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Comparative Evaluation of Several Small Mammal Species as Monitors of Heavy Metals, Radionuclides, and Selected Organic Compounds in the Environment

S. S. Talmage
B. T. Walton

Environmental Sciences Division
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ENVIRONMENTAL SCIENCES DIVISION

COMPARATIVE EVALUATION OF SEVERAL SMALL MAMMAL SPECIES AS
MONITORS OF HEAVY METALS, RADIONUCLIDES, AND SELECTED
ORGANIC COMPOUNDS IN THE ENVIRONMENT¹

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¹Submitted as a dissertation by Sylvia S. Talmage to the Graduate School of The University of Tennessee in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

TALMAGE, S. S. and B. T. WALTON. 1990. Comparative Evaluation of Several Small Mammal Species as Monitors of Heavy Metals, Radionuclides, and Selected Organic Compounds in the Environment. ORNL/TM-11605. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

The merit of using small mammals as monitors of environmental contaminants was assessed using data from the literature and results of a monitoring study at selected sites on the Oak Ridge National Laboratory (ORNL) research reservation operated for the U.S. Department of Energy in Oak Ridge, Tennessee. Heavy metals, radionuclides, and organic chemicals were included in the evaluations. In the literature review, exposure to contaminants was determined from tissue residue concentrations, biochemical assays, and cytogenetic assays. In general, there was a relationship between concentrations of contaminants in the soil and concentrations in target tissues of several species. This relationship was most obvious for the nonessential heavy metals, cadmium and lead.

Each species' suitability as a monitor for a specific contaminant or type of contaminant was evaluated and subsequently ranked. A relationship between contaminant uptake and trophic level emerged. Carnivores had the highest concentrations of contaminants, followed by omnivores and herbivores which had the lowest concentrations. Target tissues for specific contaminants were also ranked.

In the field study, ten species of small mammals were trapped at selected field and reference sites to monitor for exposure to mercury, strontium-90, and benzo[a]pyrene. Residue analyses and a hemoglobin-adduct assay were performed on several species including the shorttail

shrew (Blarina brevicauda), the white-footed mouse (Peromyscus leucopus), and the cotton rat (Sigmodon hispidus).

Accumulation of mercury in kidney tissue and strontium-90 in bone was related to the degree of contamination of the environment as well as trophic level of the species. Both shorttail shrews and white-footed mice trapped at the mercury-contaminated site had significantly higher concentrations of mercury in kidney tissue than those trapped at the reference sites. However, the mean concentration in kidney of the insectivorous shrew was nearly 33 times that of the omnivorous mouse.

Strontium-90 was present in the bone of all species trapped at the radionuclide-contaminated sites, but was highest in the herbivore, Reithrodontomys humulis, which inhabited the grassy site. For the white-footed mouse, there was a gradient effect of strontium-90 accumulation among the highly contaminated, intermediate, and uncontaminated sites.

The hemoglobin-adduct assay was evaluated as an indicator of subchronic exposure to benzo[a]pyrene in the laboratory and chronic exposure in the field. Concentrations of benzo[a]pyrene-hemoglobin adducts in C3H mice fed benzo[a]pyrene in the laboratory for seven weeks were initially high, but declined to low, barely detectable, levels with time. Concentrations of benzo[a]pyrene-hemoglobin adducts in small mammals exposed to low concentrations of benzo[a]pyrene in the field were low and variable among individuals and between species.

I. INTRODUCTION

Many potentially harmful chemicals, both natural and anthropogenic, are released to the environment each year. Although chemical analyses of soil, air, and water can tell us the concentrations of specific compounds present, they are inadequate to assess the availability and potential toxicity of contaminants to humans and other biota. These analytical techniques do not take into account critical properties that determine bioavailability of contaminants such as media-chemical interactions and the physiology and biochemistry of the organism. Moreover, toxicity assays conducted in the laboratory with single chemicals fail to account for the complexity of chemical mixtures encountered by animals inhabiting contaminated sites and, therefore, cannot be readily extrapolated to field situations. Animals that live in these environments, however, integrate contaminant exposure both spatially and temporally. Therefore, measurement of chemical contaminants and their metabolites, in vivo reaction products, and effects within the organism hold promise as a means of identifying and estimating human health risks from natural populations of animals.

Resident wildlife populations at contaminated sites can be used as surrogates for human exposure and as monitors of adverse effects on the populations themselves. Small mammals have frequently been used to monitor the presence of metals and other chemical contaminants in the environment, either directly, based on residue analysis, or indirectly, based on biochemical parameters. Small mammals can be particularly effective biomonitors of soil contaminants if the species used are abundant, easily caught, do not migrate long distances, have a wide-

spread distribution, and have generalized food habits (Beardsley et al. 1978). Good biomonitors are in equilibrium with the contaminant of interest and ideally show a graded response to a range of pollution. Their relatively short life span (generally less than one year) allows long-term monitoring of contaminants in that each new generation reflects the present type and amount of contaminants in the environment, thus increases, decreases, or the addition of new contaminants can be detected. Thus, small mammals can serve as indicators of the presence and bioavailability of chemical contaminants, and selected species can be used to measure bioaccumulation. Furthermore, knowledge of food habits and habitat may indicate routes of exposure to animals.

Biological monitoring can be used as a cost-effective survey of contaminated sites, for routine surveillance of uncontaminated sites, to provide evidence for contaminant impacts on biota, and to establish priorities for site cleanup. In preliminary studies to determine the extent of contamination, biomonitoring can be used as an alternative to extensive sampling and chemical analyses of abiotic media, particularly where a large area is involved and the origin or extent of contamination is unknown. In uncontaminated areas, where there is a potential for contamination via accidental release or migration of contaminants, small mammals can be routinely monitored over long periods of time. Since the presence of a contaminant does not necessarily involve risk to humans, evidence of impact on biota as determined by biochemical parameter changes and tissue residues will help to establish the need for site cleanup.

The primary purpose of this study was to evaluate which small mammal species are the best monitors of specific environmental contaminants. The evaluation is based on the published literature and on an analysis of small mammals trapped at several sites on the Oak Ridge National Laboratory (ORNL) Reservation in Oak Ridge, Tennessee. Studies on the uptake of heavy metals, radionuclides, and organic chemicals are reviewed in Chapter II to evaluate several small mammal species for their capacity to serve as sentinels for the presence, accumulation, and effects of various contaminants. Where several species were present at a site, a comparative evaluation was made and species are ranked for their capacity to serve as monitors of specific contaminants. Food chain accumulation and food habits of the species are used to establish a relationship with suitability as a biomonitor. In addition, tissue-specific concentration factors were noted in order to establish target tissues. Life histories, habitat, and food habits are reviewed in order to make generalizations concerning the ability of similar taxa to serve as biomonitors. Finally, the usefulness of several small mammal species as monitors of three contaminants -- benzo[a]pyrene, mercury, and strontium-90 -- present on or near the ORNL facilities was investigated. Sentinel species for these contaminants are discussed in terms of the conclusions drawn from the literature review. This study is presented in Chapter III.

II. REVIEW OF THE USE OF SMALL MAMMALS AS MONITORS OF CHEMICAL CONTAMINANTS

Introduction

The earliest biomonitoring studies, reported in the early 1970s, involved measurement of metals and persistent organic compounds in tissues and whole bodies of animals collected after accidental releases of contaminants or field applications of chemical pesticides. Most of these residue analyses made no attempt to relate tissue concentrations to potential health effects. Studies performed within the last six years have tried to document health effects at sites where complex mixtures of chemicals are present.

Heavy metals are the most commonly evaluated environmental contaminants in biomonitoring studies. Studies on heavy metals are of several types: (1) reports of metal concentrations in animals from only one location, (2) correlations of tissue concentrations with environmental concentrations, (3) monitoring a site through time, (4) concentrations in animals collected along a gradient of pollution, and (5) comparisons of concentrations in animals from reference and contaminated sites or sites where contamination is suspected. These studies provide information on background concentrations of contaminants and correlations of tissue concentrations with environmental concentrations.

Study Species

In order to evaluate species-specific uptake of contaminants, information on the diet, habitat, and activity of the monitored species is necessary. The selection of an appropriate or optimal species for a monitoring study is restricted to those present at a site. When the ideal species is not present at a specific site, closely related species or a species occupying a similar niche may be used. Alternatively, a particular species, known to be a good biomonitor, can be placed in enclosures at the site and the uptake or effect of the contaminants can be compared to that at a reference area. Life history information on the genera and species most often discussed in the literature is provided here. The most commonly trapped small mammals belonged to three families: Soricidae (shrews), Cricetidae (mice, rats, lemmings, and voles), and Muridae (Old World mice and rats). All small mammal species discussed in the literature review are listed in Table 1.

Soricidae (shrews)

Blarina brevicauda, the shorttail shrew, occurs throughout most of the eastern half of the United States and the southern regions of adjacent Canadian provinces (Hall 1981, George et al. 1986). In East Tennessee this species occupies areas with high log and stump density, hard ground, few shrubs, and dense overstory (Kitchings and Levy 1981). Blarina brevicauda nests underground; runways usually parallel the surface and are located in the top ten cm of soil. They also burrow through rotten logs and often use the runways of other rodents. The

Table 1. Species and common names of small mammals trapped in monitoring studies.

Family/Species	Common Name
Soricidae	
<u>Blarina brevicauda</u> (Say)	northern shorttail shrew
<u>Sorex araneus</u> (Linnaeus)	common shrew
<u>Sorex cinereus</u> (Baird)	masked shrew
<u>Cryptotis parva</u> (Say)	least shrew
<u>Microsorex hoyi</u> (Zimmermann)	Hoy's pigmy shrew
Cricetidae	
<u>Reithrodontomys humulis</u> (Audubon and Bachman)	eastern harvest mouse
<u>Reithrodontomys megalotis</u> (Baird)	western harvest mouse
<u>Peromyscus leucopus</u> (Rafinesque)	white-footed mouse
<u>Peromyscus maniculatus</u> (Wagner)	deer mouse
<u>Peromyscus polionotus</u> (Wagner)	oldfield mouse
<u>Sigmodon hispidus</u> (Say and Ord)	hispid cotton rat
<u>Microtus pennsylvanicus</u> (Ord)	meadow vole
<u>Microtus pinetorum</u> (LeConte)	pine vole
<u>Microtus ochrogaster</u> (Wagner)	prairie vole
<u>Microtus agrestis</u> (Linnaeus)	field vole
<u>Microtus arvalis</u> (Pallas)	common vole
<u>Clethrionomys glareolus</u> (Schreber)	bank vole
<u>Clethrionomys gapperi</u> (Vigors)	Gapper's red-backed vole
<u>Oryzomys palustris</u> (Harlan)	marsh rice rat
Muridae	
<u>Apodemus sylvaticus</u> (Linnaeus)	field mouse
<u>Mus musculus</u> (Linnaeus)	house mouse
<u>Rattus norvegicus</u> (Berkenhout)	Norway rat
<u>Rattus rattus</u> (Linnaeus)	black rat
<u>Rattus exulans</u>	Polynesian rat
Zapodidae	
<u>Zapus hudsonius</u> (Zimmerman)	meadow jumping mouse
Sciuridae	
<u>Spermophilus richardsoni</u> (Sabine)	Richardson's ground squirrel
<u>Spermophilus variegatus</u> (Erxleben)	rock squirrel
<u>Spermophilus columbianus</u> (Ord)	Columbian ground squirrel
<u>Spermophilus tridecemlineatus</u> (Mitchell)	13-lined ground squirrel
<u>Sciurus carolinensis</u> (Gmelin)	gray squirrel.
Talpidae	
<u>Talpa europea</u> (Linnaeus)	mole

home range averages 2.5 ha. Earthworms are the major diet item, but millipedes, insects and occasionally mice and voles are also eaten. In a population from New Brunswick, Canada, the major diet items by volume were earthworms (32%), slugs and snails (22%), insect larvae (19.5%), and adult insects (16.5%) (Whitaker and French 1984). Most shrews eat the equivalent of their body weight in food per day (8 g). In the northern part of their range, the breeding season lasts from early February to September. Gestation requires 21 to 22 days and weaning occurs by 25 days after birth. Litter size is four to six. Life-span is approximately one year. Population density varies from year to year and populations occasionally crash (George et al. 1986). Depending on the quality of the habitat, density varies from 3 to 30/ha. In the wild, B. brevicauda is a solitary territorial species (Nowak and Paradiso 1983). Blarina brevicauda can be distinguished from other shrews by its short tail and slate gray color. This species was trapped in several studies cited in the literature review and at ORNL.

Sorex araneus, the common shrew, is found over most of Europe except for Ireland and parts of the Iberian peninsula (van den Brink 1968). This species is commonly found in various types of habitats: woods, grasslands, marshlands, dunes, and open areas. Sorex araneus is active day and night, tunneling through the loose accumulation of litter and also burrowing underground in search of food. In England, S. araneus occupies a well-defined home range of approximately 2,800 m² and is aggressive toward other members of its species (Nowak and Paradiso 1983). Like most shrews, it eats approximately its own body weight (8 g) of food per day. Adult Coleoptera and Lumbricidae are the

dominant prey in the average diet, with insect larvae, harvestmen (Phalangids), slugs and snails also important (Rudge 1968, Pernetta 1976). Seasonal fluctuations in diet occur, depending on prey availability. The nest is usually above ground and under cover. Sorex araneus occupies a niche similar to that of B. brevicauda in the United States (Quarles et al. 1974).

Sorex cinereus, the masked shrew, is widely distributed over Canada and the northern United States with southern extensions in the mountain areas (Burt, 1976). It is found in moist situations in forests, open country, and brushland. This small, long-tailed shrew eats more than its own weight in food each day. A nest of dry leaves is built under trees, logs, or brush. Another member of the family Soricidae, the least shrew, Cryptotis parva, is found in grass-covered areas throughout the southeastern United States (Whitaker 1974), largely overlapping the distribution of B. brevicauda. Although not commonly trapped, there is a surprising amount of information concerning predation on this species, especially by owls. Cryptotis parva inhabits grassy, weedy, and brushy fields. It eats earthworms, snails, beetles, and spiders.

Although the Soricidae are found throughout most of North America, shrews were not commonly trapped in monitoring studies. The reasons for low trapping success were not discussed in the literature, but could be due to low numbers and lack of suitable trap bait. Traps were usually baited with seeds or oatmeal, whereas, all shrews are insectivorous. Their solitary and territorial, aggressive habits may also account for low densities.

Cricetidae (mice, rats, and voles)

The subgenus Reithrodontomys (harvest mice) consists of nine species (Spencer and Cameron 1982), four of which are distributed across slightly overlapping sections of the United States (Burt 1976). These very small brown mice resemble house mice, but can be distinguished from other mice by their grooved upper incisors. The eastern harvest mouse, R. humulis, is the only species found in the southeast. It inhabits old fields, marshes, and wet meadows (Burt 1976). In monitoring studies the eastern harvest mouse was trapped only at ORNL. The western harvest mouse, R. megalotis, is found from the Great Lakes to the Pacific coast. Grasslands provide habitat where seeds and insects are the primary food items. Nests are usually on or above the surface of the ground (Burt, 1976).

Burt (1976) describes 14 species of Peromyscus distributed throughout North America. The deer mouse P. maniculatus, has a broader distribution than P. leucopus, the white-footed mouse, and overlaps with this species over much of its range. Several of these closely related species have similar food habits but slightly different habitat requirements (Brown 1964).

Peromyscus leucopus is distributed from southern Canada through the eastern half of the United States to Colorado and Mexico. The habitat of P. leucopus is primarily brushy fields and deciduous woodlands, although it is also present in open, swampy areas (Kitchings and Levy, 1981; Lackey et al., 1985). Individuals occupy nest sites above the ground or at ground level in rock piles, logs, stumps, under trees, and in ground burrows. Home range varies seasonally, but

averages 0.1 ha. Peromyscus leucopus is an opportunistic feeder -- seeds, insects, and green vegetation comprise most of the diet (Brown 1964). Litter size averages four, with several litters per year. Gestation time is 22 to 32 days, and young are weaned after 20 days. In the southern part of its range, the breeding season is year round. Life span in the wild is probably one year. Peromyscus leucopus was trapped in several monitoring studies and was abundant at ORNL.

Sigmodon hispidus, the cotton rat, is found throughout most of the southeastern and southcentral United States (Cameron and Spencer 1981). It commonly occurs in grass-dominated habitats where it nests in burrows or in dense clumps of grass or brush. Home range averages 0.35 ha for the species, however, males range further than females. Home ranges of the latter do not overlap. Cotton rats feed almost exclusively on grasses; seasonal consumption of insects is a notable exception. In warmer parts of their habitat, breeding occurs year round. Litter size varies from one to fifteen with a mean size of four to five. Gestation lasts 27 days; weaning takes place at 10 to 15 days; maturation occurs at one to three months. This species was trapped in several monitoring studies and at ORNL.

Microtus spp. (voles) are found throughout Canada and the United States in areas of good grass cover. Burt (1976) lists 16 species of Microtus, including the pine vole, which is often placed in the genus Pitymys. According to the literature, the most widely distributed and commonly caught species are the meadow vole (M. pennsylvanicus), the prairie vole (M. ochrogaster), and the pine vole (M. pinetorum). Voles may be active day and night, and serve as food for larger predators.

Several species of Microtus, especially M. pennsylvanicus, were trapped in monitoring studies; both M. ochrogaster and M. pinetorum were trapped at ORNL, but were not abundant.

Microtus pennsylvanicus is found in low, moist meadows and in high grasslands with abundant vegetation throughout Canada and in the northern and eastern United States (Reich 1981). It is often present in orchards with grassy undergrowth. The meadow vole is sympatric with several other species of small mammals. Nests are built above or below ground with runways along the surface of the ground. Voles are active any time of the day. Meadow voles are primarily herbivorous, eating grasses, sedges, seeds, grains, bark, and occasionally insects. The home range is one-tenth to one acre. Populations fluctuate with two to five year intervals. Life span in the wild is one to three years. Breeding is year-round.

Microtus pinetorum, the pine vole, is found in the eastern half of the United States in a wide variety of habitats including deciduous forests, meadows, and orchards where densities may be quite high (Smolen 1981). Nests occur beneath tree roots or stumps and surface burrows are constructed through the loose litter or as shallow surface burrows. Pine voles are primarily herbivorous, eating the sprouts, fruit, and roots of grasses, wild flowers and many economically important crops. The home range is limited to the burrow system. As is the case with M. pennsylvanicus, populations fluctuate widely from year to year.

