with layers of logs harvested from the local forests. According to MacMillan (2000), the heaps were "*as large as city blocks, as wide as city streets, and as high as six feet.*" Heaps commonly burned for weeks at a time, releasing thick smoke containing undiluted oxides of sulfur, arsenic, particulates, and fluorides. Furthermore, smoke lingered in the valley for days in the wintertime during inversions. By-products of these localized mining and smelting activities included accumulation of waste piles, dumps, and tailings throughout the community, along Silver Bow Creek, and within the Clark Fork watershed (U.S. Environmental Protection Agency 2005).

Cancer Statistics in Silver Bow County

Butte is located in Silver Bow County, the only county in Montana that was assigned a "priority 1" index by the National Cancer Institute in 2004 (U.S.

Table 1. Cancer Rates from National Cancer Institute for 1997-2001.

Area	Annual Death Rate from Cancer per 100,000 people	Higher or lower than National Rate	Annual Percent Change in Death Rate from Cancer (95 % CI)	Rising or Falling Trend
Silver Bow County	**238.6** (218.3, 260.6)	**Higher**	**+3.2** (0.4, 6.1)	**Rising**
Montana	195.0 (191.0, 199.0)	Lower	-0.6 (-1.0,-0.2)	Declining
United States	199.8 (199.6, 200.0)	-	-1.1 (-1.2, -1.0)	Declining

Reference: National Cancer Institutes of Health 2004

National Institutes of Health 2004). Priority 1 indicates an area where the annual death rate from cancer is above the U.S. rate, and an area that also exhibits a rising trend of deaths from cancer (Table 1). The annual death rate from cancer for Silver Bow County between the years of 1997 and 2001 was 238.6/100,000 people compared to rates for Montana and the U.S. of 195.0/ and 199.8/100,000 people, respectively. In addition, trends showed that the annual rate of cancer death was rising in Silver Bow County but declining in the rest of Montana and the rest of the U.S.

In addition to data from the National Cancer Institute, a request from the Montana Department of Public Health and Human Services (MDPHHS) in 2001 prompted the Agency of Toxic Substances and Disease Registry (ATSDR) to conduct a health consultation, and the report was released in December of 2003 (Dearwent and Gonzalez 2002). Cancer incidences in Silver Bow County for the years 1979 to 1999 were compared to data from the entire state of Montana, and to the United States as a whole. Six types of cancer (urinary bladder, kidney, liver, lung, prostate, and skin) were chosen as those most often linked to arsenic exposure. Based on the average standardized incidence ratios (SIRs), Silver Bow County had higher cancer rates than the rest of Montana, and higher rates than the rest of the U.S., in at least one age group for nearly all six types of cancer. The only exception was for prostate cancer for which Silver Bow County dropped below the national rate, but even in that case the incidence of prostate cancer for Silver Bow County was dramatically higher than the rest of Montana.

Cancer data described above were averaged over the whole county. However, a variety of mining and smelting operations over the years resulted in levels of soil contamination with extreme spatial variations throughout the area. Spatial variations in residential exposure and health effects throughout Silver Bow County are likely. However to date, incidence of cancer or of other health problems has not been investigated on neighborhood scales with the purpose of relating environmental exposure to health effects.

Superfund Operable Units

Within the Clark Fork Basin Superfund complex, four units are listed on the NPL: Silver Bow Creek/Butte Area Site, Montana Pole Site, Anaconda Smelter Site, and Milltown Reservoir Sediment Site. Operable units (OUs) are separate components within an NPL site, and the Butte Priority Soils Operable Unit (BPSOU), a major focus of this study, is one of seven operable units within the Silver Bow Creek/Butte Area Site. Other operable units within the Silver Bow Creek/ Butte Area include Butte Mine Flooding, West Side Soils, Active Mining Area, Streamside Tailings, Rocker Timber Treating and Framing Plant, and Warm Spring Ponds OUs (U.S. Environmental Protection Agency 2005).

The remedial investigation/feasibility study (RI/FS) of the BBPSOU focused on contaminants in soils, mine waste, surface water, and alluvial groundwater in the urban area (U.S. Environmental Protection Agency 2005). One important feature of the BPSOU is the inclusion of inhabited residential areas of Butte and Walkerville. Although some remediation has been performed since 1988 (U.S. Environmental Protection Agency, 2005), residents in this area have been directly exposed to the mining-related contaminants in the air, soil, water, and dust for more than a century.

Mining-Related Contaminants of Concern and Health Effects

According to the EPA's proposed remedy for the BPSOU, the contaminants of concern (COCs) in the soils are arsenic, lead, and mercury (U.S. Environmental Protection Agency 2005). Adverse health effects in humans have been documented for these three elements (Table 2). Exposure to arsenic, lead, and mercury generally can cause minor problems, e.g., a sore throat, and major problems, e.g., nervous system disorders, cardiovascular diseases, and cancer.

Table 2. Table of Health Effects for Arsenic, Lead, and Mercury.

Contaminant	Health Effects	References
Arsenic	Sore throat, irritated lungs, nausea, vomiting, decreased red/white blood cell counts, abnormal heart rhythm, damage blood vessels, pins and needles, darkening of skin, wart/corns on palms/soles/torso, redness/swelling of skin, and lung/skin/bladder/liver/kidney/prostate cancers.	ATSDR 2005, IRIS 2005a, Klaassen 2001
Lead	Decreased reaction time, weakness in fingers/wrists/ankles, memory and intelligence effects, anemia, blood disorders, organs/nervous system effects, and damaged kidneys/ reproductive systems.	ATSDR 2005, IRIS 2005b, Klaassen 2001
Mercury	Death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects, respiratory, cardiovascular, and gastrointestinal effects.	ATSDR 2005, IRIS 2005c, Klaassen 2001

Historically, metals have been recognized primarily for their acute effects to humans exposed in the workplace, but more recently, deeper investigation into the subacute and chronic effects has evolved. Assigning responsibility for long-term toxic impact of metals has been extremely difficult because cause-and-effect relationships are hard to establish when the endpoint lacks specific symptoms. Such symptoms relate to metal exposure (Klaassen 2001) and additive effects from exposure over time to multiple contaminants at once. Research in Butte, however, has potential to improve our understanding of chronic exposure to a handful of mining-related contaminants and associated health risks in humans and in other species.