Microtus agrestis, the short-tailed vole, is found in moist areas in Northern Europe except for Ireland; it is missing from most of

Southern Europe (van den Brink 1968). The primary habitats are pastures, fields, and open woods; rarely are they found under closed canopies. Although the burrow system of this species is underground, the runways occur above ground. The nest is usually above ground, also. Short-tailed voles, which are exclusively herbivorous, feed on above ground vegetation such as the shoots and leaves of fine-leaved grasses (Evans 1973). According to Quarles et al. (1974), M. agrestis occupies a niche similar to that of the North American species M. pennsylvanicus.

Redback voles, Clethrionomys spp., are found in forested areas in both North America and Europe. Clethrionomys glareolus, the bank vole, is found in the British Isles and most of Europe except for a crescent-shaped area around the Mediterranean Sea (van den Brink 1968). The bank vole is found mainly in deciduous woods, but also in hedges, bushes, edges of woods, and parkland. Most authors noted that this species was trapped almost exclusively in areas with cover. Of 39 C. glareolus trapped by Jefferies et al. (1973), 38 were caught on field edges, hedges, dike and grass borders; only one was caught on a tilled field. Superficial burrow systems are built in dry areas. This species is active during the night, but often spends time above ground during the day. Populations are known to fluctuate periodically. As an herbivore, this species feeds primarily on the seeds and leaves of perennial trees and shrubs (Watts 1968, Jefferies and French 1972). Leaves of woody plants, including dead leaf material during the winter, make up a large portion of the diet during most of the year; insects are eaten only when they are abundant.

A related species, the Gapper's redback vole, Clethrionomys gapperi, is found in Canada and parts of the northern United States, extending south into the Rocky and Appalachian Mountains (Merritt 1981). This red-backed vole is an omnivorous, opportunistic feeder, with diet reflecting availability of foods. The primary diet item is vegetation, with fungi, seeds, and arthropods eaten seasonally. Clethrionomys gapperi occupies forest habitat with abundant litter; it may also be found in clear-cut areas and old fields.

Family Muridae (Old World mice and rats)

Apodemus sylvaticus, the wood mouse, is distributed throughout Europe except for part of the Baltic countries (van den Brink 1968). It is abundant in open country and woodland, and in Britain is common to a variety of habitats including tilled fields, grassy areas, hedge rows, woods, and dunes (Kikkawa 1964, Jefferies et al. 1973). The wood mouse digs shallow burrows, but occasionally builds nests above ground. Like most Muridae, it does not hibernate. It is primarily a nocturnal forager, feeding on grass, seedlings, buds, fruit, nuts, snails, and insects. Seeds (endosperm) are the most important food item year round, with insects and earthworms eaten in appreciable quantities in spring and a small amount of green leaves in early spring (Watts 1968). It ranges widely in hedge and field habitats, and occasionally changes its home range area (Kikkawa 1964, Pollard and Relton 1970). Quarles et al. (1974) point out that the habitat and omnivorous food habits of the wood mouse put it in a similar niche to the North American species P. leucopus. Apodemus sylvaticus was the most commonly trapped small mammal in British monitoring studies.

The only species of old world Muridae found in the United States, Mus musculus, Rattus norvegicus, and Rattus rattus are closely associated with man and his dwellings and are rarely found in open fields (Burt 1976, van den Brink 1968). These species do not hibernate. All three of these species are prolific, breeding year round and are often found in colonial nests. They are omnivorous, eating anything edible. Members of the family Muridae were rarely collected in monitoring studies except in urban areas. Both Mus musculus and Rattus norvegicus were present at ORNL, but were not abundant.

Other Families

The meadow jumping mouse (Zapus hudsonius) was trapped in only one study (Smith and Rongstad 1982). This omnivorous species is found in various types of habitats, but usually feeds in low meadows (Burt 1976). Its distribution includes southern Canada and the north-central and northeastern United States. Squirrels and chipmunks (Sciuridae) and moles (Talpidae) were not commonly trapped in numbers large enough to draw conclusions about monitoring suitability.

Metal Contaminants

A search of the literature revealed 33 studies that addressed the use of small mammals as monitors of environmental metal contamination (APPENDIX A). All of these studies used tissue or whole-body residue analyses as a measure of contaminant exposure. Whole-body and tissue concentrations for both reference and contaminated sites are listed. In addition, concentrations in soil, vegetation, and invertebrates, when available, are also provided.

In order to compare tissue concentrations of metals among studies, all concentrations were converted to dry weight using the conversion factors of several researchers as well as those developed by the authors during the present study. Jefferies and French (1972) dried liver and body samples of three species at 90°C for 72 hours in order to obtain water content. The resulting multiplication factors, used to convert wet weight concentrations to dry weight concentrations, were 3.48, 3.67, and 3.40 for liver tissue of M. agrestis, C. glareolus, and A. sylvaticus, respectively. Multiplication factors for whole bodies were 3.00, 3.16, and 3.20 for the three species, respectively. According to Roberts and Johnson (1978) and Roberts et al. (1978), multiplication factors for freeze-dried whole bodies of M. agrestis, A. sylvaticus, and S. araneus were 3.09, 3.10, and 3.09, respectively. For oven-dried samples of muscle, bone, liver, and kidney of R. norvegicus, conversion factors were 2.38, 1.33, 2.70, and 2.08, respectively (Way and Schroder 1982). Wet weight to dry weight conversion factors for kidneys of species trapped on the ORNL reservation were 2.50 for R. humulis (n=3) and 3.53 for P. leucopus (n=3), and S. hispidus (n=2). These values were obtained by drying paired kidneys to a constant weight at 100°C. For species for which no information was available, the average conversion factors of 3.12 for whole body and 3.5 for soft tissues were used.

Arsenic

Very few studies addressed the relationship between arsenic (a nonessential element) concentrations in soil and vegetation and concentrations in small mammals. Whole-body concentrations in two

species from reference sites were ≤ 1 ug/g (Smith and Rongstad 1982). Concentrations in tissues of small mammals at reference and lead-arsenate-treated orchards were not given, but concentrations in soil were 3 and 31 ug/g, respectively (Elfving et al. 1978, Haschek et al. 1979). Of seven crops sampled at the orchards, arsenic was concentrated by a factor of seven in carrots and millet from the orchard soil compared to the corresponding crops at the reference site. It was not concentrated over the soil value in several other crops. Too little animal tissue was available to analyze for arsenic at this site.

Sharma and Shupe (1977) found no relationship between arsenic concentrations in soil and vegetation and concentrations in the livers of rock squirrels (Spermophilus variegatus) from different uncontaminated areas in the western United States. The mean arsenic concentration in five composite samples of Peromyscus maniculatus from a zinc-copper mine was not different from that of reference animals (<0.1 ug/g) (Smith and Rongstad 1982). However, two composite samples of Blarina brevicauda from this site did show a difference; whole-body concentrations at the reference and mine sites were <0.1 and 0.6 ug/g, respectively. Soil and vegetation concentrations were not given. No conclusions concerning uptake or sentinel species could be made from these few studies.

Cadmium

Cadmium is usually found in mineral ores in association with other metals such as lead, zinc, and copper. Extraction and separation of the heavy metals results in extensive metal contamination in the vicinity of mine sites and smelting operations. In uncontaminated

areas, cadmium is present in the earth's crust at an average concentration of 0.2 ug/g (Hammond and Beliles 1980). Cadmium concentrations of 0.8, 1, and 2 ug/g were measured in soil at reference sites in the monitoring studies cited in Table 2. In the following studies analysis for cadmium was by atomic absorption spectroscopy.

Whole-body concentrations of cadmium in most species of small mammals from reference sites averaged <1 ug/g (0.1 to 1.4 ug/g) (Table 2). Shrews averaged slightly higher than other animals, ranging from 1.2 to 4 ug/g. Except for two studies, concentrations in liver and kidney of most animals ranged from <0.1 to 1.3 and 0.0 to 5 ug/g, respectively. Reasons for the high concentrations in S. araneus at the reference site in one study (Hunter and Johnson 1982) and Talpa europa (mole) in another study (Ma 1987) are not possible to ascertain. Many of the data points in Table 2 are based on only one or two studies.

In general, there was a positive relationship between concentrations of cadmium in soil and concentrations in soft tissues of small mammals. Several species were trapped at the most highly contaminated site, a derelict lead-zinc mine in Wales, where cadmium concentrations in the surrounding surface soil averaged 92 ug/g (Johnson et al. 1978, Roberts and Johnson 1978). Whole-body and/or tissue levels were higher at the mine site than the reference site for four species trapped: A. sylvaticus, C. glareolus, M. agrestis, and S. araneus. There were apparent species differences in accumulation which the authors related to the diet of each species. Average whole-body concentration was lowest in M. agrestis (0.6 ug/g) and lower than in its diet of cover vegetation (4 ug/g). Average whole-body concentration was highest in

Table 2. Concentrations of cadmium in tissues of small mammals trapped at reference sites.^a

Species	Mean cadmium concentrations (ug/g dry weight)		
	Whole body	Liver	Kidney
<u>Apodemus sylvaticus</u>	0.3	0.5-0.9	1.7-2.2
<u>Blarina brevicauda</u>	0.9-1.2		
<u>Clethrionomys glareolus</u>	0.2		
<u>Microtus agrestis</u>	0.1-0.9	0.3-1.1	0.3-5.0
<u>Microtus pennsylvanicus</u>	<0.4	0.0-1.5	0.0-5.6
<u>Peromyscus leucopus</u>		0.4	0.6
<u>Peromyscus maniculatus</u>	<0.4-1.4		
<u>Rattus norvegicus</u>		0.2	0.6
<u>Sorex araneus</u>	1.2-4.0	2.9-25.4	4.1-25.7
<u>Sorex cinereus</u>	0.7		
<u>Spermophilus variegatus</u>		2-27	
<u>Talpa europea</u>		30	59

^aData compiled from Andrews et al. 1984, Anderson et al. 1982, Anthony and Kozlowski 1982, Beardsley et al. 1978, Hunter and Johnson 1978, Johnson et al. 1978, Ma 1987, Roberts and Johnson 1978, Schlesinger and Potter 1974, Sharma and Shupe 1977, and Smith and Rongstad 1982.

S. araneus (40 ug/g) and higher than in its diet of invertebrates (19 ug/g).

At a refinery site which included a copper-cadmium alloy plant, cadmium concentrations in tissues of A. sylvaticus, M. agrestis, and S. araneus decreased with distance away from the plant (Hunter and Johnson 1982). The same pattern was true for surface soil; unwashed litter, grass, and cover vegetation; and invertebrates. Concentrations in unwashed litter and vegetation were always less than that of the surface soil. Concentration factors in invertebrates, compared to their estimated diets, ranged from 1.1 to 5.4. Carnivorous invertebrates contained higher concentrations than herbivorous invertebrates.

The three species of small mammals collected at the copper-cadmium alloy plant have distinct dietary patterns (Hunter and Johnson 1982). Examination of stomach contents showed that Sorex araneus ate primarily invertebrates at the top of the invertebrate food web. Microtus agrestis ate primarily green stems and leaves of grasses and A. sylvaticus ate seeds and fruit. The latter two foodstuffs contained lower concentrations of cadmium than the grasses. Total-body concentration of cadmium:diet for S. araneus was 2.2 (27.5:12.5); total body concentrations for the other two species (<1 ug/g for A. sylvaticus and 1.7 ug/g for M. agrestis) were less than diet concentrations.

The same pattern was true at the lead-zinc mine complex although tissue concentrations were higher in A. sylvaticus than in M. agrestis (Roberts and Johnson 1978). Suspension of mine wastes by wind at the derelict sites may have been less important than continuous emissions at the copper-cadmium alloy plant for deposition on grasses, thus resulting in the higher tissue concentrations for A. sylvaticus than for M. agrestis at the lead-zinc mine .

At a site where municipal effluent was used to irrigate the land, little accumulation of cadmium took place (Anthony and Kozlowski 1982). Concentrations were significantly higher than reference values for only kidney tissue of P. leucopus (0.7 ug/g compared to 0.2 ug/g); concentrations in liver and kidney tissue of M. pennsylvanicus from the reference site were higher than those from the contaminated site. The effluent sludge mixture contained 5 ug/g cadmium.

Over a wide range of exposures, cadmium has a strong affinity for soft body tissues, particularly the kidney and liver (Hammond and

Beliles 1980). At low doses the concentration in the kidney is approximately ten times that in the liver. At high exposures the liver concentration may exceed that in the kidneys. In addition to whole-body concentrations, only the kidney and liver concentrations clearly differentiated between the reference and contaminated sites in these studies. For most species and sites, kidney was the primary target organ; the tissue concentration pattern was kidney > liver > muscle > heart > bone > brain. However, for S. araneus the primary target tissue was the liver. Concentrations of cadmium in liver of S. araneus in these studies averaged 236 ug/g at the contaminated site compared to a reference value of 2.9 (Andrews et al. 1984) and 280 ug/g compared to a reference value of 25 (Hunter and Johnson 1982).

The relationship between concentrations of cadmium in soil and concentrations in soft tissues of small mammals can be seen most clearly in A. sylvaticus, the species for which most data were available (Table 3). Highest cadmium concentrations were measured in kidney tissue and ranged from approximately 2 ug/g at reference sites where soil concentrations ranged from 1 to 2 ug/g to nearly 40 ug/g at the most contaminated site where the soil concentration was 92 ug/g. The tissue:soil relationship was of a lesser magnitude for liver tissue and was not clear for whole-body residues. Fewer data were available for other species, but the same relationship held true.

There may be an age-dependent accumulation of cadmium. However, only one study investigated this relationship. Schlesinger and Potter (1974) showed a relationship between body weight and cadmium accumulation for one species, B. brevicauda, but not for P. maniculatus.

Table 3. Relationship between concentrations of cadmium in soil and tissues of Apodemus sylvaticus.^a

Cadmium concentrations (ug/g dry weight)			
Soil	Whole body	Liver	Kidney
92	2.6	9.9	39.7
46	0.8	4.4	18.0
11	1.0	2.5	10.3
8.5		1.5	7.4
3.1		1.4	5.5
2 ^b	0.3	0.9	1.7
1 ^b	0.3	0.5	1.7
1 ^b	0.3	0.7	2.2
1 ^b		0.5	1.5

^aData compiled from Hunter and Johnson 1982, Johnson et al. 1978, and Roberts and Johnson 1978.

^bReference sites

Chromium

Chromium is present in the earth's crust at a concentration of 200 ug/g. Trivalent chromium is an essential element in animals. Absorption by the body is low, about 1% (Hammond and Beliles 1980).

Uptake of chromium by small mammals was investigated at two sites where sewage effluent was applied to land, but no relationship between soil contamination and tissue uptake could be made (Beardsley et al. 1978, Anthony and Kozlowski 1982). Beardsley et al. (1978) compared concentrations in several tissues of M. agrestis from laboratory stock and reference and sewage-contaminated areas. For some tissues, concentrations were highest in the field-collected reference animals (liver, 1.1 ug/g; kidney, 0.5 ug/g; concentrations were similar in other tissues). At the other waste-water site, concentrations in the liver and kidney of P. leucopus from the

contaminated site were the same as concentrations in animals from the reference site, whereas concentrations in M. pennsylvanicus collected at the reference site were higher than in those from the treated site. The treated site had received 5.0 to 7.5 cm/week of effluent, containing 121 ug/g chromium, for 14 years (Anthony and Kozlowski 1982). The amounts of chromium in soil, invertebrates, and vegetation were not given. Although these two studies were negative for chromium uptake, they provide too few data to draw any definitive conclusions concerning uptake of chromium.

Cobalt

Cobalt is present in the earth's crust at a concentration of 23 ug/g. It is an essential element in mammalian systems as a constituent of Vitamin B₁₂ (Hammond and Beliles 1980). One study (Anthony and Kozlowski 1982) also addressed the uptake of cobalt by small mammals at the site where sewage effluent was applied to field and forest areas. The mixture contained 57 ppb cobalt and had been applied for 14 years. Microtus pennsylvanicus and P. leucopus captured at reference sites contained similar concentrations of cobalt: <1 ug/g in liver tissue and 1-2 ug/g in kidney tissue. There were no significant differences between concentrations of cobalt in liver and kidney tissues of mice and voles from the reference and sprayed areas.

Copper

Copper, present in the earth's crust at a concentration of 45 ug/g, is an essential element for hemoglobin synthesis and oxidative

enzymes. The liver is involved in copper homeostasis. With increasing dietary levels of copper, the liver, kidney, bone marrow, and hair become important storage organs (Hammond and Beliles 1980).

At reference sites copper concentrations in several tissues of eight species appeared to be remarkably similar (Table 4). With the exception of the insectivores, S. araneus and T. europea, which were slightly higher, concentrations ranged from 9 to 19 ug/g of tissue. Greatest variation was in kidney tissue, ranging from 11 to 19 ug/g. Whole-body concentrations averaged 12 ug/g. A study by Anthony and Kozlowski (1982) is not included in Table 4 because reference values for the kidney and liver were greatly elevated compared to the other studies. Standard deviations of the mean were high in this study. Analyses in all studies were by atomic absorption spectroscopy.

Environmental contamination by copper results from mining and refinery operations and from application of sewage to land. Nine species captured at such sites showed varying accumulation of copper. Copper concentrations were significantly elevated in liver tissue of A. sylvaticus (23.7 ug/g), kidney and hair of M. agrestis (22.6 and 24.2 ug/g, respectively), and liver and hair of S. araneus (56.1 and 77.8 ug/g, respectively) collected at a copper refinery where surface soil values averaged 2,480 ug/g (Hunter and Johnson 1982). The degree of contamination was highest in the shrew and lowest in the mouse. Highest concentrations were found in the liver of two of the species.

Whole-body concentrations were significantly higher in P. maniculatus and M. pennsylvanicus trapped at a zinc-copper mine than

Table 4. Concentrations of copper in tissues of small mammals trapped at reference sites.^a

Species	Copper concentrations (ug/g dry weight)		
	Whole body	Liver	Kidney
<u>Apodemus sylvaticus</u>		12.9	13.5
<u>Blarina brevicauda</u>	9-10.6		
<u>Microtus agrestis</u>		13.4-16	10.8-19
<u>Microtus pennsylvanicus</u>	11.9	17.3	15.6
<u>Peromyscus maniculatus</u>	10-13.4		
<u>Sorex araneus</u>		31.1	22.8
<u>Sorex cinereus</u>	12.8		
<u>Talpa europea</u>		23	25

^aData compiled from Anderson et al. 1982, Anthony and Kozlowski 1982, Beardsley et al. 1978, Hunter and Johnson 1978, Ma 1987, Schlesinger and Potter 1974, and Smith and Rongstad 1982.

at a reference site, with concentrations higher in P. maniculatus than in M. pennsylvanicus (Smith and Rongstad 1982). Relative to reference values, uptake was greater in P. maniculatus than in M. pennsylvanicus or B. brevicauda. In another study, there were no differences in liver and kidney concentrations between reference and effluent-sludge disposal sites for M. pennsylvanicus and P. leucopus (Anthony and Kozlowski 1982). Concentrations were generally higher in tissues of animals from the reference sites.

Since copper is well regulated by the body, it is not a candidate to be monitored by small mammals except at sites that are suspected to be extremely highly contaminated as shown in the study by Hunter and Johnson (1982). At this site both S. araneus and M. agrestis showed elevated concentrations when uptake by several different tissues was considered. Relative to reference concentrations, concentrations in both grass and invertebrates were high, averaging 153 and 375 ug/g in

different types of vegetation (the food of M. agrestis) and 310, 343, and 568 ug/g in different types of invertebrates (the food of S. araneus).

Lead

Although the average concentration of lead in the earth's crust is 15 ug/g, concentrations at reference sites in monitoring sites were higher, 78 and 96 ug/g. Lead is a nonessential element in mammalian systems. Most lead enters the body through ingestion and ninety percent of the total body burden is found in the bone. Higher than average body concentrations are also found in the kidney and liver. Since the excretion rate is low, the lead burden increases with age. In addition to body and tissue burdens, evidence of exposure to high concentrations of lead can be seen by the production of intranuclear inclusion bodies in the proximal tubule cells of the kidney, inhibition of delta-amino levulinic acid dehydratase (ALAD), and renal edema (Goyer et al. 1970, Hammond and Beliles 1980).

In all studies, lead in tissue and whole-body samples of small mammals was analyzed by atomic absorption spectrometry following acid digestion (wet oxidation). Tissues were processed wet, freeze-dried, or dried to a constant weight at 100°C. Concentrations were reported on a dry weight basis.

Concentrations of lead in tissues of several species of small mammals collected at reference sites varied considerably (Table 5). Concentrations in bone, kidney, liver, and whole body ranged from 5 to 25, <1 to 13, 1 to 12, and 2 to 18 ug/g, respectively. The wide range of concentrations for reference areas makes it difficult to estimate a

Table 5. Tissue and whole body concentrations of lead in small mammals from reference sites.^a

Species	Lead (ug/g dry weight)			
	Bone	Kidney	Liver	Whole Body
<u>Apodemus sylvaticus</u>	12-25	<0.4-13	3.5-9	3-4
<u>Blarina brevicauda</u>				3-17
<u>Clethrionomys glareolus</u>		5	8-12	5-8
<u>Microtus agrestis</u>	6	6-9	4	5-9
<u>Microtus ochrogaster</u>				3
<u>Microtus pennsylvanicus</u>		4-13	1-2	2-18
<u>Mus musculus</u>	9.3	3.4	1.9	4.6
<u>Peromyscus leucopus</u>		13	3	2.6-6.4
<u>Peromyscus maniculatus</u>	5-6	2-3	1.1	3-9
<u>Rattus norvegicus</u>	10-14	4-6	1-3	
<u>Reithrodontomys megalotis</u>				3
<u>Sorex araneus</u>				3
<u>Sorex cinereus</u>				3
<u>Talpa europea</u>	22	9		

^aData compiled from Anthony and Kozlowski 1982, Beardsley et al. 1978, Chmiel and Harrison 1981, Clark 1979, Elfving et al. 1978, Gardner et al. 1978, Getz et al. 1977, Goldsmith and Scanlon 1977, Haschek et al. 1979, Jefferies and French 1972, Johnson et al. 1982, Ma 1987, Mierau and Favara 1975, Mouw et al. 1975, Quarles et al. 1977, Roberts and Johnson 1978, Roberts et al. 1978, Scanlon 1977, Smith and Rongstad 1982, Welch and Dick 1975, and Williamson and Evans 1972.

baseline concentration for any species or any tissue. The differences may be due to numerous biological and environmental factors including the natural variability of the lead content of the soil and vegetation, diet of the species, season of the year, and age and sex of the animals.

A number of studies have shown the effect of automobile exhaust on lead concentrations in small animals living various distances from highways of different traffic densities. In general, the concentrations of lead in soil, vegetation, invertebrates, and selected tissues of mammals correlate with the traffic density of the highway (Jefferies

and French 1972, Welch and Dick 1975, Goldsmith and Scanlon 1977, Getz et al. 1977, Scanlon 1979). A compilation of the data on traffic density and lead concentrations in several tissues of P. maniculatus, the species for which most data were available, shows this relationship (Table 6). Although the relationship holds true for all of the tissues, it is most dramatic for bone. Concentrations ranged from approximately 5 ug/g in bone at untravelled reference sites to 106 ug/g at a major highway. Concentrations of lead in soil were not provided for these sites. In addition to the relationship with traffic density, there was also a gradient effect of tissue lead concentrations with distance from the highway (Williamson and Evans 1972, Quarles et al. 1974). All species had highest lead concentrations at sites adjacent to heavy-use highways.

In other studies the elevated concentrations of lead in tissues reflected elevated concentrations in soil, vegetation, and invertebrates. The greatest environmental contamination with lead occurred at abandoned lead-zinc mines in Great Britain (Johnson et al. 1978, Roberts and Johnson 1978, Roberts et al. 1978). At one such site, lead concentrations in soil averaged 14,010 ug/g; the highest whole-body concentration of lead was found in M. agrestis (140.4 ug/g) (Roberts and Johnson 1978). The highest reported mean tissue concentration of lead was in bone of A. sylvaticus at an abandoned smelter waste heap, 672 ug/g (Johnson et al. 1978). There were general increases in all tissues except muscle at these contaminated sites compared to tissues of animals from respective reference sites. There was also accumulation in vegetation and invertebrates compared to respective reference

Table 6. Relationship between traffic density and tissue concentrations of lead in Peromyscus maniculatus.^a

Vehicles/day	Tissue concentrations (ug/g dry weight)			
	Whole body	Liver	Kidney	Bone
38,000		4.6	23	106
19,800		3.3	8.5	52
19,600	5.5	3.5	7.9	24.6
18,500		1.3	3.9	27
9,500		0.8	1.7	8.6
4,200		0.7	2.6	5.1
1,360	3.7	1.7	9	8
340	2.4	1.8	3	6.4
Reference		1.1	3.3	4.8
Reference	2.8	1.1	1.8	5.7

^aData compiled from Getz et al. 1977, Mierau and Favara 1975, and Welch and Dick 1975.

sites, with greater uptake in vegetation (249/29 ug/g) than in invertebrates (82/22 ug/g).

The most data on tissue concentrations in relation to soil concentrations were available for A. sylvaticus (Table 7). Concentrations at reference sites for this species were: whole body, 3 to 4 ug/g; liver, 3.5 to 9 ug/g; kidney, <1 to 13 ug/g; and bone, 11.5 to 25 ug/g. Concentrations in several tissues of this species collected at contaminated sites were elevated, but did not reflect differences in environmental lead levels. Bone, followed by kidney, were the target tissues for lead.

At sewage-sludge treated areas, concentrations in soil were not reported, but are presumably lower than in the above studies. Significant differences between reference and contaminated sites were seen for some tissues, but, in general, uptake was low (Beardsley et al. 1978, Anthony and Kozlowski 1982, Anderson et al. 1982). There were

Table 7. Relationship between lead concentrations in soil and tissues of Apodemus sylvaticus.^a

Soil	Lead concentrations (ug/g dry weight)			
	Whole body	Liver	Kidney	Bone
14,010	43.1	13.0	39.2	189
8,430	26.7	11.7	46.6	352
4,030		12.1	65.2	672
150		12		
120		9.5	6.5	
96 ^b	2.9	7.9	12.7	11.5
78 ^b	3.8	5.4	9.4	21.1
Reference		9.0	5.0	
Reference	3.5	6.1		
Reference		3.5	<0.4	25

^aData compiled from Chmiel and Harrison 1981, Johnson et al. 1978, Roberts and Johnson 1978, Roberts et al. 1978, and Williamson and Evans 1972.

^bReference sites

significant differences in selected tissues of rats (R. norvegicus) collected in urban and rural areas (Mouw et al. 1975, Way and Schroder 1982).

At an abandoned orchard that had been treated with lead-arsenate for insect control, lead was still present in soil several years later (218 ug/g), and accumulated in several tissues of voles and mice (Elfving et al. 1978, Haschek et al. 1979). The authors related uptake to the degree of subsurface feeding and movement of the three species, with concentrations in pine voles > meadow voles > white-footed mice, but there were too few data to draw firm conclusions.

Although bone, kidney, and liver, in that order, are the primary target tissues for lead accumulation, most studies measured whole body concentrations. Because inhalation may be an important route of uptake

adjacent to heavily traveled roads, one study (Getz et al. 1977) also measured concentrations in the lung tissue. Concentrations were higher in lung tissue for B. brevicauda and R. megalotis from the contaminated area, but statistical analyses were not performed on this tissue. Much higher concentrations were found in bone and kidney tissue.

In addition to tissue residues, exposure to lead may be monitored by observing changes in cellular structures and enzyme levels. The most sensitive index of lead poisoning is the presence of intranuclear inclusion bodies in the epithelial cells of the proximal tubules of the kidney. These bodies are composed of protein and lead and may be formed following a single dose of lead (Hammond and Beliles 1980). In experimental studies, renal lead levels as low as 13 ug/g have been associated with cellular alterations (Goyer et al. 1970).

Intranuclear inclusion bodies were found in the proximal tubule cells of 20/23 rats caught in urban areas and in none of the rats caught in rural areas by Mouw et al. (1975). Lead concentrations in the kidney were 30.2 ug/g in urban rats and 1.5 ug/g in rural rats. Urban rats also exhibited excess kidney weight and had reduced ALAD in the kidney and red blood cells.

Intranuclear inclusion bodies were also found in M. agrestis trapped on spoil heaps of two abandoned lead-zinc mines (Roberts et al. 1978). No nuclear alterations were present in the tissues of animals from matched control sites. Whole body lead concentrations averaged 128.4 and 135.9 ug/g at the two mine sites; concentrations in renal tissue were not given, and no consistent relationship between whole body and kidney concentrations could be made from the reported values.

Renal edema was observed in M. agrestis and A. sylvaticus from sites where kidney and bone concentrations were very high (46.6 ug/g and 352 ug/g for kidney and bone, respectively) in A. sylvaticus; concentrations for M. agrestis were not given.

Meadow voles and pine voles (M. pennsylvanicus and M. pinetorum) from lead-arsenate treated orchards had lesions in the epithelial cells of the proximal convoluted cells of the kidney (Elfving et al. 1978, Haschek et al. 1979). The cells were enlarged, degenerating, and contained enlarged nuclei with intranuclear inclusion bodies. There was also arrested development in the long bones. Lead concentrations in meadow voles trapped in the orchard ranged from 14 to 41 ug/g in kidney and from 73 to 303 ug/g in bone. Two pine voles contained 27 and 30 ug/g in kidney and 306 and 401 ug/g in bone.

Lead poisoning was not observed in populations of deer mice (P. maniculatus) living adjacent to a major highway in Colorado (Mierau and Favara 1975). Kidney and bone concentrations in this population averaged 8.5 and 52.1 ug/g, respectively. Lead poisoning was not investigated in other roadside populations. Data from all of the above studies agree with the laboratory study of Goyer et al. (1970) which showed that the lowest concentration of lead in the kidney that produces renal changes is 13 ug/g.

Manganese

Beardsley et al. (1978) investigated the concentrations of manganese (an essential element) in several tissues of M. agrestis trapped at a sewage-sludge treated area, an unpolluted grassland, and a laboratory stock of this species. Concentrations in vegetation at the

reference and polluted sites were 38 and 183 ug/g, respectively.

Differences in median concentrations of manganese in several tissues of the vole were small and erratic and no conclusions could be drawn.

Mercury

There is a scarcity of data on mercury exposure of small mammals at contaminated sites. Mercury was analyzed by atomic absorption spectroscopy and values were reported as total mercury; only one study made a distinction between inorganic and organic mercury. In three studies, residues of mercury in whole bodies and tissues of A. sylvaticus, B. brevicauda, and C. glareolus from reference areas were ≤ 0.1 ug/g. Concentrations in samples of surface soil and grass (ug/g dry weight) and earthworms (ug/g wet weight) at a reference site were 0.11, 0.10, and 0.04, respectively (Bull et al. 1977). Hammond and Beliles (1980) cite 0.5 ug/g as an average value for the earth's crust. In the above study (Bull et al. 1977), concentrations within 0.5 km of a chlor-alkali plant were 3.8, 4.0, and 1.3 for these three compartments, respectively, showing no uptake over soil concentrations by vegetation or invertebrates. Conversely, small mammals living within 0.5 km of the plant accumulated mercury in their tissues, with slightly higher concentrations in the mouse, A. sylvaticus, than in the vole, C. glareolus. The highest concentrations were in the muscle of A. sylvaticus (3.43 ug/g) and in the kidney of C. glareolus (1.24 ug/g). For the two species caught in this study, tissue concentrations were statistically higher in hair, kidney, and liver of A. sylvaticus and in brain and hair of C. glareolus from the contaminated site. Although methyl mercury is not emitted by the plant, some of the total mercury

(<10%) in the tissues of the animals was in the form of methyl mercury. Of several species of small mammals trapped at an active zinc-copper mine, only a composite sample of two B. brevicauda had concentrations higher than two reference animals (Smith and Rongstad 1982). Concentrations in soil, vegetation, and invertebrates were not given.

Concentrations of total mercury in small mammals trapped in fields sown with wheat treated with an organomercury fungicide were higher following sowing, but declined with time (Jefferies and French 1973, Jefferies and French 1976, Westlake et al. 1980). Residues were detected up to several months after planting. Apodemus sylvaticus, which inhabited both the open field and the surrounding pastures and woodland, fed immediately on the seed as indicated by increased residues during the two weeks after sowing. Clethrionomys glareolus was caught almost exclusively on the grass borders of the fields and M. agrestis was caught only in short grass. These habitat patterns are reflected in the low tissue concentrations of these two species. Since A. sylvaticus was the only species that lived on or ventured into the treated fields, a comparative evaluation of accumulation could not be made.

Nickel

The accumulation of this nonessential element was investigated at an active zinc-copper mine (Smith and Rongstad 1982) and at field and forest sites sprayed with sewage-sludge effluent containing 75 ug/g nickel (Anthony and Kozlowski 1982). Soil concentrations at neither site were given. The average concentration in soil at uncontaminated sites is 80 ug/g. Whole-body concentrations of animals collected at

uncontaminated areas ranged from 2.2 ug/g for the jumping mouse (Z. hudsonius) to 6.2 ug/g for the deer mouse (P. maniculatus). Concentrations in the liver and kidney of P. leucopus and M. pennsylvanicus were similar (2.0-15 ug/g). Nickel did not accumulate in mice, shrews, or voles at either site; in all cases, reference concentrations were higher than concentrations at the contaminated sites.

Zinc

Zinc, a ubiquitous element in the environment, is an essential trace element. It is present in the earth's crust at a concentration of 65 ug/g. In mammalian systems, zinc is involved in enzyme functions, protein synthesis, and carbohydrate metabolism (Hammond and Beliles 1980).

The average concentrations in whole-body samples of nine species of small mammals captured at reference sites ranged from 96 to 143 ug/g and averaged 108 ug/g (Table 8). Averages in liver ranged from 120 to 199, and averages in kidney ranged from 104 to 192 ug/g. There were too few data for some species and too much variation among studies to make specific statements about concentrations in tissues of a particular species.

Dry-weight body burdens for four species were elevated one- to two-fold at an abandoned lead-zinc mine relative to reference concentrations (Johnson et al. 1978, Roberts and Johnson 1978). But body burdens for M. agrestis (191.6 ug/g), A. sylvaticus (107.3 ug/g), S. araneus (141.1 ug/g), and C. glareolus (123.4 ug/g) were lower than soil (21,000 ug/g), vegetation (340 ug/g) and invertebrate (220-270 ug/g) concentrations and thus were less than their estimated diets.

Table 8. Concentrations of zinc in tissues of small mammals trapped at reference sites.^a

Species	Zinc concentrations (ug/g dry weight)		
	Whole body	Liver	Kidney
<u>Apodemus sylvaticus</u>	96-112	133	158
<u>Blarina brevicauda</u>	96		
<u>Clethrionomys glareolus</u>	103-143		
<u>Microtus agrestis</u>	121	120	104
<u>Microtus pennsylvanicus</u>	107	138-199	115-192
<u>Peromyscus leucopus</u>		161	140
<u>Peromyscus maniculatus</u>	106		
<u>Sorex araneus</u>	96-111		
<u>Zapus hudsonius</u>	107		

^aData compiled from Anthony and Kozlowski 1982, Beardsley et al. 1978, Johnson et al. 1978, Roberts and Johnson 1978, and Smith and Rongstad 1982.

The increase in body burden was significant in only M. agrestis (reference 121.2 ug/g), but the increase was only 50% in contrast to a 17-fold increase in the vegetation on which it feeds.

Radionuclides

Biomonitoring may be a critical technique for determining the presence of leaks at radioactive waste sites, but literature on this subject is scarce (Table 9). Kaye and Dunaway (1962) measured bioaccumulation of radionuclides in P. leucopus and S. hispidus at several contaminated sites at ORNL. They did not include a reference site in this preliminary study. They found that body burdens were higher than those expected from radioactive fallout, but too few animals of either species were caught to draw conclusions about sentinel species. Tissues of muskrats (Ondatra zibethica), rabbits (Sylvilagus

Table 9. Radionuclide concentrations in carcasses and tissues of small mammals collected at radioactively contaminated sites.