Because of the health hazards associated with arsenic and lead, residential preliminary remediation goals (PRGs) were developed for the BPSOU. The initial PRG for lead in the BPSOU soil was 1200 ppm based on a risk assessment published in 1994, but the value increased to 1575 ppm in 2003. The PRG for arsenic in soil and house dust was set at 250 ppm, a threshold that equates to a cancer risk of 1 in 10, 000 (U.S. Environmental Protection Agency 2005). These PRGs are less conservative than PRGs set for other Superfund sites; in fact, EPA Region 9 lists a PRG of 0.39 ppm for arsenic in residential soils (U.S. Environmental Protection Agency Region 9 2004), and the Montana Department of Environmental Quality (DEQ) recently published their action level in surface soil as 40 ppm based on 209 native soil samples collected in unimpacted areas throughout Montana (Department of Environmental Quality 2005). Unfortunately, no reliable research has been performed to date to document whether the cleanup actions based on these elevated PRGs in Butte have been effective at reducing residential exposure.

Sentinel Species for Studying Exposure

According to O'Brien et al. (1993), *"Sentinels are organisms in which changes in known characteristics can be measured to assess the extent of environmental contamination and its implications for human health and to provide early warning of those implications."* Over the years, many animals have been used as sentinel species to advance the field of toxicology (O'Brien et al. 1993). Heyder and Takenaka (1996) noted that small mammals have been used as primary sentinel species in most laboratory studies, but they also concluded that dogs (*Canis familiaris*) were preferred for evaluating pulmonary responses to air

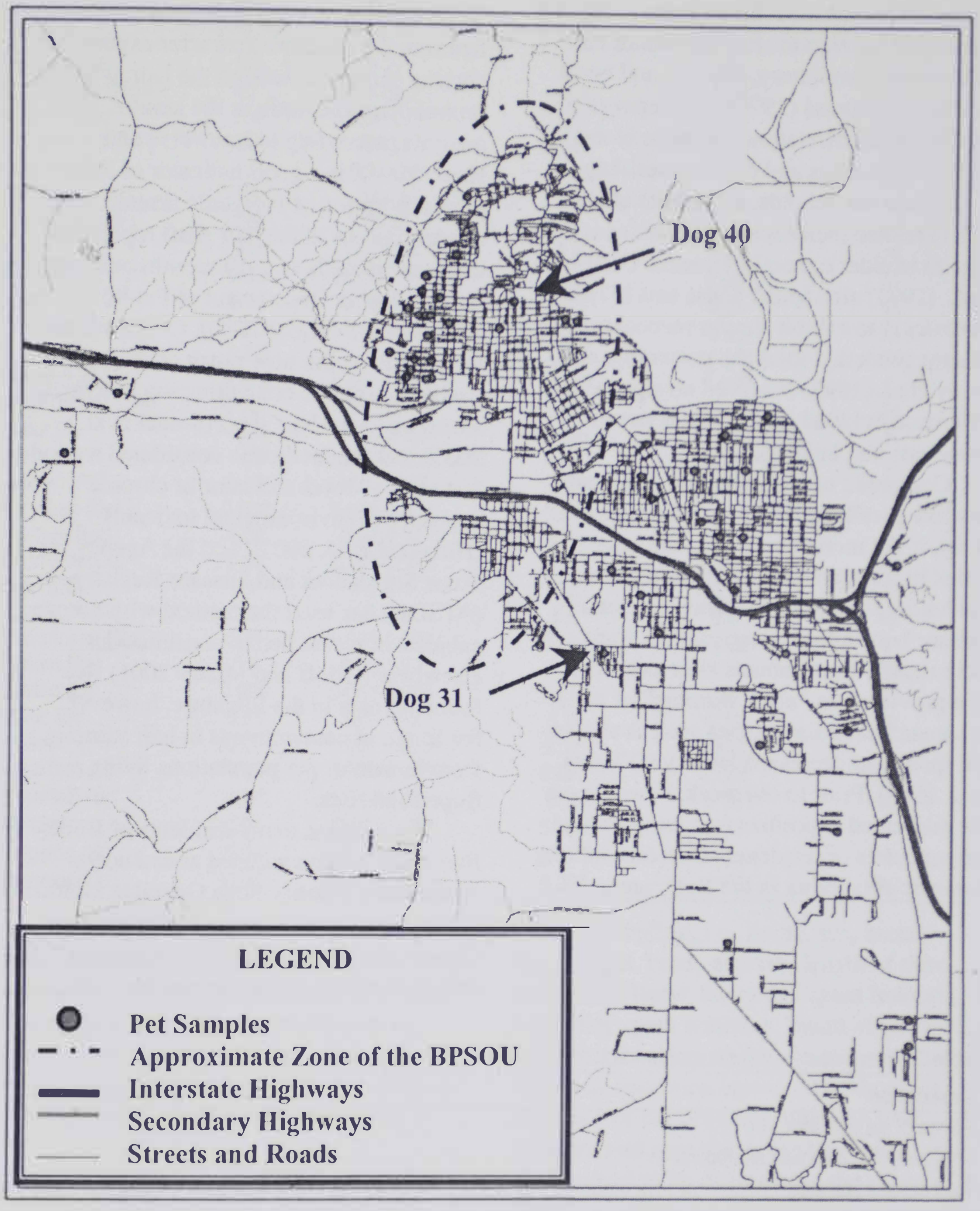

Figure 1. Map of Butte showing residence locations for the Butte pets sampled during the 2004 Hair Sampling Campaign. (Dogs 31 and 40 are discussed in the text.)

pollutants in controlled laboratory settings. Calderon-Garciduenas et al. (2001a, 2001b) expanded on this idea and examined dogs residing in Mexico to study effects of air pollution on respiratory, cardiac, and brain pathology. Thomas (1976) and Berny et al. (1995) found correlations between blood lead levels in dogs and children residing in the same households, and Hayes et al. (1981) related incidence of bladder cancer in dogs to bladder cancer in humans. O'Brien et al. (1993) also found house cats (*Felis domesicus*) to exhibit similar responses as humans to methyl mercury poisoning, and Berny et al. (1995) observed no significant difference in blood lead levels between house cats and humans.

Compared to other species, dogs and cats live parallel to humans and are most likely, if not more, exposed to the same toxins in soils, water, and house dust in a residential setting. In addition, dogs and cats are known to develop clinical signs more rapidly than humans after exposure, thus providing an "early warning" of threat to human health for reasons such as shorter life spans and decreased latency (O'Brien et al. 1993). Prior to our work, however, no one addressed exposures to mining-related contaminants or incidence of disease among domestic pets living in the Butte area.

Hair Analysis for Studying Chronic Exposure

The body has physiological mechanisms that remove contaminants after exposure such as excretion through the hair, which immobilizes the toxin in the keratin. Hair analysis, according to Lauwerys and Hoet (2001), is a good indicator of long-term absorption of inorganic arsenic, and concentrations in the hair shaft represents exposure periods of 1-10 months and longer (United States Department of Public Health and Human Services 2000). Controversial views exist about how much of the hair analysis might be contamination externally adsorbed to the hair shaft (Siedel et al. 2001), but the method is considered a good "screening" level indicator of chronic exposure to environmental toxicants (Hinwood et al. 2003), and the Agency for Toxic Substances and Disease Registry (ATSDR) has used the method with human subjects in recent health consultations elsewhere (Orloff and Mistry 2001). We found no data in the literature, however, for levels of contaminants in hair samples from human or pet populations living on Superfund sites.

In summary, many residents of Butte live inside a contaminated zone known as the Butte Priority Soils Operable Unit. Although cancer statistics for Silver Bow County are elevated when compared to rest of Montana and the rest of the U.S.,

Table 3. Summary of Information for Pets in the 2004 Hair Sampling Campaign.

Description	Number
Range of pet numbers	1-101
Total pets with complete lab reports	99
Total cat hair samples	7
Total dog hair samples	93
Pets sampled that resided in Butte	90
Pets sampled that resided elsewhere	10
Range of ages of pets (yr)	0.2-16
Average age of pets (yr)	6
Range of weight of pets (kg)	0.01-0.73
Average weight of pets (kg)	0.27
Number of male pets	57
Number of female pets	43
Pets residing within the Butte Priority Soils Operable Unit	48
Pets residing outside of the Butte Priority Soils Operable Unit	51

Table 4. Concentration Statistics of Elements for Hair Samples Collected in 2004.

Element	RfC (mg %)	Range (mg %)	Average (mg %)	Standard Deviation (mg %)	≥RfC (%)
Calcium (Ca)	129	13-272	93	55	26
Magnesium (Mg)	27	1.6-50.2	18.1	11.7	22
Sodium (Na)	205	9.6-1014	281	186	58
Potassium (K)	62	2-211	44	41	20
Copper (Cu)	1.7	0.9-10.3	1.7	1.3	23
Zinc (Zn)	20	14-41	18	3	11
Phosphorus (P)	35	16-46	31	6	20
Iron (Fe)	9.9	1-144.4	9.7	17.3	22
Manganese (Mn)	0.33	0.018-6.426	0.569	0.864	42
Chromium (Cr)	0.12	0.05-0.69	0.11	0.08	15
Selenium (Se)	0.16	0.09-0.49	0.22	0.08	72
Boron (B)	0.59	0.01-16.8	0.93	1.97	34
Cobalt (Co)	0.026	0.001-0.067	0.008	0.012	8
Molybdenum (Mo)	0.022	0.003-0.272	0.026	0.035	33
Sulfur (S)	4885	3726-5433	4466	326	9
Uranium (U)	0.02	0.000-0.0296	0.002	0.004	2
Arsenic (As)	0.02	0.01-1.397	0.065	0.149	76
Beryllium (Be)	0.002	0.001-0.003	0.001	0.0004	3
Mercury (Hg)	0.04	0.01-0.04	0.01	0.01	0
Cadmium (Cd)	0.02	0.001-0.318	0.014	0.045	8
Lead (Pb)	0.2	0.1-4.1	0.3	0.5	26
Aluminum (Al)	3.2	0.6-75.1	6.0	9.4	49
Germanium (Ge)	0.04	0.005-0.022	0.008	0.002	0
Barium (Ba)	0.4	0.02-1.93	0.24	0.29	16
Lithium (Li)	0.008	0.001-0.286	0.018	0.034	53
Nickel (Ni)	0.25	0.01-2.07	0.18	0.31	20
Platinum (Pt)	0.02	0.001-0.006	0.001	0.0005	0
Vanadium (V)	0.06	0.008-0.154	0.029	0.028	8
Strontium (Sr)	0.54	0.02-1.3	0.18	0.18	10
Tin (Sn)	0.04	0.001-0.09	0.020	0.02	8
Tungsten (W)	0.04	0.0003-0.073	0.005	0.011	2
Zirconium (Zr)	0.06	0.003-0.36	0.068	0.065	36

residential exposure and health problems have not been investigated on neighborhood scales. Our research introduces a new type of screening tool using domestic pets as sentinel species with hair sampling and analysis to study incidental, chronic contact with contaminants in the environment.

Experimental Methods

Our sampling campaign was conducted during February and March of 2004. We sampled ~ 100 pets, and the protocol consisted of the following components: 1) documentation, 2) hair sample collection, 3) hair sample analysis, 4) data entry, and 5) data analysis. Regarding documentation, pet owners were asked a series of standard questions about their dog or cat at the time of sampling, i.e., name, sex, breed, age, weight, home address, length of time residing in Butte, hours/day spent indoors, hours/day spent outdoors, brand of pet food, source of drinking water, etc. We collected each hair specimen between the shoulder and neck of the pet using stainless steel scissors, and care was taken to avoid cross contamination by disinfecting the scissors between collections. Specimen size was at least 150 mg, and immediately following collection, each sample was cataloged and placed in a sealed envelope specifically provided by the testing laboratory, Trace Elements, Incorporated (4501 Sunbelt Drive, Addison, TX 75001). Hair specimens were mailed to the laboratory within several days of collection.