Radionuclide	Species	Site	Concentration (Bq/g)	Tissue	Observation	Reference
Cesium-137	<u>Peromyscus leucopus</u>	Waste site	0.26	Carcass	Body burden above background; no reference animals	Kaye and Dunaway 1962
	<u>Peromyscus maniculatus</u>	Waste site	2.13	Carcass	Significant difference between sites	Arthur et al. 1987
		Reference site	0.01	Carcass		
	<u>Sciurus carolinensis</u>	Land use: park residential school cemetery	0.32	Muscle	No association between land use and socio-economic stratum of area	Jenkins et al. 1980
			0.54	Muscle		
			1.42	Muscle		
			0.38	Muscle		
<u>Sigmodon hispidus</u>	Waste site	2.13	Carcass	Body burden above background; no reference animals	Kaye and Dunaway 1962	
Cobalt-60	<u>Peromyscus leucopus</u>	Waste site	0.59	Carcass	Body burden above background; no reference animals	Kaye and Dunaway 1962
	<u>Sigmodon hispidus</u>	Waste site	1.08	Carcass	Body burden above background; no reference animals	Kaye and Dunaway 1962
Ruthenium-106	<u>Peromyscus leucopus</u>	Waste site	41.96	Carcass	Body burden above background; no reference animals	Kaye and Dunaway 1962
	<u>Sigmodon hispidus</u>	Waste site	2.31	Carcass	Body burden above background; no reference animals	Kaye and Dunaway 1962
Strontium-90	<u>Peromyscus leucopus</u>	Waste site	1.48	Carcass	Body burden above background; no reference animals	Kaye and Dunaway 1962
	<u>Peromyscus maniculatus</u>	Waste site	26.67	Carcass	Significant difference between sites	Arthur et al. 1987
		Reference site	0.02	Carcass		
<u>Sigmodon hispidus</u>	Waste site	23.12	Carcass	Body burden above background; no reference animals	Kaye and Dunaway 1962	

floridanus), and a cotton mouse (S. hispidus) were analyzed for different radionuclides. Strontium-90 concentrations were highest in bone, whereas cesium-137 was present in all soft tissues. They did find large variations in bioaccumulation among individuals of a single species.

Arthur et al. (1987) measured radionuclide concentrations in tissues of deer mice (P. maniculatus), the most abundant species at the Subsurface Disposal Area of the Idaho National Engineering Laboratory. Deer mice were contaminated with radionuclides in areas where the surface soil was contaminated and in areas where the waste was covered with 0.6 m soil. Exposure at the latter site was presumably due to burrowing activity since the two dominant plant species on this site, crested wheatgrass and Russian thistle, were not contaminated. Concentrations of strontium-90 and cesium-137 in carcasses were significantly different from concentrations at the reference site. Concentrations of americium-241, plutonium-238, and plutonium-239 + 240 in carcasses were all less than 1 Bq/g. No other tissue, except lung, was analyzed for radionuclides. Other species were present at the site, but were not analyzed. Small mammals were also trapped at sites contaminated with actinide elements (Garten et al. 1987, Halford 1987), but tissue concentrations were generally below 1 Bq/g of tissue.

Organic Contaminants

Many organic chemicals are not susceptible to either biotic or abiotic degradation and consequently are persistent in the environment. These compounds tend to be lipophilic and may also accumulate through

food chains. Thus, potential hazards to wildlife exist due to the toxicity of these chemicals.

In addition to residue analyses, a variety of biochemical and cytogenetic assays can be applied to determine the presence and effects of persistent organic compounds. Where complex mixtures are present, such as at hazardous waste sites, a number of nonchemical-specific assays, originally developed for laboratory studies, have been applied.

Many of the present chemicals of concern, such as insecticides, were developed for their toxicity and persistence in the environment. Unfortunately, many insecticides are not selective, and nontarget species may be affected. Chlorinated hydrocarbon insecticides that are persistent in the environment and may be potentially harmful to wildlife include DDT and its metabolites, dieldrin, Kepone, mirex, and the hexachlorocyclohexanes (Menzer and Nelson 1980).

Organochlorine insecticides are being replaced, in many instances, by organophosphate and carbamate pesticides. Most of the latter compounds are cholinesterase inhibitors and the problems of persistence and chronic toxicity seen with the organochlorines have been replaced with the problem of acute toxicity and less selectivity. Because the transient nature of organophosphate insecticides as well as their higher potency make the measurement of residues difficult, biochemical assays such as cholinesterase inhibition have been used to measure effects on wildlife populations. Other biochemical assays such as mixed function oxidase induction have been applied to monitor exposures of several species, including fish (Jimenez et al. 1987), but have not yielded repeatable results when applied to natural populations of

terrestrial animals (Rattner and Hoffman 1987). Biochemical markers such as the formation of contaminant adducts with deoxyribonucleic acids (DNA) or hemoglobin have been investigated in the laboratory (Calleman 1984, Shugart 1985) and are potentially useful for field studies. Cytogenetic assays have been applied at sites where mixtures of unidentified chemicals are present. Residue analyses and biochemical assays applied to small mammals exposed to organic contaminants and hazardous wastes are summarized in Table 10. Most analyses were performed on a wet weight basis.

DDT and metabolites.

DDT is an environmentally stable organochlorine pesticide. Residues of DDT and DDD and DDE, its major metabolites, are ubiquitous. The residues are highly lipophilic and concentrate through food chains (Murphy 1980). Although the Environmental Protection Agency has restricted the use of DDT, residues persist in the environment. Accumulation in small mammals is of concern because small mammals such as Microtus spp. serve as prey for raptors and DDE-induced egg-shell thinning has been linked to reproductive failure in these birds (Kendall 1982).

Environmental persistence is evidenced by the presence of measurable residues in mice, voles, and shrews nine years after single applications to forests in Maine at a rate of 0.89 kg/ha (1 lb/acre) (Dimond and Sherburne 1969). Pooled samples of shrews (M. hoyi, B. brevicauda, and Sorex sp.) contained an average of 15.58 ug/g wet weight of DDT residues during the year of application and 1.18 ug/g nine years later. Pooled samples of mice (Peromyscus sp.) and voles

Table 10. Organic contaminants assayed in small mammals at field and hazardous waste sites.

Contaminant	Site	Species	Assay	Observation	Reference
DDT and metabolites	Treated forest	<u>Blarina brevicauda</u> <u>Sorex</u> sp. <u>Microsorex hoyi</u>	Whole-body residues	Residues persisted for nine years following treatment; 15.6 ug/g during first year, 1.2 ug/g during ninth year.	3
		<u>Clethrionomys gapperi</u> <u>Peromyscus</u> sp.	Whole-body residues	Residues persisted for nine years following treatment; 1.1 ug/g during first year, 0.0 ug/g during ninth year.	
	Background	<u>Blarina brevicauda</u> <u>Microtus pennsylvanicus</u> <u>Sorex cinereus</u>	Whole-body residues	Residue concentrations low: 0.01-0.03 ug/g.	11
	Field plots	<u>Blarina brevicauda</u> <u>Microtus pennsylvanicus</u>	Whole-body analysis	Residues were concentrated in shrews (17.6 ug/g in adults and 23.0 ug/g in subadults) compared to residues in stomach contents (13.4 and 13.0 ug/g, respectively). Residues were not concentrated in voles (adults: 5.1 ug/g in whole body and 6.0 in stomachs.	5
	Agricultural areas	<u>Peromyscus</u> spp.	Liver residues	Concentrations ranged from 0.006 to 0.929 ug/g and were highly correlated with soil concentrations.	10
		<u>Sigmodon hispidus</u>	Liver residues	Only two animals caught; concentrations of 0.102 and 0.940 ug/g reflected soil concentrations.	

Table 10. (continued)

Contaminant	Site	Species	Assay	Observation	Reference
Dieldrin	Wheat field	<u>Apodemus sylvaticus</u>	Whole-body residues	Residues increased by a factor of 68 following sowing of seeds dressed with dieldrin; decrease in residue with time.	7,8
		<u>Clethrionomys glareolus</u>	Whole-body residues	Residues increased following sowing of seeds dressed with dieldrin,	
Kepone	50 km downstream of manufacturing site	<u>Peromyscus leucopus</u>	Liver residues	Concentrations significantly greater at river site (2.74 ug/g) than at reference site (0.16 ug/g)	17
Organophosphate pesticides	Wheat field	<u>Apodemus sylvaticus</u>	Plasma, brain, liver cholinesterase	Cholinesterases were inhibited for up to six months.	21
	Treated forests	<u>Spermophilus columbianus</u>	Brain cholinesterase, brain residues	Brain cholinesterase inhibited up to 33% in 3/9 animals collected within six days post-spray, residues detectable in 4/10 animals; no inhibition in 3 animals collected 25-26 days post-spray.	23
	Treated fields	<u>Microtus pennsylvanicus</u>	Population size, recruitment, survival, body weight, reproductive activity	No significant difference in any parameter studied.	9

Table 10. (continued)

Contaminant	Site	Species	Assay	Observation	Reference
Polychlorinated biphenyls	Transformer salvage company	<u>Peromyscus</u> sp.	Liver and muscle residues	Concentration ratios of factory/reference areas were 4.7 for muscle and 3.1 for muscle.	6
		<u>Microtus</u> sp.	Liver and muscle residues	Concentrations for factory and reference areas: liver, 7.0 and 4.7 ug/g, respectively; muscle, 4.6 and 2.6 ug/g.	
		<u>Spermophilus tridecemlineatus</u>	Liver and muscle residues	Concentrations for factory and reference areas; liver, 8.7 and 1.0 ug/g, respectively; muscle, 2.9 and 0.90 ug/g.	
	Hazardous waste site	<u>Blarina brevicauda</u>	Carcass residues	Only one animal trapped; residue: 166 ug/g.	20
		<u>Microtus pennsylvanicus</u>	Carcass residues	Concentration range: 0.1 to 4.1 ug/g.	
		<u>Peromyscus leucopus</u>	Carcass residues	Concentration range: not detected to 3.0 ug/g.	
Mirex	Treated fields	<u>Blarina brevicauda</u> <u>Mus musculus</u> <u>Oryzomys palustris</u> <u>Sigmodon hispidus</u>	Whole-body residues	Residues higher in shrews (0.10 to 1.89 ug/g) than in mice and rats.	22
	Treated fields	<u>Mus musculus</u> <u>Rattus rattus</u> <u>Rattus exulans</u>	Carcass residues	Temporary increase in residues following treatment.	1

Table 10. (continued)

Contaminant	Site	Species	Assay	Observation	Reference
2,3,7,8-Tetrachloro-dibenzo-p-dioxin	Factory explosion	<u>Microtus arvalis</u>	Whole-body residues	Voles accumulated concentrations of same order of magnitude as those in soil (0.07 to 49 ng/g).	9
	Military test area	<u>Peromyscus polionotus</u>	Liver residues, liver histology, reproduction	Concentrations ranged from 0.3 to 2.9 ug/g and were related to soil concentrations; liver/soil concentration factors ranged from 6 for females to 18 for males. No significant pathological lesions or reproductive effects.	2, 18
Complex mixtures	Petrochemical waste site: petrochemicals, PCBs, metals	<u>Peromyscus leucopus</u>	Chromosomal aberrations	Lesions/cell and aberrant cells/individual were significantly elevated at the contaminated site compared to the reference site.	12, 13, 14
		<u>Sigmodon hispidus</u>			
	Hazardous waste site	<u>Peromyscus leucopus</u>	Cytogenetic assays	Significant increase in bone marrow micronucleated polychromatic erythrocytes (PCEs); significant depression in bone marrow mitotic index and percent PCEs at contaminated area compared to reference area. No changes in average generation time or sister chromatid exchange frequency.	19
	Hazardous waste site: lindane, chlorobenzene, benzylchlorides, dioxin	<u>Microtus pennsylvanicus</u>	Mortality rate, organ toxicity, tissue residues	Significant differences in life expectancy; liver, adrenal, seminal vesicle weights; and concentrations of some chlorinated hydrocarbons in fat tissue between the reference and contaminated sites.	16

Table 10. (continued)

Contaminant	Site	Species	Assay	Observation	Reference
	Chemical plants	<u>Mus musculus</u>	Sister chromatid exchange frequency	Values were inversely proportional to distance to the nearest chemical/ industrial area.	15

References

1. Bevenue et al. 1975
2. Cockerham and Young 1981
3. Dimond and Sherburne 1969
4. Fanelli et al. 1980b
5. Forsyth and Peterle 1984
6. Greichus and Dohman 1980
7. Jefferies et al. 1973
8. Jefferies and French 1976
9. Jett et al. 1986
10. Laubscher et al. 1971
11. Lincer and Sherburne 1974
12. McBee 1985
13. McBee et al. 1987
14. McBee and Bickham 1988
15. Nayak and Petras 1985
16. Rowley et al. 1983
17. Terman and Huggett 1980
18. Thalken and Young
19. Tice et al. 1987
20. Watson et al. 1985
21. Westlake et al. 1980, 1982
22. Wolfe and Norment 1973
23. Zinkl et al. 1980

(C. gapperi) contained 1.06 ug/g during the first year and 0.04 ug/g nine years later. The two groups collected from an untreated area contained 0.30 and 0.03 ug/g, respectively. DDE residues in voles (M. pennsylvanicus) and shrews (B. brevicauda and S. cinereus) in the Ithaca, New York, area were 0.01 and 0.03 ug/g, respectively (Lincer and Sherburne 1974). It was not reported when this area or nearby areas had been treated. In a third field study, total residues of DDT were detected at concentrations ranging from 0.006 to 0.929 ug/g wet weight in whole bodies of P. leucopus and were related to soil concentrations of 3.6 to 6,700 ug/g (Laubscher et al. 1971).

Following treatment of field plots with ³⁶Cl-DDT, concentrations of total DDT residues were greater in shrews (B. brevicauda, 17.6 ug/g wet weight) than in voles (M. pennsylvanicus, 5.1 ug/g) (Forsyth and Peterle 1984). Whole body residues were higher in juveniles than in adults and increased with increasing body fat content. Whole body residues were greater than residues in stomach contents of B. brevicauda, thus showing bioaccumulation; this was not true for M. pennsylvanicus.

Dieldrin

As part of an investigation of the food chain transfer of chlorinated organic compounds to carnivorous birds in Britain, a study of the dieldrin residues of their small mammal prey was made (Jefferies et al. 1973, Jefferies and French 1976). Until 1974, both an organomercury fungicide and the insecticide dieldrin were commonly used to treat cereal seeds before sowing. Dieldrin residues were measured in small mammals before and up to two months after sowing. Results showed that

the field mouse (A. sylvaticus), which inhabited the open fields and the grassy areas surrounding the fields, fed immediately on the seeds. This resulted in elevated concentrations one day after sowing. The bank vole (C. glareolus) was restricted in its habitat to the field edges, resulting in lower, but erratic concentrations of residues compared to the field mouse.

Kepone

The pesticide, Kepone, was released to the James River, Virginia, and the Chesapeake Bay through careless manufacturing practices at a small chemical plant in Hopewell, Virginia (Menzer and Nelson 1980). Monitoring with two populations of the white-footed mouse (P. leucopus), one located on an island in the James River, 50 km downstream of the plant and the other located 4.8 km inland of the river, established that Kepone has moved into the terrestrial environment (Terman and Huggett 1980). Kepone was found in all of the mice on the island. Significantly lower concentrations were found in 70% of mice trapped at a reference site 4.8 km inland. No detectable amount was present in a laboratory-reared individual of this species. Concentrations in other compartments were not measured, and thus comparisons with other species could not be made.

Mirex

Mirex has been used for fire ant control in the southeastern United States. Mirex is persistent in the environment; residues in soil, water, and vegetation were found to be constant over a period of 300 days (Val Valin 1968, Murphy 1980). Acute toxicity to wildlife is

low. Mirex residues were monitored in wildlife in Mississippi for one year following an aerial application (Wolfe and Norment 1973). Whole-body residues in several species were related to food habits, with the shrew (B. brevicauda) having the highest concentrations (up to 1.89 ug/g wet weight). Herbivores generally had lower concentrations than omnivores.

Mirex residues were also monitored in several small mammals (M. musculus, R. rattus, and R. exulans) before and after aerial application to pineapple fields in Hawaii (Bevenue et al. 1975). Prior to application, residues for all species were <0.3 ug/g wet weight. Within three months of application, residues ranged up to 9.4 ug/g in the Polynesian rat (R. exulans) and then declined to background values by the tenth month. Thus, mirex appears to lend itself to only short-term biomonitoring.

Organophosphate Pesticides

The use of biochemical assays to complement residue analyses is an important technique for the investigation of the effects of newly developed pesticides on wildlife. Westlake et al. (1980, 1982) used the field mouse (A. sylvaticus) as an indicator species to investigate the effect of the insecticides chlorfenvinphos and carbophenothion on wildlife. Following sowing of seed dressed with these chemicals, the pattern and persistence of esterase inhibition in several tissues reflected the persistence of these insecticides in the field. Likewise, Zinkl et al. (1980) found that brain cholinesterase was depressed in birds and two species of squirrels after an aerial application of acephate. At this level of application, the inhibition of cholines-

terase in birds was close to the toxic level, but the depression in squirrels was much lower.

Polychlorinated Biphenyls (PCBs)

PCBs are exceptionally environmentally persistent chlorinated hydrocarbons that have been used as insulating materials in electrical capacitors and transformers and in plasticizers, fire retardants, hydraulic and lubricating fluids, and heat exchangers. The large amounts manufactured, their widescale use, and chemical stability have led to worldwide contamination. They have contaminated the environment through industrial discharges, movement from disposal sites, and use of oil wastes containing PCBs as a dust control measure on parking lots and roads. PCBs are lipophilic which, in addition to their stability, leads to bioaccumulation in fat tissue (Menzer and Nelson 1980, Watson et al. 1985). In the laboratory, administration of PCBs has led to reproductive failure in P. leucopus (Linzey 1987).