Trace Elements, Incorporated is a

licensed, certified clinical laboratory. They specialize in analysis of human hair, mainly for nutritional purposes and are regularly inspected by the Clinical Laboratory Division of the Department of Health and Human Services. The laboratory personnel at Trace Elements, Incorporated use a state-of-the-art Sciex Elan 6100 for the inductively coupled plasma-mass spectrometry (ICP-MS) measurements. They factor extensive quality assurance and quality control (QA/QC) checks into all analytical procedures (Trace Elements, Incorporated 2000), and we submitted split samples with each batch of specimens as an external check.

Lab reports from Trace Elements contained concentrations for 32 elements, and they assigned a concentration equal to the instrument detection limit in the event that a concentration was below detection. Concentration units on each report were mg percent (mg of the element/100 g hair), and 1mg percent (mg %) = 10 ppm. Upon receipt of the lab reports, we entered concentrations for each pet into a spreadsheet, and at least one person checked data entry for accuracy.

The laboratory reports also listed reference concentrations (RfCs) for the elements. Reference ranges were established from a study of healthy dogs including all common breeds (Trace Elements, Incorporated 2005). A single reference level was given for toxic elements, e.g., uranium, arsenic, beryllium, etc., although nutritional elements, e.g., calcium, magnesium, etc. had a lower reference limit and an upper reference limit that described a zone of preferred concentrations. For example, the RfC for arsenic was 0.02 mg percent, but an acceptable RfC range for calcium was 41-129 mg percent. Concentrations above these RfCs are not necessarily toxic, but the values can be considered "guidelines for comparison with reported test values" (Trace Elements, Incorporated 2005).

Results and Discussion

General Information

Background data gathered during the sampling campaign included a suite of information for the study (Table 3). Pet numbers ranged from 1 to 101, but one young puppy (#34) did not have enough hair to add up to a 150-mg sample, and one number (#55) was not used as a pet number,

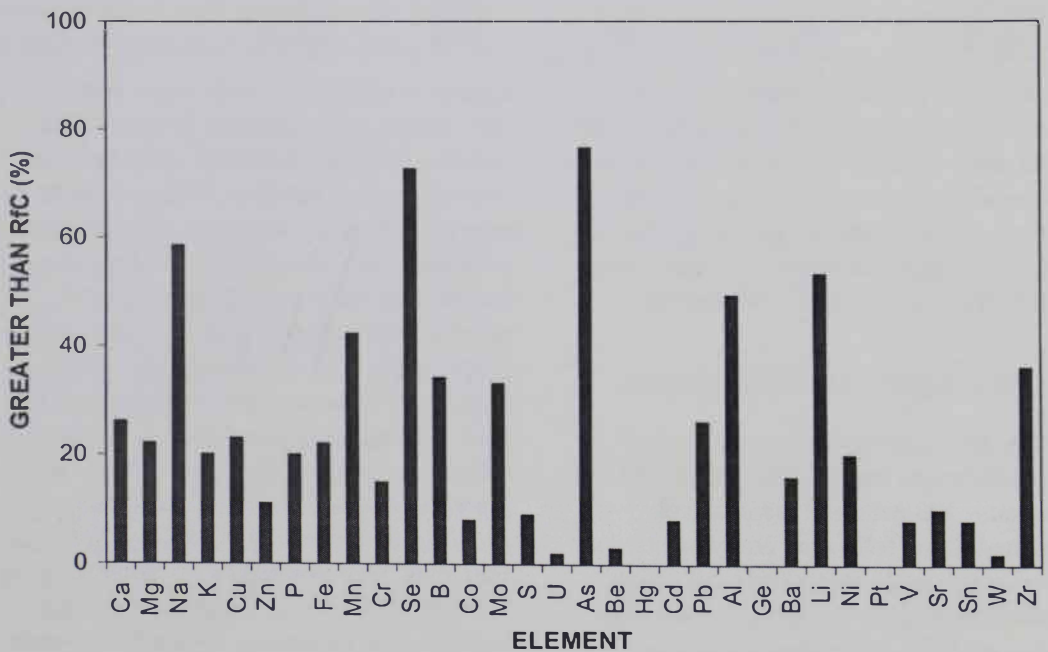

Figure 2. Percent of samples in the 2004 campaign where the concentration exceeded the reference level for each element.

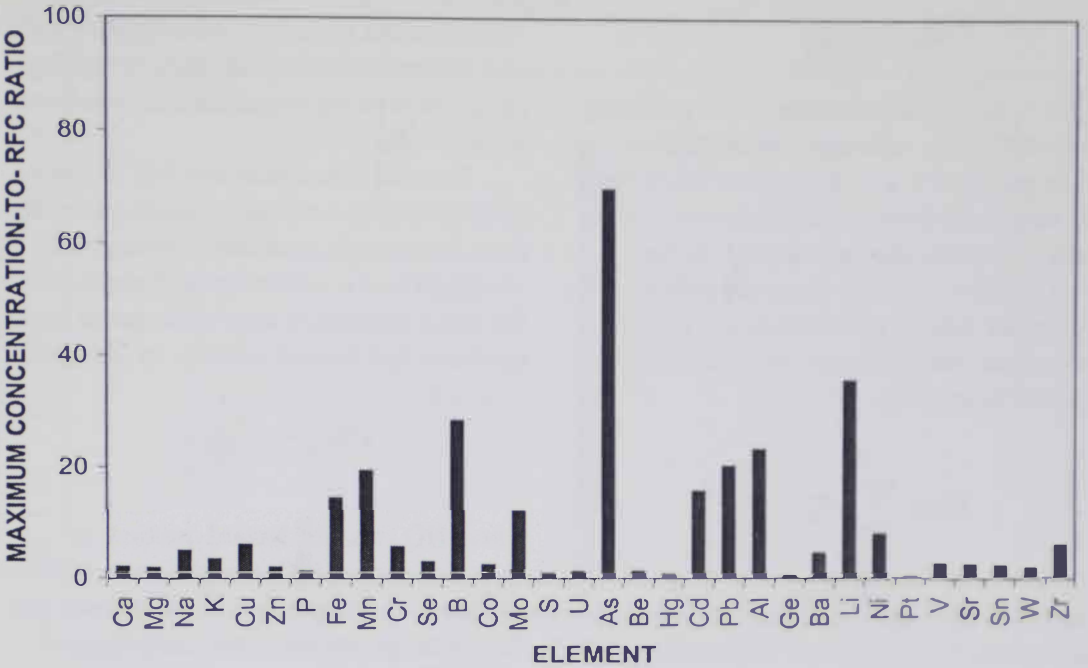

Figure 3. Ratio of the maximum concentration to the reference concentration for each element.