Small mammals have been used to monitor the movement of PCBs from two contaminated sites, one in South Dakota and one in New York. PCBs were measured in the area surrounding two electrical transformer salvage companies near Colman, South Dakota (Greichus and Dohman 1980). Concentrations were highest in soil (<0.025 to 46 ug/g), lowest in vegetation (0.3 to 2.2 ug/g) and earthworms (1.96 ug/g), and intermediate in small mammals. Concentrations ranged from 2.07 to 17.2 ug/g in liver tissue of small mammals and 2.89 to 6.87 in muscle. The highest concentration was in the liver of the mouse (Peromyscus sp.), but too few animals of other species were caught to make meaningful comparisons. PCBs were detectable in small mammals trapped in an area

believed to be relatively free of contamination and ranged up to 4.7 ug/g in liver; this value appears to be high when compared to undetectable amounts in several animals trapped at a contaminated waste site in New York state (Watson et al. 1985). The study does indicate that PCBs are accumulating in small mammals over the concentrations found in their sources of food.

PCBs also moved from soil into the food chain at a hazardous waste site in New York state where 452 tons of liquid waste containing PCBs and other organic compounds were dumped (Watson et al. 1985). As a dust control measure, an access road to the site had been repeatedly treated with oil containing PCBs. Ranges of PCBs in several media were: soil, 5.5 to 6,300; vegetation, 0.3 to 2.0; invertebrates, 0.1 to 60; and small mammal carcasses, not detected to 166 ug/g wet weight. Except for the high value in a single shrew (166 ug/g), concentrations were variable but similar in the mouse (P. leucopus) and vole (M. pennsylvanicus). The nondetectable values in some of the animals indicate recent immigration or activity away from the area of contamination. A reference area was not investigated.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

In the past, TCDD was a contaminant of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). 2,3,7,8-Tetrachlorodibenzo-p-dioxin has entered the environment through the extensive use of 2,4,5-T in agriculture to control broad-leaf weeds and as a consequence of the explosion of a chemical plant in Sevesco, Italy in 1976. During the 1960s, TCDD was extensively used in military defoliation operations in Southeast Asia. More recently, it has been shown to be produced during

the combustion of fossil fuels and incineration of municipal wastes. It is extremely toxic to some species, causing degenerative changes in the liver and thymus and altering serum enzyme concentrations (Menzer and Nelson 1980). In humans it has been found to cause chloracne and is a teratogen.

Following an explosion at a chemical plant in Italy, a large area around the plant was contaminated with TCDD; thousands of small animals died within a few weeks (Fanelli et al. 1980a). Four years later, measurements in the common vole (M. arvalis) and other animals showed that TCDD was still accumulating in wildlife (Fanelli et al. 1980b). Whole-body concentrations of voles ranged from 0.07 to 49 ppb (average 4.5 ppb); contamination of the soil ranged from 0.01 to 12 ppb (average 3.5 ppb). Values at reference sites were not given, but presumably should be below the level of detection.

Aerial spraying equipment for the defoliation operation in Southeast Asia was tested at Eglin Air Force Base in Florida (Thalcken and Young 1981). The test area was sprayed with 73,000 kg of 2,4,5-T during the period 1962-1970. Although no residues of the herbicide were present eight years later, residues of TCDD were still present. Animals were abundant on the site, and tissue residues were monitored in the beach mouse (P. polionotus). There was a close relationship between soil and liver concentrations of TCDD. Bioconcentration factors (liver/soil) (wet weight) ranged from six to 18. There were no histopathological differences between liver tissues of mice from the contaminated and reference sites (Cockerham and Young 1981).

Complex Mixtures

Biomonitoring at sites contaminated with a complex mixture of chemicals, especially when the chemicals are unidentified, is difficult. Biochemical analyses for a variety of chemicals can be time-consuming and expensive. In addition, measuring body burdens of specific chemicals does not take into account the potential for interactions within the organism. At such sites a variety of physiological and biochemical assays can be employed. In three studies, cytogenetic or genotoxic assays were applied to field populations of small mammals at sites containing complex mixtures of waste chemicals.

Monitoring chromosomal aberrations in small mammals at a petrochemical waste disposal site was used as a natural model for the analysis of environmental mutagenesis (McBee 1985, McBee et al. 1987, McBee and Bickham 1988). From 1961 to 1985 the site had been used for fire-fighting training exercises. Retention ponds on the site contained a mixture of oil, grease, partially combusted hydrocarbon compounds, PCBs, hexachlorobenzene and a variety of metals. Two species of small mammals, the white-footed mouse (P. leucopus) and the cotton rat (S. hispidus), were trapped in sufficiently large numbers to allow statistical analyses of differences in chromosome aberrations between the contaminated site and a reference site. Using several assays (karyology, G-band karyology, and flow cytometry), all of which detect DNA damage, differences between the populations at the contaminated and reference sites were found for both species in one or more of the assays. Peromyscus leucopus had significantly more chromosomal lesions per cell than S. hispidus, suggesting it might be

of DNA damage. Using the Ames assay, an earlier study had indicated that the water in the waste pond was mutagenic to Salmonella typhimurium (Brown and Donnelly 1982).

Tice et al. (1987) examined only one species, P. leucopus, for genotoxic and cytotoxic damage at a U.S. EPA Superfund site in Camden County, New Jersey. They examined bone marrow cells for micronucleated polychromatic erythrocytes, sister chromatid exchange, average generation time, and mitotic index and peripheral blood for percent polychromatic erythrocytes. A significant increase in bone marrow micronucleated polychromatic erythrocytes was found in addition to a significant depression in both bone marrow mitotic index and percent polychromatic erythrocytes in peripheral blood at the hazardous waste site compared with a control site. There were no alterations in average generation time of cells or sister chromatid exchange frequency. They also compared the field-caught specimens with their laboratory colony of P. leucopus and found a significant increase in mitotic index in the laboratory-reared stock.

Nayak and Petras (1985) also used sister chromatid exchange frequency to compare wild-caught and laboratory-reared populations of mice, M. musculus. Sister chromatid exchange values were higher in wild-caught mice than in the laboratory population and the values in wild-caught mice were inversely proportional to distance between the site of capture and the nearest industrial area. Field-caught mice maintained in the laboratory for nine or more months had sister chromatid exchange values similar to those of the laboratory stock.

Rowley et al. (1983) used field voles (M. pennsylvanicus) to assess risk of hazardous chemicals to wildlife at a waste site in Love Canal, New York. Field voles were chosen because they were the most common species at the site and in the surrounding area. The site contains over 6,900 tons of lindane, 2,000 tons of chlorobenzenes, 2,400 tons of benzylchlorides, and 200 tons of trichlorophenol contaminated with dioxin. A variety of assays were used as endpoints: mean life expectancy, organ toxicity, and tissue residues. The investigators found significant differences in population density; mean life expectancy; liver, adrenal, and seminal vesicle weights; and concentrations of some chlorinated hydrocarbons in fat tissue between the contaminated and reference sites.

In follow-up studies, mice and rats were exposed to the Love Canal soil or extracts of the soil under laboratory conditions. Mice exposed by direct contact with the soil had increased body and liver weights (Silkworth et al. 1984). Pregnant rats administered extracts of the soil by gavage showed effects on maternal health and fetal development (Silkworth et al. 1986). Benzene hexachlorides, tetrachloroethanes, and TCDD were identified in the soil and extracts.

Evaluation of Study Species as Monitors

Based on the studies discussed in the previous three sections, an evaluation of the suitability of each species as a sentinel species for specific contaminants can be made (Table 11). This assessment of each species as a biomonitor is based on the extent of accumulation, significant differences between means of residues in animals from

Table 11. Evaluation of small mammal species as biomonitors of environmental contaminants.

Contaminant	Site	Species	Tissue/Assay	Value as Biomonitor	Reference
Metals					
arsenic	zinc-copper mine	<u>Blarina brevicauda</u>	whole body	+	Smith and Rongstad 1982
		<u>Peromyscus maniculatus</u>	whole body	-	
cadmium	lead-zinc mine	<u>Sorex araneus</u>	whole body	++	Johnson et al. 1978, Roberts and Johnson 1978
		<u>Apodemus sylvaticus</u>	kidney > liver > whole body	++	
		<u>Clethrionomys glareolus</u>	kidney > liver > whole body	++	+
		<u>Microtus agrestis</u>	kidney > liver > whole body		
zinc-copper mine		<u>Blarina brevicauda</u>	whole body	++	Smith and Rongstad 1982
		<u>Peromyscus maniculatus</u>	whole body	+	
		<u>Microtus pennsylvanicus</u>	whole body	-	
mine waste site		<u>Sorex araneus</u>	liver > kidney > whole body	++	Andrews et al. 1984
		<u>Microtus agrestis</u>	kidney > liver, whole body	+	
copper-cadmium plant		<u>Sorex araneus</u>	liver > kidney > hair	++	Hunter and Johnson 1982
		<u>Microtus agrestis</u>	kidney > liver > hair	++	
		<u>Apodemus sylvaticus</u>	kidney > liver > hair	+	
metal smelter		<u>Talpa europea</u>	kidney • liver	++	Ma 1987
sewage farm		<u>Microtus agrestis</u>	kidney > liver	+	Beardsley et al. 1978
sewage-sludge farm		<u>Microtus pennsylvanicus</u>	kidney > liver	+	Anderson et al. 1982
waste-water-irrigated site		<u>Peromyscus leucopus</u>	kidney > liver	+	Anthony and Kozlowski 1982
		<u>Microtus pennsylvanicus</u>	kidney, liver	-	

Table 11. (Continued)

Contaminant	Site	Species	Tissue/Assay	Value as Biomonitor	Reference
	urban areas	<u>Rattus norvegicus</u>	liver > kidney	+	Way and Schroder 1982
chromium	sewage farm	<u>Microtus agrestis</u>	carcass > kidney > liver	-	Beardsley et al. 1978
	waste-water-irrigated site	<u>Peromyscus leucopus</u> <u>Microtus pennsylvanicus</u>	kidney, liver kidney, liver	- -	Anthony and Kozlowski 1982
cobalt	waste-water-irrigated site	<u>Peromyscus leucopus</u>	kidney, liver	-	Anthony and Kozlowski 1982
		<u>Microtus pennsylvanicus</u>	kidney, liver	-	
copper	copper smelter	<u>Sorex araneus</u>	hair > liver > kidney > whole body	+	Hunter and Johnson 1982
		<u>Microtus agrestis</u>	hair > kidney > whole body	+	
		<u>Apodemus sylvaticus</u>	liver > whole body	-	
zinc-copper mine		<u>Peromyscus maniculatus</u>	whole body	+	Smith and Rongstad 1982
		<u>Microtus pennsylvanicus</u>	whole body	+	
		<u>Blarina brevicauda</u>	whole body	-	
	sewage farm	<u>Microtus agrestis</u>	liver > kidney	+	Beardsley et al. 1982
	waste-water-irrigated site	<u>Microtus pennsylvanicus</u>	liver, kidney	-	Anthony and Kozlowski 1982
		<u>Peromyscus leucopus</u>	liver, kidney	-	
	sewage-sludge treated site	<u>Microtus pennsylvanicus</u>	liver, kidney	-	Andrews et al. 1982

Table 11. (Continued)

Contaminant	Site	Species	Tissue/Assay	Value as Biomonitor	Reference
lead	roadside	<u>Sorex araneus</u>	kidney > liver	+	Williamson and Evans 1972
		<u>Clethrionomys glareolus</u>	kidney, liver	+	
		<u>Apodemus sylvaticus</u>	liver	+	
		<u>Microtus agrestis</u>	kidney, liver	+	
	roadside	<u>Microtus agrestis</u>	whole body > liver	++	Jefferies and French 1972
		<u>Clethrionomys glareolus</u>	whole body > liver	+	
		<u>Apodemus sylvaticus</u>	whole body > liver	+	
	roadside	<u>Peromyscus maniculatus</u>	bone > kidney > liver	++	Welch and Dick 1975
	roadside	<u>Peromyscus maniculatus</u>	bone > kidney > liver	++	Mierau and Favara 1975
	roadside	<u>Blarina brevicauda</u>	bone > whole body > kidney > liver	++	Getz et al. 1977
		<u>Cryptotis parva</u>	whole body	++	
		<u>Reithrodontomys megalotis</u>	bone > muscle > lung > whole body	++	
		<u>Microtus ochrogaster</u>	bone > muscle > kidney	+	
		<u>Mus musculus</u>	bone > kidney > whole body	+	
		<u>Peromyscus maniculatus</u>	bone > kidney	+	
	roadside	<u>Blarina brevicauda</u>	whole body	++	Goldsmith and Scanlon 1977
		<u>Peromyscus leucopus</u>	whole body	+	
	roadside	<u>Blarina brevicauda</u>	whole body	++	Quarles et al. 1977
		<u>Microtus pennsylvanicus</u>	whole body	++	
		<u>Peromyscus leucopus</u>	whole body	+	
	roadside	<u>Blarina brevicauda</u>	whole body	++	Clark 1979
<u>Microtus pennsylvanicus</u>		whole body	+		
<u>Peromyscus leucopus</u>		whole body	+		

Table 11. (Continued)

Contaminant	Site	Species	Tissue/Assay	Value as Biomonitor	Reference
	roadside	<u>Sorex araneus</u>	liver, kidney, bone	++	Chmiel and Harrison 1981
		<u>Apodemus sylvaticus</u>	liver, kidney, bone	+	
	lead-zinc mine	<u>Microtus agrestis</u>	bone > whole body > kidney > liver	++	Roberts and Johnson 1978, Roberts et al. 1978
		<u>Clethrionomys glareolus</u>	bone > whole body > kidney > liver	++	
		<u>Apodemus sylvaticus</u>	bone > kidney > whole body > liver	++	
		<u>Sorex araneus</u>	whole body	+	
	zinc-copper mine	<u>Peromyscus leucopus</u>	whole body	+	Smith and Rongstad 1982
		<u>Microtus pennsylvanicus</u>	whole body	+	
		<u>Blarina brevicauda</u>	whole body	+	
	lead-arsenate-treated orchard	<u>Microtus pinetorum</u>	bone > kidney > liver	++	Elfving et al. 1978, Haschek et al. 1979
		<u>Microtus pennsylvanicus</u>	bone > kidney > liver	++	
		<u>Peromyscus leucopus</u>	bone > kidney > liver	+	
	waste-water-irrigated	<u>Peromyscus leucopus</u>	kidney > liver	+	Anthony and Kozlowski 1982
		<u>Microtus pennsylvanicus</u>	kidney > liver	-	
	sewage-sludge-treated	<u>Microtus agrestis</u>	bone > carcass > kidney	-	Beardsley et al. 1978
	sewage-sludge-treated	<u>Microtus pennsylvanicus</u>	liver, kidney	-	Anderson et al. 1982
	metal smelter	<u>Talpa europea</u>	kidney, liver	-	Ma 1987
	urban areas	<u>Rattus norvegicus</u>	bone > kidney > liver	++	Mouw et al. 1975
	urban areas	<u>Rattus norvegicus</u>	bone > kidney > liver	+	Way and Schroder 1982

Table 11. (Continued)

Contaminant	Site	Species	Tissue/Assay	Value as Biomonitor	Reference
manganese	sewage farm	<u>Microtus agrestis</u>	kidney, liver, bone	-	Beardsley et al. 1978
mercury	chlor-alkali plant	<u>Apodemus sylvaticus</u>	hair > kidney > liver	+	Bull et al. 1977
		<u>Clethrionomys glareolus</u>	hair > brain > kidney	-	
	zinc-copper mine	<u>Blarina brevicauda</u>	whole body	-	Smith and Rongstad 1982
		<u>Peromyscus leucopus</u>	whole body	-	
	mercury-dressed seed	<u>Apodemus sylvaticus</u>	whole body	+	Jefferies et al. 1973, Jefferies and French 1976
		<u>Clethrionomys glareolus</u>	whole body	-	
	mercury-dressed seed	<u>Apodemus sylvaticus</u>	whole body	+	Westlake et al. 1980
zinc	lead-zinc mine	<u>Microtus agrestis</u>	whole body	++	Johnson et al. 1978, Roberts and Johnson 1978
		<u>Sorex araneus</u>	whole body	+	
		<u>Clethrionomys glareolus</u>	whole body	+	
		<u>Apodemus sylvaticus</u>	bone > whole body > kidney > liver	+	
	zinc-copper mine	<u>Peromyscus maniculatus</u>	whole body	+	Smith and Rongstad 1982
		<u>Blarina brevicauda</u>	whole body	+	
		<u>Microtus pennsylvanicus</u>	whole body	-	
	sewage farm	<u>Microtus agrestis</u>	carcass > liver > kidney	-	Beardsley et al. 1978
	sewage-sludge field	<u>Microtus pennsylvanicus</u>	liver, kidney	-	Anderson et al. 1982
	waste-water-irrigated site	<u>Peromyscus leucopus</u>	liver, kidney	-	Anthony and Kozlowski 1982
		<u>Microtus pennsylvanicus</u>	liver, kidney	-	

Table 11. (Continued)

Contaminant	Site	Species	Tissue/Assay	Value as Biomonitor	Reference
Radionuclides					
Sr-90, Cs-137	radioactive waste site	<u>Peromyscus maniculatus</u>	carcass	+	Arthur et al. 1987
Organic Compounds					
DDT and metabolites	DDT-treated forest	<u>Microsorex hoyi</u>	whole body	++	Dimond and Sherburne 1969
		<u>Blarina brevicauda</u>			
		<u>Sorex sp.</u>			
		<u>Peromyscus spp.</u>			
	background concentrations	<u>Clethrionomys gapperi</u>	whole body	+	Lincer and Sherburne 1974
		<u>Sorex cinereus</u>	whole body	+	
		<u>Blarina brevicauda</u>	whole body	+	
	experimental field plots	<u>Microtus pennsylvanicus</u>	whole body	-	Forsyth and Peterle 1984
		<u>Blarina brevicauda</u>	whole body	++	
	agricultural areas		<u>Peromyscus leucopus</u>	liver	+
dieldrin	dressed seeds	<u>Apodemus sylvaticus</u>	whole body	+	Jefferies et al. 1973, Jefferies and French 1976
		<u>Clethrionomys glareolus</u>	whole body	-	
kepone	contaminated river	<u>Peromyscus leucopus</u>	liver	+	Terman and Huggett 1980

Table 11. (Continued)

Contaminant	Site	Species	Tissue/Assay	Value as Biomonitor	Reference
mirex	treated areas	<u>Blarina brevicauda</u>	liver > whole body	+	Wolfe and Norment 1973
		<u>Peromyscus leucopus</u>	whole body	+	
		<u>Sigmodon hispidus</u>	liver > whole body	-	
OP pesticides	dressed seed	<u>Apodemus sylvaticus</u>	cholinesterase inhibition	+	Westlake et al. 1980, 1982
PCBs	hazardous waste site	<u>Blarina brevicauda</u>	whole body	++	Watson et al. 1985
		<u>Microtus pennsylvanicus</u>	whole body	+	
		<u>Peromyscus leucopus</u>	whole body	+	
	transformer salvage company	<u>Spermophilus</u> sp.	liver > muscle	++	Greichus and Dohman 1980
		<u>Peromyscus</u> sp.	liver > muscle	+	
		<u>Microtus</u> sp.	liver > muscle	+	
dioxin	accidental release	<u>Microtus arvalis</u>	whole body	+	Fanelli et al. 1980b
	treated areas	<u>Peromyscus polionotus</u>	liver	+	Thalke and Young 1981
complex mixtures	hazardous waste site	<u>Microtus pennsylvanicus</u>	mortality rate, organ toxicity tissue residues	+	Rowley et al. 1983
	hazardous waste site	<u>Peromyscus leucopus</u> <u>Sigmodon hispidus</u>	chromosomal aberrations chromosomal aberrations	+ -	McBee 1985, McBee et al. 1987

Table 11. (Continued)

Contaminant	Site	Species	Tissue/Assay	Value as Biomonitor	Reference
	hazardous waste site	<u>Peromyscus leucopus</u>	cytogenetic endpoints		Tice et al. 1987
			micronuclei formation	+	
			mitotic index	+	
			% polychromatic erythrocytes	+	
			cell generation time	-	
			sister chromatid exchange	-	
	chemical plants	<u>Mus musculus</u>	sister chromatid exchange	+	Nayak and Petras 1985

Key: ++ (excellent), + (good), - (unsatisfactory)

reference and contaminated sites, as well as observations by the respective authors. Where accumulation was high (several orders of magnitude above accumulation at reference sites), bioconcentration above soil values was evident, or differences between contaminated and reference sites were statistically significant, species were ranked as excellent monitors (++). Where differences in accumulation between contaminated and reference sites were apparent, but statistics were not provided or too few animals were trapped to make comparisons, species were ranked as good monitors (+). Where there were no differences between contaminated and reference sites or where data was insufficient, the notation (-) is used. Where several species were studied at the same site, a comparative evaluation was made, with species ranked in order of decreasing ability to serve as biomonitors. Not all studies employed the primary target tissues, but where several tissues were studied, they are ranked in order of contaminant concentration. For arsenic, chromium, cobalt, manganese, and nickel, not enough information was available to make such determinations.