so 99 complete lab reports were available. Ten of the animals were cats, and 90 were dogs. Pet age in the study ranged between 0.2 and 16 years with an average of 6 years, and weight of the pet ranged 0.01-0.73 kg with an average of 0.27 kg. Of the dogs, 57 were males and 43 were females. In addition, 48 of the dogs resided within the envelope of the Butte Priority Soils Operable Unit (BPSOU) while 51 lived outside of the BPSOU boundary. Figure 1 shows the residence locations for the pets living in Butte.

General Statistics

We observed a wide range of concentrations in the dataset (Table 4), but mercury, germanium, and platinum were the only elements that did not show levels above reference concentrations. Arsenic concentrations in 76 percent of the samples exceeded the RfC value of 0.02 mg percent, and sodium, manganese, selenium, aluminum, and lithium exceeded the RfCs in 58, 42, 72, 49, and 53 percent of the samples, respectively (Table 4 and Fig. 2). In addition, lead concentrations in 26 percent of the samples were higher than the RfC.

As a worst-case scenario, the maximum arsenic concentration was 69.9 times higher than the reference level (Fig. 3). Several other elements also appeared to have dramatically-elevated maximum concentrations, such as iron, manganese, boron, molybdenum, cadmium, lead, aluminum, and lithium. Some of these elements may have nutritional sources, and others may be environmental. At this stage of the research, we did not try to distinguish between the two sources, but the mining-related contaminants, e.g., such as arsenic, aluminum, lead, etc., were elevated in many of the samples. We will examine these and additional data to identify nutritional versus environmental sources in future analyses.

Risk Analysis

In the field of risk assessment for air toxics (U.S. Environmental Protection Agency 2004), a non-cancer hazard quotient (HQ) is calculated for each air pollutant (i) as follows:

$$HQ_i = \frac{C_i}{RfC_i}$$

where C_i is the concentration of air pollutant i, and RfC_i is the reference concentration for air pollutant i. Concentrations below the RfC threshold should result in no adverse health effects (excluding cancer), so the target HQ is < 1.0 for a single air pollutant. Also in the field of risk assessment for air toxics, multiple pollutants are addressed via a hazard index (HI):

$$HI = \sum_{i=1}^{i=N} HQ_i$$

where N is the total number of air pollutants. This approach assumes that health effects are additive from exposure to multiple contaminants at once. Again, the target HI is < 1.0, so it is possible to have individual HQ values < 1.0 but still exceed the target HI.

Similar calculations of hazard values are utilized for human health risk assessments on Superfund sites (U.S. Environmental Protection Agency 1989). Instead of air concentration units, however, intake values and reference doses (with units of mg/[kg day]) are used for contaminants in soil and water media.

No established protocol was available for performing a risk assessment on results from hair sample analyses; consequently, we developed a simple method employing the same commonly used concepts of hazard quotients and hazard indices. In our case, however:

$$HQ_{ij} = \frac{C_{ij}}{RfC_i}$$

where HQ_{ij} was the hazard quotient of element i for pet j; C_{ij} was the concentration of element i in the hair sample of pet j; and RfC_i was the reference concentration for element i.

We also defined two hazard indices. A pet hazard index (HI_j) was calculated for each pet by summing the hazard quotients across the elements:

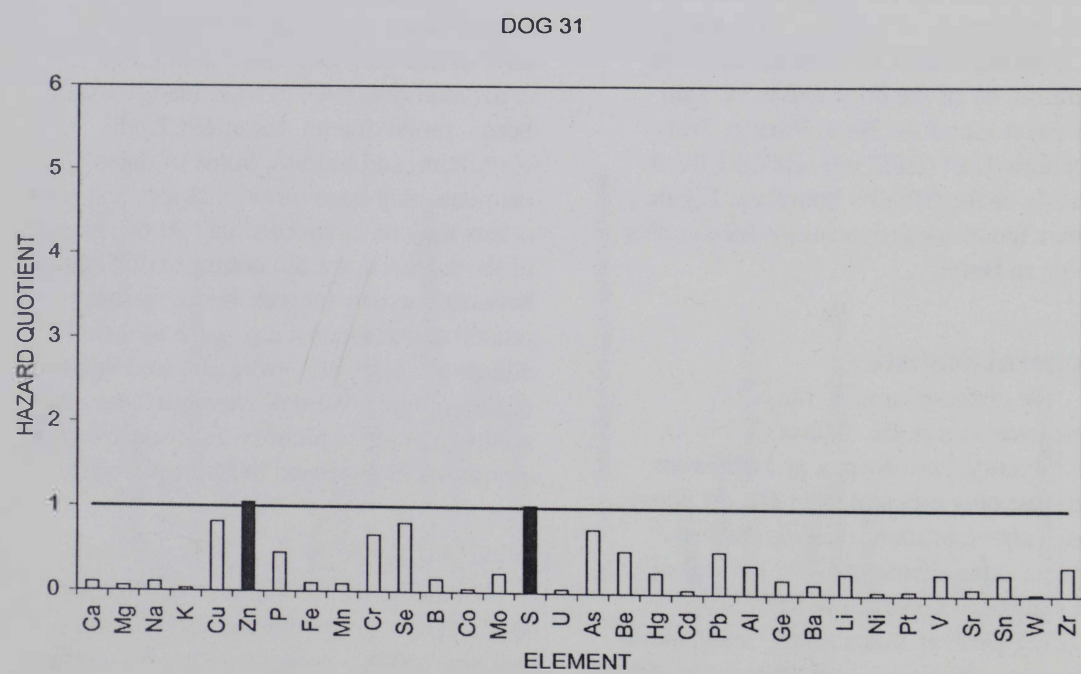

Figure 4. Hazard quotient (HQ) graph for Dog 31. White shading indicates HQ values < 1.0, and black shading corresponds to HQ values ≥ 1.0.