Food is the major source of heavy metal, radionuclide, and organic contamination for these species, and degree of tissue contamination can generally be related to concentrations in the diet. In the following discussions the general term shrews refers to members of the genera Blarina and Sorex; mice refers to the genera Peromyscus and Apodemus, and voles refers to the genera Microtus and Clethrionomys.

Cadmium in the environment is primarily associated with soil. It was not shown to accumulate in vegetation; concentrations in vegetation were always less than those of soil. The concentration factor of

soil:invertebrates was approximately 1. Cadmium is present in invertebrates such as earthworms and insects, the primary food items of carnivorous species. Accumulation of cadmium in small mammals was generally highest in the kidney and this tissue appears to be the most suitable for monitoring studies. For the mouse and vole, concentration factors of kidney:soil or kidney:invertebrates were similar and ranged from 0.5 to 1.5. An exception was at a copper-cadmium alloy plant where cadmium was deposited directly on the vegetation. Here concentration factors of kidney:soil and kidney: vegetation were 2.7 and 11.7, respectively, for the vole. For the shrew, accumulation was greatest in the liver, followed by the kidney. Concentration factors for the liver and kidney compared to soil or invertebrates ranged from 15 to 33. It is interesting to note that the mean concentrations of cadmium in the kidney and liver of the shrew at the reference site were greater than the mean concentrations for the other two species at the contaminated site. Cadmium also accumulated above soil and invertebrate concentrations in the liver and kidney of the mole, a predator of invertebrates, especially earthworms.

Copper in animal tissues accumulated above reference concentrations at highly contaminated sites. An exception was one sewage-treated area where copper in the liver of the vole M. agrestis was more than twice that at a reference site and copper in herbage, the main foodstuff, was also more than twice reference values. At the active copper smelter site, the copper concentration in vegetation was up to 47 times that of vegetation from a reference site while copper in the kidney of M. agrestis was higher by only a factor of two and the same

as reference values in liver tissue. Copper accumulation was greatest in the shrew (S. araneus) followed by the vole (M. agrestis) and the mouse (A. sylvaticus) in decreasing order of tissue accumulation. The copper concentration of invertebrates was higher than that of vegetation, 343-569 ug/g compared to 153-375 ug/g, and the shrew eats a greater weight of food than the vole, making the copper intake much greater for the shrew. Reference values were also high for shrews. Copper translocation to the seeds of vegetation was low, making the diet of the mouse A. sylvaticus the lowest in copper of the three species trapped at this site. Concentrations of copper in soil, vegetation, and invertebrate compartments were not measured at the zinc-copper mine. Based on these few studies, copper uptake was well regulated and where accumulation took place, choosing a monitoring species depended on the degree of contamination, route of deposition, and food concentrations. Liver and kidney were the primary target tissues for copper.

At roadside sites where lead is deposited on both soil and vegetation, all species collected were usable as monitors for this metal. There was a positive relationship between traffic density and tissue or whole-body accumulation and an inverse relationship between distance from the road and tissue or whole-body concentrations. For sites at which shrews were present, highest tissue concentrations were present in these species compared to mice and voles. At several roadside sites, shrews were more abundant than mice, indicating that roadside grassy areas are suitable habitat for shrews but not mice. The ranking of species as biomonitors at roadside sites can be corre-

lated with food habits. The high food intake of shrews and the concentration of lead in insects, its primary food items, contribute to the high body burdens. Lead from automobile exhaust is deposited on the grasses, the primary food of Microtus spp., contributing to their positive ranking as monitors. The grassy edge along roadsides is not the primary habitat of the mice, Peromyscus spp. and A. sylvaticus and their lower body burdens may reflect the short time they spend in this part of their home range.

At other sites contaminated with lead, shrews were either not abundant or not considered for study. At a site where they were present, lead levels were higher in voles than in shrews or mice, reflecting the high uptake of lead by grassy vegetation compared to invertebrates and seeds. In two studies in urban areas, rats were used as monitors of lead contamination, indicating the usefulness of this species in areas where other small mammals are usually absent.

Although lead preferentially accumulates in bone, only half of the studies used bone as the monitoring tissue. Where bone was used, the highest accumulation value of all tissues was reported, 672 ug/g in bone of A. sylvaticus at a metal smelter waste site (Johnson et al. 1978). Whole body, kidney, and liver tissues were also suitable tissues for biomonitoring at all but sewage-treated sites.

At two sites where mercury was present, either as emissions from a chlor-alkali plant or as mercury-dressed seeds, the body burdens in mice and voles were similar. The area around the chlor-alkali plant was not highly contaminated, and although both species showed statistically significant increases compared to their respective reference

areas, a site with a higher degree of contamination might produce a more definitive result. An orchard treated with phenylmercuric acetate likewise had a low concentration of mercury in the soil and animal tissues were not processed for mercury. Mercury was analyzed in whole bodies and hair, kidney, and liver tissues, but too few studies were available to choose one target tissue.

Zinc, an essential element, is regulated in living organisms and thus is not usually a suitable contaminant for biomonitoring. At derelict mine sites where the mean soil zinc concentration was 21,000 ug/g, total body burdens were highest for M. agrestis, followed, in order, by S. araneus, C. glareolus, and A. sylvaticus. Zinc concentrations were higher in cover vegetation, the diet of the vole, than in invertebrates. Whole-body concentrations at contaminated sites were never more than a factor of 1.6 greater than those at reference sites. At a zinc-copper mine where concentrations were not measured in soil or diet compartments, mean whole-body concentrations were about two times greater at the contaminated site for three species, with concentrations in P. maniculatus being slightly higher and less variable than in M. pennsylvanicus. Only two B. brevicauda were trapped. Most studies used whole body measurements; zinc did not bioconcentrate in liver or kidney tissues.

Deer mice were the only species trapped in sufficient numbers at radioactive waste sites in a study to evaluate their usefulness as monitors. This species was a good monitor of both strontium-90 and cesium-137. Although carcass concentrations were used in this study, some sources indicate that other tissues, such as bone for strontium

and soft tissues for cesium, might be better targets for radionuclide monitoring.

Higher concentrations of DDT and its metabolites were present in shrews than in voles at an experimental field plot and in areas previously treated with DDT for pest control. Shrews accumulated DDT residues in excess of concentrations in their stomach contents at the field plots, but had lower concentrations than in one of their primary food items, Coleoptera, at the other site, and the residues decreased with time.

Shrews were not abundant at other sites where organic wastes were present. Thus, in spite of accumulating the highest concentrations of mirex and PCBs in two studies, their scarcity would make biomonitoring difficult if it were not for the presence of other suitable species. Several species of Peromyscus, common to diverse habitats, were reliable monitors of organic contaminants including kepone, TCDD, mirex and some complex mixtures. Peromyscus leucopus was present in numbers great enough for statistical analyses at two sites contaminated with complex mixtures. At both sites they gave positive results in cytogenetic assays. Additional investigations with this species, utilizing biochemical assays as well as tissue residues are in order. Thus, a successful small-mammal monitoring program requires experience and knowledge regarding target mammals and contaminants in order to design and implement a meaningful program.

As discussed, food was the major source of contaminants at most of the study sites. Feeding group or trophic level of the species collected were generally positively related to the degree of tissue

contamination. Thus, while the potential for monitoring at a site depends to some degree on the chemical and biological characteristics of the contaminant and the species present at the site, a generalization about food habits and capacity to biomonitor can be made. A classification of trophic level of the small mammals studied and value as a biomonitor as established in Table 11 indicates that insectivorous small mammals (shrews) were the best sentinels for all contaminants (Table 12). Metals and organic compounds accumulate in this trophic level by direct food intake such as ingesting soil-containing earthworms, and through food chain accumulation by ingesting contaminant-rich carnivorous beetles and spiders. Shrews were not tested in cytogenetic assays. Omnivorous species such as mice and rats were suitable for many contaminants. Herbivorous species such as voles were in many cases unsuitable except when contaminants were accumulated by or deposited directly on vegetation in aerosol form such as emissions from refineries, smelters, and automobile exhaust.

Limitations to biomonitoring

As noted throughout the foregoing discussions, there are several problems with the use of small mammals as biomonitors. The most obvious drawback is the limited population sizes, including the lack of a suitable biomonitoring species, at some sites. Insectivorous species such as the Soricidae (Sorex and Blarina) are widely distributed, but they are generally more selective in their habitat preference than are the Cricetidae or Muridae, and are either far less abundant than other species or more difficult to trap. In many studies highest contaminant concentrations were recorded in the Soricidae, but only one or two

Table 12. Relationship of diet to biomonitoring suitability of small mammals collected at contaminated sites.

Contaminant/Assay	Trophic Level		
	Insectivorous	Omnivorous	Herbivorous
Heavy Metals/ Residue analysis			
Soil adsorbed	++	+	-
Aerosol deposits	++	+	+
Organic Compounds/ Residue analysis			
	++	+	+
Complex Mixtures/ Cytogenetic assays			
		+	

Key: ++ = excellent, + = good, - = unsatisfactory.

specimens were trapped, making statistical analysis impossible. Also, the Cricetidae and Muridae species can be raised and tested in the laboratory, while the carnivorous shrews are more difficult to maintain.

Although not noted in the tables, there was often an extremely high variability of endpoints (high standard deviations of the mean) stemming from uneven distribution of the contaminant or a small area of contamination with varying home range overlap of the contaminated area. To some degree, age, sex, and season of the year may play a part in bioaccumulation. In the few cases where these parameters were considered, results were usually equivocal. At mine sites and other sites where a mixture of metals were present, antagonistic action between the metals may take place. An antagonistic action between cadmium and zinc

has been suggested, but such actions are not well documented (Hammond and Beliles 1980).

Not all species are good sentinels. Food habits and habitat of each species to a large degree determine contact with the contaminant. The nonbiological matrix in which the contaminant is present - air, water, or soil - as well as the contaminants's presence in biota that are links in food chains - vegetation and invertebrates - are important considerations for determining the most appropriate species.

Advantages of biomonitoring

The most important advantage of biomonitoring is that critical information can be obtained on the sources, bioavailability, and impact on biota of chemical contaminants. Furthermore, biomonitoring can be more cost-effective than analysis of a large number of soil, water, and vegetation samples. Uncontaminated sites and sites with the potential for contamination can be routinely monitored for evidence of contaminant migration. Finally, biomonitoring can help establish priorities for site cleanup.

For organic compounds, including undefined, complex mixtures, life history studies and cytogenetic assays using an abundant, widely distributed, opportunistic feeder such as the genus Peromyscus appears to be a promising line of investigation for evaluation of exposure to hazardous wastes. The use of residue analyses are also of value, but they do not demonstrate the effect of the contaminant on the organism or population. Further investigation using cytogenetic assays as an indicator of hazard assessment is needed. Also the usefulness of

abundant, widely distributed herbivorous genera, such as Microtus, as monitors needs further investigation.

An asset to the use of insectivorous and herbivorous species instead of the carnivorous shrews is the ease with which they can be raised and investigated in the laboratory. In several cases laboratory colonies were used to confirm results of investigations in the field. Tice et al. (1987) compared cytogenetic endpoints among wild P. leucopus from reference and contaminated sites and laboratory colonies. More laboratory investigations to support effects observed in the field, such as the association between lead exposure and intranuclear inclusion bodies, would allow a better assessment of potential effects at a site, and would help to establish criteria for prioritization of hazardous sites for cleanup.

III. BIOMONITORING STUDIES AT ORNL

Introduction

As summarized in Chapter II, the data on biomonitoring of many contaminants such as heavy metals, radionuclides, and organic chemicals is sparse. The situation at ORNL provides a unique opportunity for such studies in that several sites on or near the ORNL reservation are contaminated with the three types of contaminants considered. The area also has a diverse rodent fauna that can be used to investigate comparative uptake and accumulation of these contaminants.

Organic chemicals, including polycyclic aromatic hydrocarbons (PAHs), released to the environment are of concern because many of them, such as benzo[a]pyrene (BaP), are known carcinogens. Suess (1976), using data from 1966-1969, estimated the yearly emissions of BaP in the United States to be 1300 tons, the greatest contribution coming from coal combustion. BaP levels tend to be higher in urban than suburban areas. A review of the literature on benzo[a]pyrene concentrations in the environment reveals that little research on its fate in the terrestrial environment has been done (Edwards 1983).

Although PAHs such as BaP are both lipophilic and persistent, they generally do not accumulate to a large extent in mammals. BaP is rapidly metabolized to more than twenty metabolites in mammalian systems. Some BaP metabolites have been shown to covalently bind to cellular macromolecules including DNA and protein (Koreeda et al. 1978, Gelboin and T'so 1978) and as such are a potential indicator of PAH exposure. These binding properties also provide a convenient approach

to isolation and measurement of the metabolites (Rahn et al. 1982). Of the BaP metabolites formed, two isomeric diol epoxides are very active in binding to DNA and hemoglobin. Upon acid hydrolysis of tissues, the diol epoxide adducts are released as tetrols. These tetrols can be separated by high performance liquid chromatography (HPLC) and detected by fluorescence analysis. There is a dose-response relationship between the amount of BaP applied to the skin of mice and the occurrence, 24 hours later, of BaP adducts to DNA and hemoglobin (Shugart 1985).

Unfortunately, results of laboratory studies in which large doses of a chemical are administered over a short period of time are of questionable value for extrapolation to the long-term low-level exposure that is likely to occur in the environment. There are no published investigations of the titers of BaP adducts in small mammals ingesting BaP as part of their diet in their natural habitat. Application of the above method to small mammals collected in the field would allow such measurements to be made.

Elevated levels of mercury in the environment occur as a direct result of human activity. The major sources of mercury releases to the environment are combustion of fossil fuels; mining and reprocessing of gold, copper, and lead; operation of chlor-alkali plants; and disposal of batteries and fluorescent lamps (National Research Council 1978, Eisler 1987). As a result of these localized environmental increases, elevated concentrations of mercury (>3.9 ug/g) are present in biological samples. As discussed in Chapter II, elevated mercury concentrations have been documented in small mammals in the vicinity of a chlor-

alkali plant (Bull et al. 1977), but concentrations in all tissues were below the 3.9 ug/g that Eisler (1987) considered evidence of contamination.

Mercury has no known biological function and its presence in living organisms is potentially hazardous. The toxic effects of mercury, especially methyl mercury, are well documented (Hammond and Beliles 1980). Except for seeds treated with alkyl mercury fungicides, most mercury released to the environment is in the inorganic form. While inorganic mercury poisoning is of less concern than methyl mercury poisoning, elemental or inorganic mercury released to the environment can be biomethylated, especially under anaerobic conditions.

The primary effect of exposure to inorganic mercury is on the central nervous system where it can produce neuropsychiatric symptoms such as tremors (Hammond and Beliles 1980). The kidney is the primary target organ for inorganic mercury (Hg^{2+}) where it probably causes tubular obstruction.

Mercury can bioconcentrate in organisms and biomagnify through food chains (Eisler 1987). Concentrations in animal tissue from uncontaminated sites average <0.07 ug/g (National Research Council 1978). As noted in Chapter II, there are only a few studies available on mercury concentrations in tissues of small mammals. Most of these studies report concentrations from only one location and only occasionally report concentrations in the soil or vegetation where the animals were captured. Little or no mention is given to the effects of mercury on wild mammals (Wren 1986a).

Over the past three decades, a large amount of radioactive waste material has been generated from nuclear research and technology. Appropriate storage of this material is of present and future concern. Improper storage or burial of such waste, particularly if it comes into contact with fluctuating water tables or migrates into nearby surface waters, may result in widespread environmental contamination. Small mammals may come into contact with contamination through surface contact with soil and water and through their burrowing activity. They may also serve as a means of radionuclide transport from the disposal area, both through emigration and as prey for larger animals.