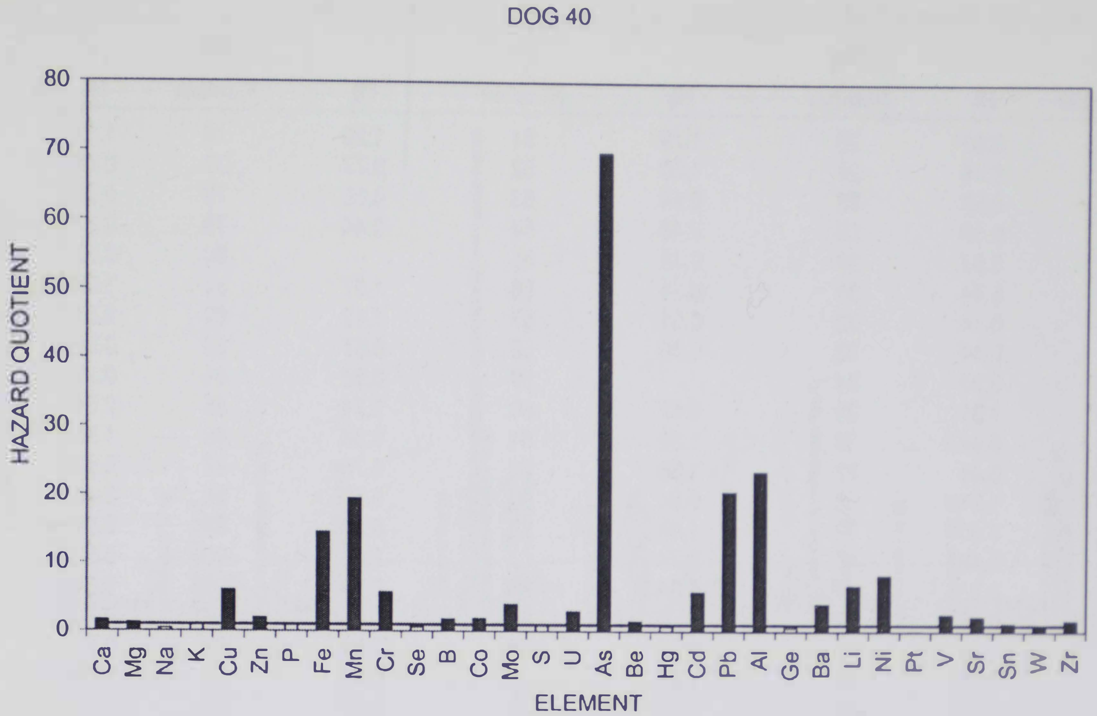

Figure 5. Hazard quotient (HQ) graph for Dog 40. White shading indicates HQ values < 1.0, and black shading corresponds to HQ values ≥ 1.0.

$$HI_j' = \sum_{i=1}^{i=N} HQ_{ij}$$

where N was 32 elements, and an element hazard index (HI_i') was calculated for each element by summing the hazard quotients across the pets:

$$HI_i' = \sum_{i=1}^{i=M} HQ_{ij}$$

where M was the total number of pets. The hazard indices were then normalized by the number of elements and pets as follows:

$$HI_j = \frac{\sum_{i=1}^{i=N} HQ_{ij}}{N}$$

and

$$HI_i = \frac{\sum_{j=1}^{i=M} HQ_{ij}}{M}$$

where the target value was 1.0 for both HI_j and HI_i. Pets with HI_j values ≥ 1.0 were defined as pets of concern (POCs), and elements with HI_i values ≥ 1.0 were defined as elements of concern (EOCs).

Table 6. Element Hazard Index (HI_i) Data for the 2004 Campaign.

Element	HI_i	Element	HI_i	Element	HI_i	Element	HI_i
Calcium	0.72	Manganese	1.72	Arsenic	3.25	Lithium	2.30
Magnesium	0.67	Chromium	0.88	Beryllium	0.54	Nickel	0.73
Sodium	1.37	Selenium	1.40	Mercury	0.30	Platinum	0.05
Potassium	0.72	Boron	1.57	Cadmium	0.71	Vanadium	0.48
Copper	1.00	Cobalt	0.30	Lead	1.58	Strontium	0.51
Zinc	0.89	Molybdenum	1.20	Aluminum	1.86	Tin	0.50
Phosphorus	0.87	Sulfur	0.91	Germanium	0.19	Tungsten	0.12
Iron	0.98	Uranium	0.20	Barium	0.59	Zirconium	1.13

Table 5. Pet Hazard Index (HI_j) Data for Pets in the 2004 Campaign.

Pet Number	HI_j	Pet Number	HI_j	Pet Number	HI_j	Pet Number	HI_j
1	0.62	26	1.79	51	1.20	76	1.02
2	0.48	27	1.50	52	0.77	77	0.66
3	0.58	28	0.44	53	0.65	78	0.76
4	0.89	29	0.48	54	0.44	79	0.57
5	0.55	30	0.38	55	-	80	0.72
6	0.44	31	0.31	56	1.37	81	1.28
7	0.44	32	0.51	57	1.12	82	0.51
8	0.44	33	0.46	58	0.57	83	0.56
9	0.90	34	-	59	0.56	84	0.79
10	1.31	35	0.57	60	0.74	85	0.54
11	0.44	36	0.82	61	0.88	86	1.35
12	0.44	37	1.38	62	0.78	87	0.52
13	1.21	38	0.64	63	0.73	88	0.46
14	0.80	39	1.44	64	0.83	89	0.94
15	0.48	40	6.74	65	2.27	90	0.52
16	0.83	41	2.34	66	0.88	91	0.54
17	0.85	42	0.50	67	0.41	92	0.53
18	0.66	43	1.55	68	0.64	93	0.61
19	2.09	44	1.08	69	0.60	94	0.58
20	0.68	45	1.88	70	0.65	95	1.53
21	0.96	46	0.94	71	0.99	96	2.06
22	0.49	47	0.74	72	0.74	97	1.69
23	0.73	48	0.71	73	3.07	98	0.38
24	0.63	49	0.37	74	0.59	99	0.52
25	0.95	50	0.64	75	0.79	100	3.03
						101	1.46