Strontium-90 is a beta-emitting fission product of nuclear weapon detonations and is produced in the fuel cycle of nuclear power reactors. Because of its presence in fallout from nuclear weapons testing and its relatively long half-life (28 years), its toxicity has been extensively studied in the laboratory. Strontium, as a metabolic analog of calcium, is readily absorbed from the gastrointestinal tract and lungs, into the bloodstream and deposited in bone. Chronic ingestion results in leukemia and bone tumors in experimental animals (Hobbs and McClellan 1980). Strontium is also taken up by vegetation. Little information on the uptake and effects of strontium-90 on wildlife populations is available.

Extensive aquatic and groundwater monitoring studies have been undertaken on the ORNL reservation to determine the extent and impact of existing contamination (Boyle et al. 1982). Contaminants in sediments in the vicinity of the Department of Energy's Y-12 plant in Oak Ridge have also been characterized (Hoffman et al. 1984). These

studies provided information for selection of contaminated and reference sites. From these preliminary studies, mercury, strontium-90, and several organics including BaP were shown to be present in the soil at several sites; however, no terrestrial biomonitoring studies have been performed in recent years at these sites.

The primary objectives of this part of the study were to determine if, through tissue and residue analyses, resident small mammal populations could serve as indicators of the presence of environmental contaminants at ORNL field sites and to determine which species are the best sentinel species for specific contaminants. This investigation focused on three areas: (1) the bioavailability of the contaminants as evidenced by tissue residues and biochemical markers; (2) the evaluation of each species as a sentinel of exposure, and (3) the relationship between habitat and food habits and the consequences for biomonitoring studies. The conclusions reached in Chapter II, that highest concentrations of contaminants are found in insectivores, that omnivores are good sentinels under most conditions, and that herbivores can serve as sentinels under specialized conditions will be tested. To aid in the accomplishment of these objectives, an effort was made to document or determine experimentally the presence of these contaminants in soil and vegetation at the sites.

Another goal of the study was to assess the utility of measuring concentrations of BaP adducts bound to hemoglobin as a tool for monitoring the exposure of wildlife populations to mutagenic or carcinogenic compounds that are persistent in the environment but do not accumulate in animal tissues. Feeding studies with BaP were

conducted in the laboratory because information on the accumulation of this contaminant during subchronic exposure was not available.

Materials and Methods

All reagents were HPLC grade and were purchased from Burdick & Jackson, Muskegon, Michigan, except where otherwise noted. BaP (Gold Label, 99.9% pure) was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, and 7,10-¹⁴C BaP (29.7 mCi/mmol) was obtained from Amersham Corporation, Arlington Heights, Illinois. Both of these chemicals were used in the analysis of soil for BaP. For the subchronic feeding study with mice, purified BaP tetrols were obtained from Dr. Lee Shugart (Biology Division, Oak Ridge National Laboratory). They were prepared by the hydrolysis of the corresponding BaP diol-epoxide isomers followed by HPLC separation (Shugart et al. 1985). Female C3H mice were obtained from the colony maintained by the Biology Division at ORNL. The mice were approximately 12 weeks old and were housed under climate-controlled conditions (20°C) with free access to water and Purina 5010-C chow. Glass-distilled water was used for all analytical procedures, except in the HPLC system, where HPLC grade water (Burdick & Jackson) was used.

Laboratory Study

Groups of 20 female C3H mice, 10 per cage, were administered doses of 2, 10, or 50 ug of BaP dissolved in corn oil by intragastric intubation, twice weekly, for 7.5 weeks. A group of five controls was administered the corn oil vehicle only. Following the third dose, three mice from the 50 ug dose group were anesthetized and sacrificed

each week by exsanguination (heart puncture) and their blood was analyzed for BaP metabolites. After the fifth dose (2.5 weeks), three mice from the 10 ug dose group and after the ninth dose (4.5 weeks), four mice from the 2 ug dose group were killed and analyzed weekly for BaP-hemoglobin adducts using HPLC separation and fluorescence detection.

Description of Study Sites

East Fork Poplar Creek.

East Fork Poplar Creek (EFPC) originates at the Y-12 plant, one of three facilities in Oak Ridge managed by Martin Marietta Energy Systems, Inc., for the U. S. Department of Energy. This creek is the receiving stream for industrial effluent from the Y-12 plant and may also receive wastes as it flows through a commercial area of Oak Ridge. The creek is 23.7 km in length from New Hope Pond, a waste settling pond at the east end of the Y-12 plant, to its confluence with Poplar Creek. For much of its length, EFPC flows through the City of Oak Ridge. Near kilometer 17, about four kilometers from the Y-12 plant, the floodplain is low and the creek periodically overflows, depositing sediment. The floodplain at this point contains abundant vegetation including sneezeweed (Helenium autumnale), jewelweed (Impatiens capensis), and grasses (Sorghum halepense); there is a boxelder (Acer negundo) canopy and an old field adjacent to the creek. Animals were trapped at this site during 1986 and 1987.

From 1950 to 1963, mercury used in a lithium separation process was released from the Y-12 plant into the creek. Approximately 1,080 metric tons (1,000,000 kg) could not be accounted for at the plant and

may have been released into the creek (Bashor and Turri 1986). It was recently estimated that 80,000 kg may be present in the floodplain (Turner 1987). Analyses of the soil by researchers at Oak Ridge Associated Universities indicated that most of the mercury in floodplain sediments is present as inorganic salts (Bashor and Turri 1986). Soil concentrations of up to 2,000 ug/g have been measured on the site (Gist 1986).

Benzo[a]pyrene and other organic contaminants are also present in the floodplain (Hoffman et al. 1984). The source of BaP is uncertain--but it might have been released as runoff from coal piles or ash from the Y-12 steam plant at the same time as the mercury spills (Munger 1988). Concentrations of BaP are higher in the floodplain adjacent to the Y-12 plant than at downstream sites. Animals trapped during 1985 and 1986 were tested for BaP metabolites; animals trapped during 1986 and 1987 were analyzed for mercury residues. Radionuclides are present at extremely low levels and, according to Hoffman et al. (1984), can be excluded from consideration as important contaminants. Thus, EFPC served as a negative reference site for radionuclides in small mammals and a positive reference site for mercury and BaP.

White Oak Lake

White Oak Lake (WOL) is used as a settling lake for radionuclides and other contaminants in effluents from ORNL. Small mammals were trapped under a power line on a ridge above the lake. The right of way contains dense grass (primarily Festuca sp.) and is bordered by shrubs and deciduous trees. This site became a reference area for the EFPC

BaP studies once it became evident that only background concentrations of BaP were present. Trapping took place from June to August 1986.

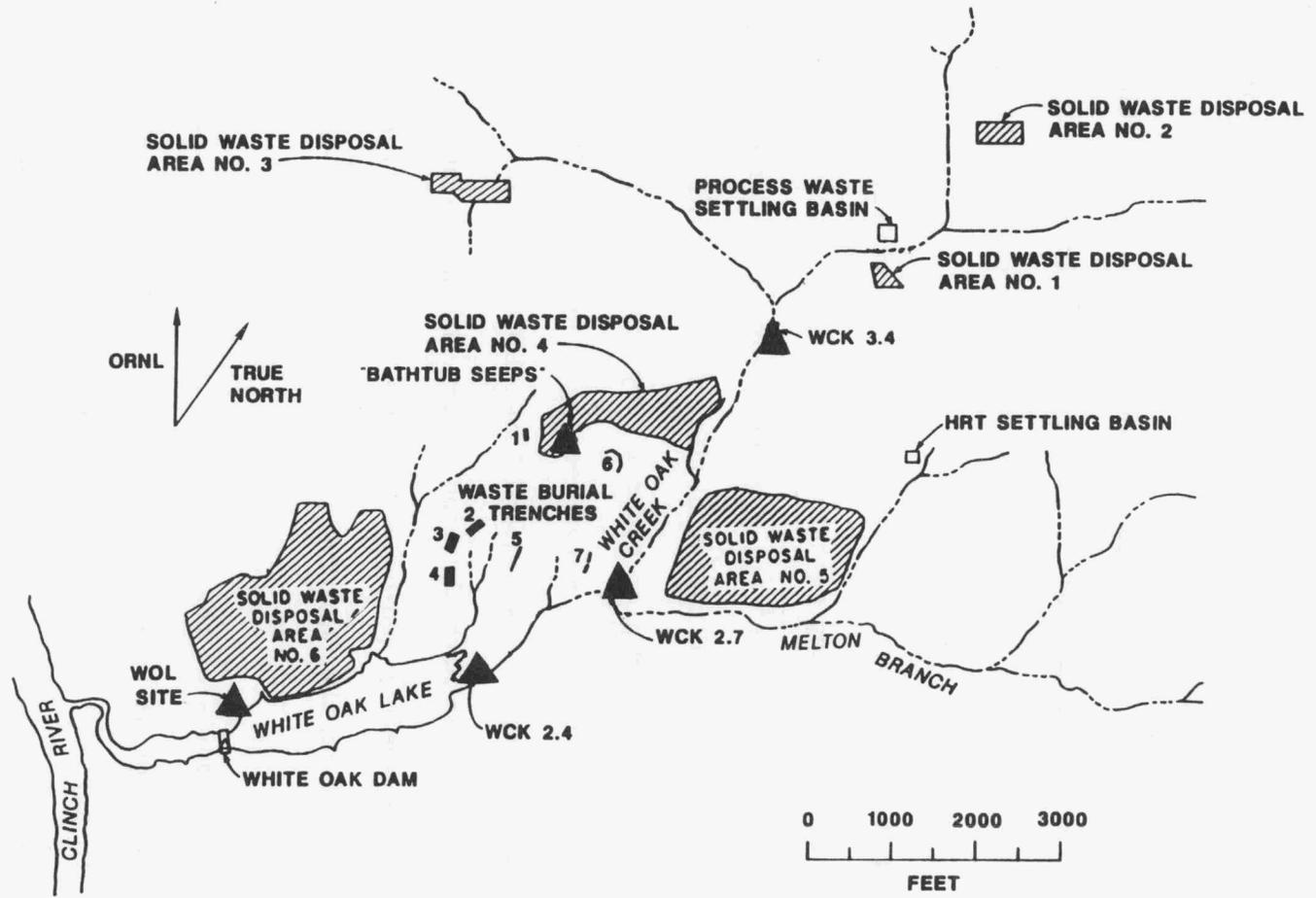
White Oak Creek

White Oak Creek (WOC) flows through the ORNL complex into WOL before entering the Clinch River. Discharges from a number of settling ponds contaminated with radionuclides (strontium-90, cesium-137, and cobalt-60) enter the stream at several locations. The stream flows near several solid waste storage areas (SWSAs) and trenches (Figure 1), where contaminated surface and ground water from these areas can enter the stream. Mercury is also present in the creek and its floodplain.

Two weirs are present on WOC downstream of the main plant and serve as convenient references for designating locations of sampling sites. The areas above the weirs are characterized by low lying flat fields covered with grassy vegetation. At the point where WOC enters WOL, the vegetation consists of shrubs with a canopy of boxelder and willow (Salix sp.). Animals were trapped at kilometer 2.1 (WCK 2.1), the point where WOC enters WOL; above Weir 2 (kilometer 2.7 [WCK 2.7]); and above Weir 1 (kilometer 3.4 [WCK 3.4]) during the spring and summer of 1987. The WCK sites contain both mercury and strontium-90.

Solid Waste Storage Area-4

Solid Waste Storage Area-4 (SWSA-4) comprises an area of approximately 10 ha on the Oak Ridge Reservation. Between 1951 and 1959 SWSA-4 was used as a low-level radioactive waste disposal site for wastes generated both on- and off-site (Melroy et al. 1986). Trenches



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Figure 1. White Oak Creek floodplain showing the solid waste disposal areas and small mammal trapping sites. Trapping locations are designated by solid triangles.

and auger holes were used for disposal of the wastes. Alpha-emitting wastes were covered with concrete, and the beta- and gamma-emitting wastes were covered with a natural soil cover (Lomenick and Cowser 1961). The burial ground was covered with uncontaminated fill and closed in 1959. It is now maintained as a grassy field. A bank covered with brush and trees separates SWSA-4 from a small tributary of WOC.

During periods of rain or high water table, the trenches at SWSA-4 fill up and overflow, producing surface contamination. This phenomenon is referred to as the bathtub effect and the seeps are referred to as "bathtub seeps." According to recent studies (Melroy et al. 1986), up to 150 Bq of strontium-90 per gram of soil (dry wt) is present at the surface. Ground level GM survey meter readings range up to 25,000 counts per minute (Figure 2) (Garten and Lomax 1987). Animals were trapped in the area of the bathtub seeps during the spring and summer of 1987 to determine whether radionuclides were taken up by resident fauna. SWSA-4 served as the negative reference area for the mercury study.

Contaminants in soils at the study sites are summarized in Table 13. Based on the study of Hoffman et al. (1984), EFPC was considered contaminated with BaP (+). Background concentrations (-) of BaP are ≤ 10 ng/g (Edwards et al. 1983). Based on the previous discussions, background concentrations of mercury (-) are < 1.0 ug/g, contaminated (+) refers to 1 to 20 ug/g, and highly contaminated (++) refers to > 20 ug/g (up to 2,000 ug/g at EFPC according to Gist 1986). Background concentrations of strontium-90 (-) are usually undetectable to very low

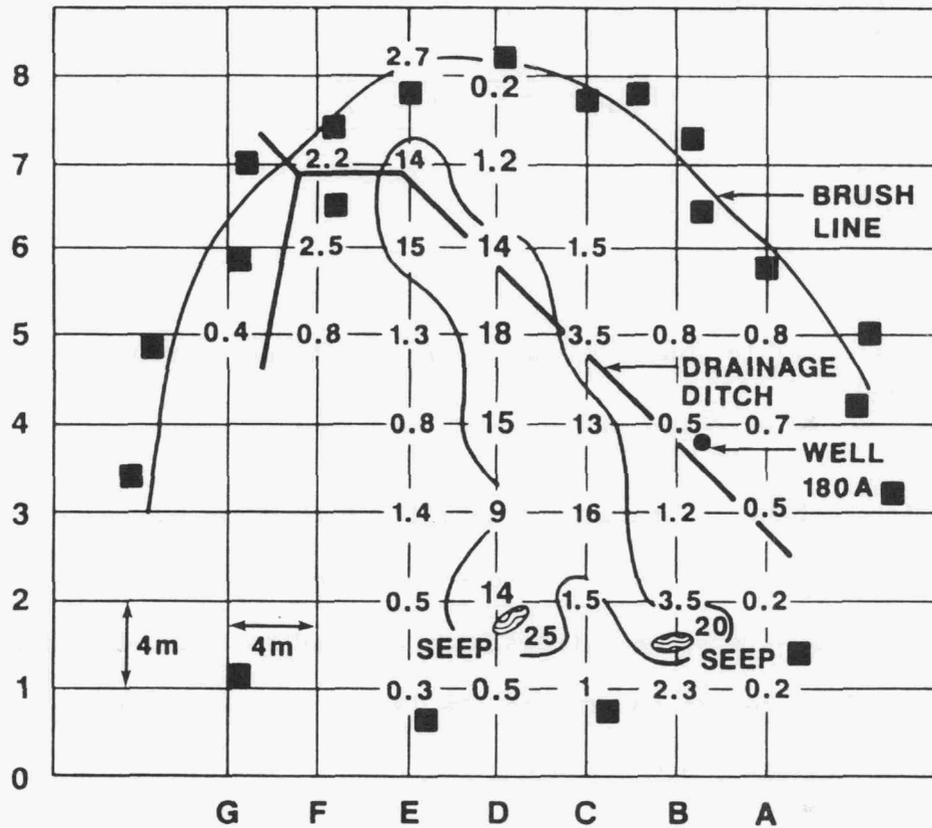


Figure 2. Solid Waste Storage Area-4 showing ground-level gamma readings, the seeps, and small mammal trapping sites. Gamma readings are in thousands of counts/minute. Traps are designated by solid squares.

(<0.008 Bq/g at EFPC according to Hoffman et al. 1984); highly contaminated sites such as SWSA-4 (++) contain up to 150 Bq/g of soil (Melroy et al. 1986). Contaminated sites (+) such as the WCK floodplain contain 2 to 11 Bq/g (Garten and Lomax 1987).

Collection of Study Animals

Small mammals were trapped using 22.9 x 7.6 x 7.6 cm Sherman live traps baited with sunflower seeds and/or fish. Larger mammals were trapped with 66 x 22.9 x 22.9 cm Tomahawk live traps placed along

Table 13. Contaminants present at the East Fork Poplar Creek (EFPC), White Oak Lake (WOL), White Oak Creek (WOC), and Solid Waste Disposal Area-4 (SWSA-4) study sites.

Site	Benzo[a]pyrene	Mercury	Strontium-90
EFPC	+	++	-
WOL	-	-	-
WOC	-	+	+
SWSA-4	+	-	++

Key: ++ (highly contaminated), + (contaminant present), - (background)

stream and lake banks. Traps were labelled with codes for each study site. Apples were found to be the best bait for larger animals such as groundhogs and muskrats. All traps were checked each morning and trapped animals were transported to the laboratory for weighing, species determination, and blood collection.

Collection of Soil Samples

Surface soil samples (0-3 cm) were collected at trap locations at all sites. At EFPC samples were also taken with a soil corer at depths up to 40 cm. Deposits of a grey to black substance, which probably included mercury salts and carbon-rich material, were visible in the cores at a depth of 20-40 cm. Sampling locations were noted by trap location or by coordinates with respect to a permanent marker (tree or grid stake) at the site. Soil was analyzed for mercury and BaP.

Analytical Methods

Benzo[a]pyrene

Soil samples were collected at the selected sites for analysis for BaP. Samples were air dried to a constant weight and sieved (2-mm

mesh) before extraction. In preliminary studies, BaP and other organics in soil and vegetation from the EFPC site were determined by the Analytical Chemistry Division at ORNL. Subsequent extractions and analyses were conducted in our laboratory using the procedures below.

BaP was separated from soil by soxhlet extraction according to EPA Method 3540 (U. S. Environmental Protection Agency 1982). All extractions were performed in duplicate. Five-gram soil samples were mixed with five grams of anhydrous sodium sulfate (to absorb water) and five grams of Ottawa sand (to promote solvent percolation), placed in a glass extraction thimble, and extracted using 150 ml of solvent placed in a 250-mL round-bottomed flask (Kimax). Preliminary analyses using 100% cyclohexane, acetone:hexane (1:1), toluene: methanol (10:1), or 100% methylene chloride as solvents showed that methylene chloride was a satisfactory solvent. Extractions were carried out for 18 hours at a temperature appropriate for the solvent(s) used. Solvent volumes were reduced to 1 mL on a Buchi Rotovapor-R rotoevaporator.