To illustrate hazard quotient method in this paper, we selected the two extreme cases from the 2004 field campaign. Dog 31 was a 13-year old male Lhasa apso that weighed 0.5 kg and lived at 3518 Oregon Avenue located outside of the southern boundary of the BPSOU (Fig. 1). Dog 40 was a 12-year old male border collie that weighed 0.25 kg and lived at 125 West Copper in the uptown area of Butte, inside the BPSOU (Fig. 1). Hazard quotients for Dog 31 were < 1.0 for all but two of the elements, and most were much less than 1.0 (Fig. 4). The HQs for zinc and sulfur were the highest but still barely above the level of concern at values of 1.05 and 1.03, respectively. When averaged over all of the elements, the HI_j for Pet 31 was 0.31, the smallest pet hazard index for the whole campaign.

Dog 40's hair sample revealed much different results from Dog 31's. Hazard quotients for Dog 40 were < 1.0 for sodium, potassium, phosphorous, selenium, sulfur, mercury, germanium, and platinum, but all of the rest of the elements exceeded the level of concern (Fig. 5). The top five HQs were 69.9, 23.5, 20.5, 19.5, and 14.6 for arsenic, aluminum, lead, manganese, and iron, respectively. When averaged over the elements, this dog had highest pet hazard index for the whole campaign, a HI_j value of 6.74.

Identification of Pets of Concern and Elements of Concern

While Dogs 31 and 40 represented the two extremes of the campaign, 25 pets were identified as pets of concern based on pet hazard indices (HI_j) ≥ 1.0 (Table 5 and Fig. 6). Ten of the POCs resided inside the BPSOU and 15 resided outside the BPSOU. This suggested that the envelope of the BPSOU might not describe a clear division of contaminated soils inside and non-contaminated soils outside the boundary.

The following eleven elements were

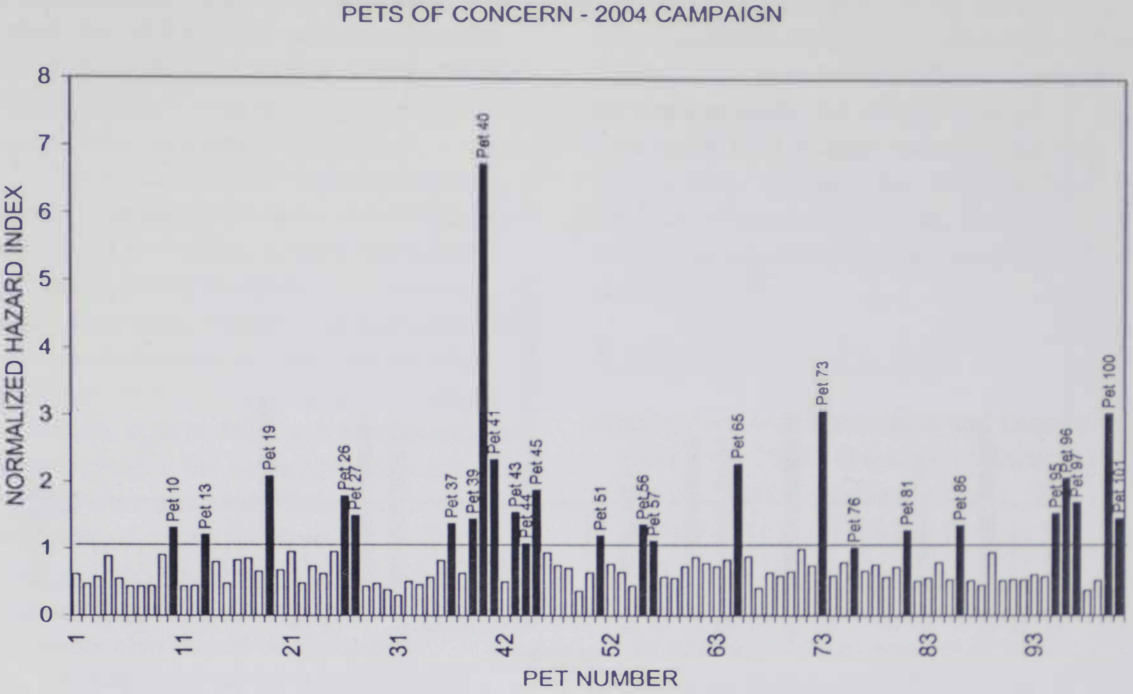

Figure 6. Hazard index (HI_j) graph for all pets in the 2004 campaign. While white shading indicates HI_j values < 1.0, black shading corresponds to HI values ≥ 1.0. Pets with HI_j values ≥ 1.0 are defined as pets of concern.

identified as elements of concern for our campaign based on element hazard indices (HI_i) ≥ 1.0: sodium, copper, manganese, selenium, boron, molybdenum, arsenic, lead, aluminum, lithium, and zirconium (Table 6 and Fig. 7). Of these elements, several may be mining-related, e.g., copper, iron, manganese, molybdenum, arsenic, lead, aluminum, etc., whereas others may be diet-related, e.g., such as sodium, selenium, boron, etc.

Based on our study, contaminant levels continue to be elevated in Butte's domestic pet population after nearly 20 years of cleanup activities. Of the mining-related contaminants, arsenic and lead stand out as major elements of concern because of possible links to health effects previously discussed. Manganese and aluminum, however, are also important because they have not been addressed in the local Superfund activities, and both are believed to play roles in serious health problems such as Parkinson's or Alzheimer's disease (Klaassen 2001).

Although these results are site specific, they illustrate the potential for developing this method into a simple screening-level tool for identifying elements in a residential setting that should be further investigated. In addition, citizens are normally hesitant about providing blood or urine samples from family members, especially from their children; however, residents in Butte were fascinated and enthusiastic about participating in a study that only required a small amount of hair from their pet.

Variability, Limitations, and Applications

As with all biological or ecological indices, our data contained variability in accumulation of the elements of concern. In this paper, we focused on introducing the method, presenting results from our field campaign, and devising a way to examine the data. Sources of variability (such as breed type, sex, age of the pet, etc.) were not discussed here but will be addressed in a future publication regarding a larger dataset.

In terms of limitations, exposure to environmental toxins is likely extreme for pets compared to exposure for humans

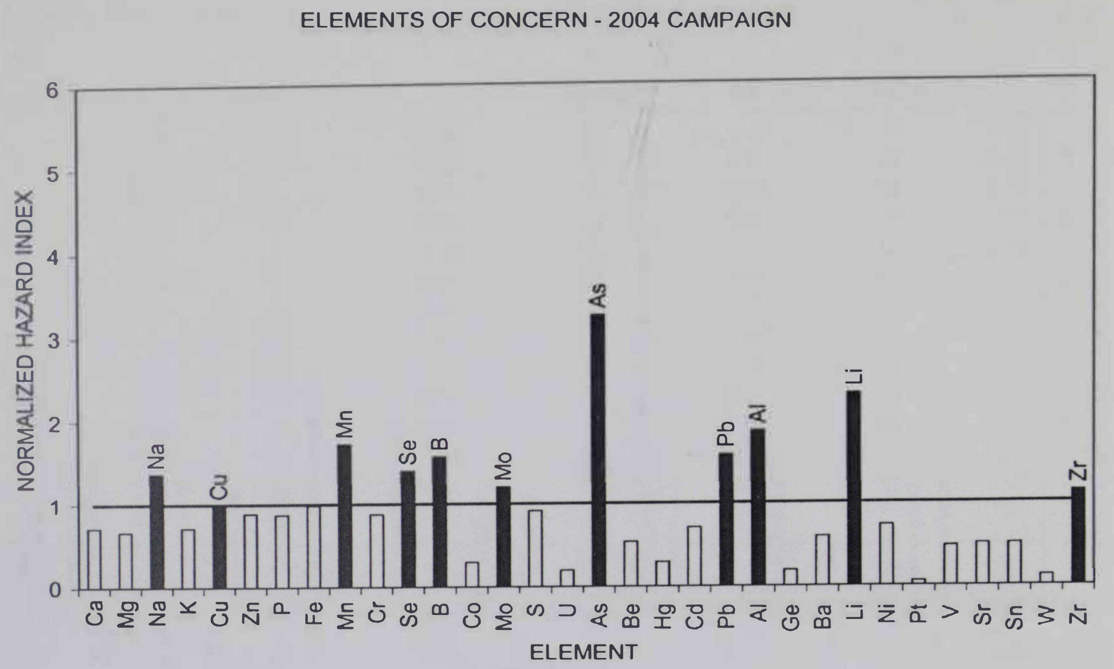

Figure 7. Hazard index (HI_i) graph for all elements in the 2004 campaign. While white shading indicates HI_i values < 1.0, black shading corresponds to $HI_i \geq 1.0$. Elements with HI_i values ≥ 1.0 are defined as elements of concern.

because of self-grooming habits and because pets have more direct contact with soil, house dust, and mud puddles; thus, we cannot assume that the method might be directly extrapolated to humans. Furthermore, our method of assigning hazard quotients and hazard indices is based on concentrations relative to reference concentrations. This is a simple, initial step toward evaluating incidental exposure to environmental contaminants, but relative toxicity of individual elements is not incorporated. Refinement of the method could include relative toxicity factors derived from data employed in the field of human health risk assessment.

Overall, our new biomonitoring technique was designed as a screening-level tool to identify contaminants that may be of concern in a community. For a residential area like Butte, the method may be useful for discovering homes or neighborhoods that might need of further investigation and/or remediation. Justification of the project involved use of dogs and cats as sentinels to protect the health of adults and children residing in a contaminated area, but protection of the health and well being of local pets is also important.

Conclusions

The purpose of this project was to develop a new way of investigating residential exposure to environmental pollutants by utilizing domestic pets as a sentinel species. We met our objectives by developing a biomonitoring technique involving pet hair samples with subsequent analysis for an array of elements, including metals. We tested the technique in the field during early 2004 using pets residing primarily in the Butte area. Results from the sampling campaign were entered into a database, and to examine the data we developed a hazard quotient technique similar to the methods used in the field of risk analysis.

We calculated a pet hazard index for each pet by summing HQs across the elements and by normalizing by the number of elements, and calculated an element hazard index for each element by summing HQs across the pets and by normalizing by the number of pets. We identified 25 pets as pets of concern based on $HI_j \geq 1.0$ of which only 10 resided inside the BPSOU. This suggested that the envelope of the BPSOU does not necessarily define a distinct zone of environmental contaminant exposure in Butte.

Likewise, the following 11 elements were identified as elements of concern based on $HI_i \geq 1.0$: sodium, copper, manganese, selenium, boron, molybdenum, arsenic, lead, aluminum, lithium, and zirconium. Of these eleven elements, only two (arsenic and lead) of the five contaminants (arsenic, cadmium, copper, lead, and zinc) commonly investigated in the Butte and Anaconda mine wastes were included. These results confirm the EPA's designation of arsenic and lead as contaminants of concern, but our study also indicated that other elements of concern probably also should be addressed.

In summary, we have introduced a new way of studying incidental exposure to environmental contaminants using pets as biosamplers. Because pets are companion animals, results from the method have implications for human health risk assessment. While this paper focused on describing the method and summarizing results from samples collected during winter of 2004, additional research is ongoing, and a subsequent paper will address data from approximately 250 more pets sampled during the past two years. Future effort will statistically compare exposure levels for pets residing inside and outside the boundary of the BPSOU and demonstrate the potential of using domestic pet hair as a tool for quantifying efficacy of remediation. A longer-term goal of the research is to identify links between concentration data of elements of concern on neighborhood scales from the biomonitoring technique with 1) concentrations of contaminants in the soils, water, and house dust, and 2) incidence of disease, including cancer, in pets and humans. Butte is the ideal community for conducting this type of research.

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