Because colored contaminants were present in the extract, cleanup steps developed by Edwards (1985) were employed to clarify the samples. The 1 mL of analyte was added to 30 mL of cyclohexane which was pipetted into a separatory funnel containing 60 mL of dimethyl sulfoxide (DMSO). BaP was extracted into the DMSO by gently inverting the funnel several times. Since the reaction is exothermic, pressure was allowed to escape periodically. The DMSO, present as the lower layer, was drained into a clean separatory funnel to which 120 mL of cyclohexane and 120 mL of water were added. Most of the interfering colored contaminants remained in the original cyclohexane. Addition of water

to the DMSO allows re-extraction of the BaP into the cyclohexane. BaP was re-extracted into the cyclohexane by shaking. The DMSO-water layer was drained off and discarded. The cyclohexane was then washed twice with 50 mL of water, placed in a flask, dried with sodium sulfate, poured into a round-bottomed flask, and reduced in volume to 3 mL using the rotoevaporator. The concentrate was filtered through silica SepPak columns (Waters Silica Sep-Paks) and dried under a stream of nitrogen. The samples were dissolved in 1 ml of 100% methanol and filtered through a Cameo nylon filter into 1-mL brown bottles (Shamrock Glass Co.) for storage and analysis.

Twenty-uL samples were injected into a HPLC system (Perkin-Elmer) equipped with a fluorescent detector (Perkin-Elmer LS Series 4) and a Hewlett-Packard Integrator. Samples were eluted with 100% methanol; detector settings were excitation, 355 nm and emission, 410 nm. BaP was quantified using peak heights of standard solutions.

In order to determine the percent recovery of BaP using the described method, soil samples were spiked with ^{14}C -BaP and extracted. Samples were counted on a Tricarb 2000 CA Liquid Scintillation Counter. Because recoveries were consistent for soil samples from a specific site, but varied among soil types, the organic carbon content of the soils was determined as a critical variable that may influence the adsorption of organic compounds by sediments and soils (Means et al. 1980). Total carbon, which closely approximates the organic carbon content of soil, was measured on a LECO WR 12 Carbon Determinator. No clear cut relationship was found between organic carbon content of soil and percent BaP recovery (Stout 1988). Since BaP recoveries were

consistent for soil from each site, but differed among sites, an internal ^{14}C -BaP spike was used for each soil analysis and corrections were made for percent recovery. Analyses were done in duplicate using methylene chloride as the solvent.

Benzo[a]pyrene metabolites

BaP metabolites were separated from hemoglobin and quantified according to a modification of the method of Shugart (1985). Small mammals were anesthetized with Metofane (methoxyflurane) in a closed jar. The thoracic cavity was opened and a sample of blood was taken by heart puncture using a 1-cc syringe treated with an anticoagulant (sodium heparin). Blood was taken from the tails of larger animals such as muskrats by venous puncture. The blood sample was placed in a heparinized plastic microfuge tube and centrifuged (Eppendorf Microcentrifuge) at 11,000 RPM for two minutes to isolate red blood cells (RBCs). The plasma was discarded and the cells were rinsed twice, by gentle inversion, with 2 mL of sterile saline (0.9% sodium chloride). The samples were centrifuged again and the saline was discarded. The RBCs were pipetted with water into a 15-mL conical glass centrifuge tube (total liquid volume of 4 mL) and lysed with 0.2 mL of carbon tetrachloride. The cells were vortexed intermittently over a 30-minute period to ensure lysis. The samples were then centrifuged at 20,000 RPM for 20 minutes (4°C) in 15-mL plastic centrifuge tubes to separate hemoglobin from the cell debris (Beckman TJ-6R). Three mL of the hemoglobin was then transferred to a 10-mL vial and a drop of the hemoglobin was set aside for later hemoglobin analysis. Two mL of water were added to the sample in the 10-mL vial, followed by 50 μL of

concentrated HCl (the solution darkens at this point). The samples were heated in an oven at 80°C for four hours. The acid-induced release of the diol-epoxide metabolites of BaP, covalently bound to hemoglobin as adducts, results in tetrol products.

The tetrols were separated from the hemoglobin by extraction through a series of three columns: a SepPak C₁₈ cartridge (Waters Associates) attached to a 5-cc syringe, a Bond Elute PH cartridge (Analytichem International) with a 3-cc piggyback syringe, and a column prepared from diethylaminoether cellulose (DEAE) (Whatman DE 32) in an empty Bond Elute cartridge. The DEAE was suspended in sterile water and added to the cartridge to a height of 2.5 cm. Bubbles were removed by agitation. Liquid was forced through the latter cartridge by attachment to a Micro-Vac vacuum apparatus. All columns were prewashed with 2 mL of 100% methanol, 2 mL of 20% methanol, and 2 mL of water before use. The water-suspended tetrols were added to the syringes or cartridges, the water removed by vacuum, and the cartridges were washed with two 2-mL water rinses and two 2 mL 20% methanol rinses. The tetrols were removed from the columns with two 2 mL 100% methanol washes which were collected in test tubes. The tetrols were taken to dryness under a gentle stream of nitrogen and then resuspended in 2-mL water for placement on the Bond Elut and DEAE columns. After removal from the DEAE column and drying, the tetrols were dissolved in 1 mL of methanol, filtered through a Cameo nylon filter (attached to a 1-cc glass syringe) into 1-mL brown glass bottles, which were capped with teflon lined caps.

For field-collected animals, the tetrols were separated and quantified on the same HPLC system as used for BaP in soil. A 20-uL sample was injected into a HPLC-equipped fluorescence detector. The metabolites were separated by an isocratic reversed-phase technique using a Vydac column and methanol:water (50:50) as the eluent. Detector settings were excitation, 246 nm and emission 370 nm. In the laboratory study, a DuPont 850 Liquid Chromatograph coupled with a Schoeffel FS 950 fluorometer was used. The column was an ODS 10u Zorbex. Two metabolites of BaP, the tetrols (\pm)-7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydro-benzo[a]pyrene, referred to as tetrols I-1 and II-2, are detected and serve as indicators of BaP exposure in the animals. A Hewlett-Packard Integrator was used for the graphical display. Standard solutions of tetrols were prepared and run on the system to determine retention times. Pooled blood from unexposed animals was spiked with a known amount of tetrols and taken through the process to determine percent recovery. The lower level of detection was 10 pg.

BaP tetrol values were calculated using peak heights of known standard solutions run at the same time. Values were normalized to hemoglobin content of the sample. Hemoglobin samples were prepared by a modification of the method of Sigma Chemical Company (1982). Sample absorbencies were read at 540 nm on a Beckman Spectrophotometer Acta C111.

Mercury

Air-dried soil samples and fresh kidneys from small mammals were analyzed for total mercury by the ORNL Analytical Chemistry Division.

Samples were wet digested in nitric and perchloric acids and detected as a cold vapor by atomic absorption spectroscopy.

Because the levels of mercury in tissue were unknown before analysis and because data from literature searches indicated they might be very low, kidney samples from several animals of the same species collected at EFPC (the positive reference site for Hg) were pooled to make a sample weight of one gram. When analysis indicated that mercury levels were extremely high compared to the level of detection (40 ng/g), individual kidneys were submitted for each animal. In addition, kidneys from laboratory-reared (control) mice were analyzed to establish background for uncontaminated animals. The raw data were converted to natural logarithm values and differences between reference and contaminated site means were tested using the Student's t-test.

Strontium-90

Strontium-90 concentrations in bone were obtained by detecting the Cerenkov effect from high energy beta particles produced by yttrium-90, a daughter product of strontium-90 (Haberer 1965, Larsen 1981).

Hindlimbs and crania of sacrificed animals were cleaned of muscle tissue, dried overnight at 100°C, and ashed at 900°C. Weights of wet, dried, and ashed samples were recorded. The bone samples were ground, successively digested in 4M hydrochloric and 8N nitric acid, dried, cleared with 30% hydrogen peroxide, and then dissolved in plastic vials containing 20 ml of water and a few drops of hydrochloric acid. The vials were counted for Cerenkov radiation in a Packard Tri-Carb Scintillation Spectrometer (Model 3002). The samples were corrected for a counting efficiency of 55%. The limit of detection was 0.33 Bq.

Bones from laboratory-reared mice were used as control samples; in addition, blank samples were taken through the procedure as process controls. Counts were transformed by taking square roots before parametric statistical analysis methods were applied. Differences between means were tested using the Student's t-test.

Results

Laboratory Study

Only the anti-(+)-benzo[a]pyrene metabolite (tetrol I-1) was found in blood samples of mice treated subchronically with BaP, as indicated by the single peak present at the same retention time (18 minutes) as the tetrol I-1 standard (Figure 3). A peak corresponding to Tetrol II-2 (retention time 35 minutes) was not present. In the high and middle dose groups (50 and 10 ug/treatment), the amount of BaP tetrol I-1 measured in the blood decreased over the experimental period. In the 50-ug dose group, concentrations of adduct fell from 7.23 pg/mg hemoglobin at week 2.5 to 0.50 pg/mg hemoglobin at week 7.5. The 2 and 10 ug dose groups reached equilibrium by the middle of the fifth week, at which time the concentrations were 0.25 pg/mg and 0.21 pg/mg hemoglobin, respectively (Table 14). Concentrations in corn oil intubated controls were below the limit of detection.

In addition to one accidental death, five of the 65 mice did not survive to the end of the experiment. Ten of the mice in the treated groups developed large abscesses in the area surrounding the lymph nodes of the forearms; these abscesses made walking difficult.

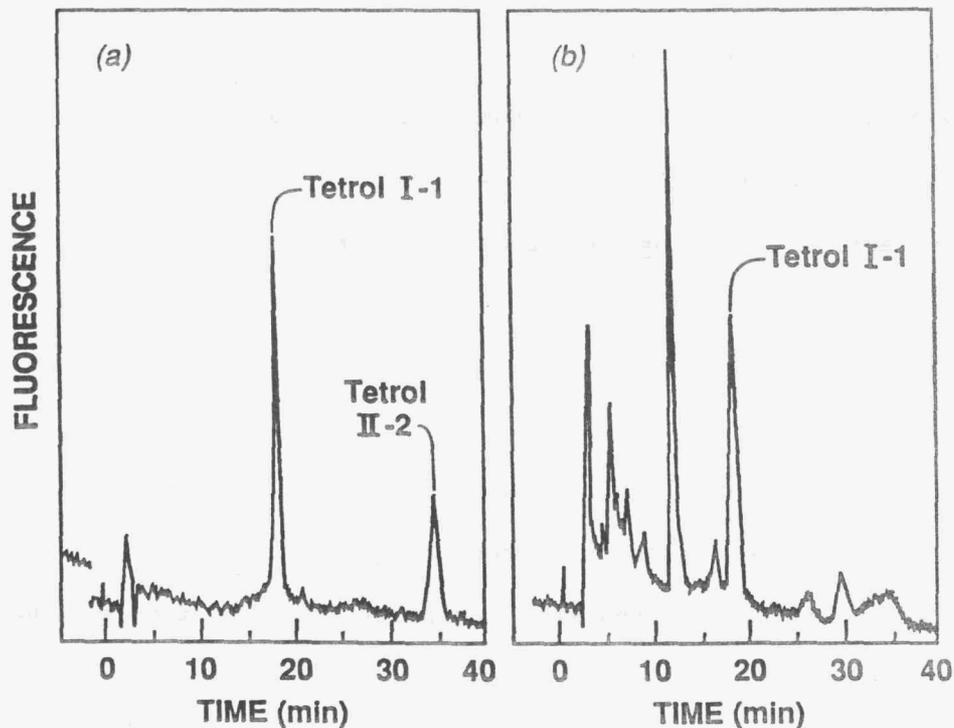


Figure 3. High performance liquid chromatography/fluorescence profiles of (a) standard solution of benzo[a]pyrene tetrols I-1 and II-2, and (b) tetrols hydrolyzed from the hemoglobin of a C3H mouse following four weeks of treatment with 50 ug of benzo[a]pyrene twice weekly.

Table 14. Weekly concentrations of benzo[a]pyrene tetrol I-1 metabolite in the blood of C3H mice fed three dose levels of benzo[a]pyrene twice weekly in the laboratory. Concentrations in pg/mg of hemoglobin.

Week	Administered Dose		
	2 ug	10 ug	50 ug
1.5	-	-	7.23
2.5	-	1.01	1.97
3.5	-	1.04	4.33
4.5	0.58	0.62	1.29
5.5	0.25	0.21	3.11
6.5	0.61	0.33	2.03
7.5	0.50	0.37	0.50

Field Studies

A total of 10 different species of mammals were captured along White Oak Creek, at the Solid Waste Disposal Area-4, above White Oak Lake, and along East Fork Poplar Creek (Table 15). The white-footed mouse, P. leucopus, was the most common species trapped and was present at all sites.

Description of Study Species

Peromyscus leucopus was the most common species collected in the vicinity of the ORNL reservation. It was found at all sites in habitats of deciduous forest, border areas, and old fields. At EFPC it was present under the boxelder canopy along the creek where vegetation was sparse in spring, but dense by late summer and fall. White-footed mice were not found in the grassy old-field, except at the bases of solitary trees. During a long dry period in summer, it and other species were trapped only close to the creek. At SWSA-4 it was not present in the mowed field, but was trapped along the brush line and in the deciduous forest. This was the only species trapped along the lower expanse of White Oak Creek. At WCK 2.1 the ground cover is similar to that of EFPC; the ground under the boxelder and sycamore canopy is damp, but not marshy. At WCK 2.7, P. leucopus was trapped in the grassy, dry floodplain adjacent to the creek and along the border of the forest, about 20 meters away. It was not common at the marshy WCK 3.4 area.

At ORNL, S. hispidus was most common at WCK 3.4 where the floodplain is low and swampy much of the year. It was also present at SWSA-4 in the fall when the grass was not mowed. Surprisingly, the

Table 15. Species and numbers of small mammals trapped at ORNL study sites during 1986-1988.

Site	Species	Common name	Number
East Fork Poplar Creek (EFPC)			
	<u>Peromyscus leucopus</u>	White-footed mouse	32
	<u>Blarina brevicauda</u>	Shorttail shrew	12
	<u>Sigmodon hispidus</u>	Cotton rat	1
	<u>Ondatra zibethica</u>	Muskrat	9
	<u>Rattus norvegicus</u>	Norway rat	2
White Oak Creek kilometer 2.1 (WCK 2.1)			
	<u>Peromyscus leucopus</u>	White-footed mouse	9
White Oak Creek kilometer 2.7 (WCK 2.7)			
	<u>Peromyscus leucopus</u>	White-footed mouse	5
White Oak Creek kilometer 3.4 (WCK 3.4)			
	<u>Peromyscus leucopus</u>	White-footed mouse	2
	<u>Sigmodon hispidus</u>	Cotton rat	4
	<u>Reithrodontomys humulis</u>	Eastern harvest mouse	1
White Oak Lake (WOL)			
	<u>Peromyscus leucopus</u>	White-footed mouse	13
	<u>Blarina brevicauda</u>	Shorttail shrew	4
	<u>Sigmodon hispidus</u>	Cotton rat	2
	<u>Microtus ochrogaster</u>	Prairie vole	1
	<u>Mus musculus</u>	House mouse	1
Solid Waste Disposal Area-4 (SWSA-4)			
	<u>Peromyscus leucopus</u>	White-footed mouse	16
	<u>Blarina brevicauda</u>	Shorttail shrew	3
	<u>Sigmodon hispidus</u>	Cotton rat	5
	<u>Reithrodontomys humulis</u>	Eastern harvest mouse	3
	<u>Microtus pinetorum</u>	Pine vole	3
	<u>Tamias striatus</u>	Eastern chipmunk	2
	<u>Mus musculus</u>	House mouse	1

cotton rat was not present at EFPC with the exception of one animal during three years of trapping. This may have been due to the dryness of the field during the summer trapping periods. No animal was trapped in the old field at a distance of greater than three meters from the boxelder stand.

In this study B. brevicauda was most common in areas with dense understory vegetation and fallen trees. At EFPC five were trapped within a few meters of a fallen log covered with honeysuckle. They were much less abundant than P. leucopus and had a greater tendency to die in the traps. Trapping success of B. brevicauda improved when sardines were added as bait to the traps.

At ORNL R. humulis was found only in grassy areas and was the only species present in the short grass surrounding the seeps at SWSA-4. Other species were caught in numbers too low to consider them as sentinel animals. Microtus pinetorum was the only true herbivore caught. Previous studies reported that pine voles were common on the Oak Ridge Reservation (Dunaway et al. 1971), but only three were trapped at SWSA-4. All three were trapped along the brush border between the grassy area and the deciduous forest. Muskrats, trapped only at EFPC, were tested for BaP metabolites but not for mercury or strontium. Muskrat dens were present (and trapping success was high) where three conditions existed: creeks banks were high; large, solitary trees having root systems that extended down the creek bank were present; and sunny, densely vegetated banks were located nearby. Muskrats slides were observed on the banks. Apples proved to be the best bait for muskrats. This species was not trap shy and the same

animal (identified by ear tag) was often trapped on consecutive nights.

Food habits of collected species range from primarily herbivorous (M. pinetorum) to primarily insectivorous (B. brevicauda). Other species such as P. leucopus are opportunistic feeders -- their diets are composed of seasonally abundant items. Food habits of the captured species are summarized in Table 16.

Table 16. Food habits of species trapped at the ORNL study sites.

Species	Herbivorous	Omnivorous	Insectivorous
<u>Microtus pinetorum</u>	X		
<u>Peromyscus leucopus</u>		X	
<u>Sigmodon hispidus</u>		X	
<u>Reithrodontomys humulis</u>		X	
<u>Blarina brevicauda</u>			X

Benzo[a]pyrene

Results of soil analyses show that BaP was present at all three sampling locations with the highest concentrations at EFPC (70 ng/g) and the lowest along WCK (5 ng/g) (Table 17). The chromatographic profile of soil from EFPC shows the complex nature of the chemicals present (Figure 4). Based on a retention time identical to that of the BaP standard in Figure 4(a), 4.00 minutes, the highest peak in Figure 4(b) was identified as BaP in the field sample. The other compounds in the profile have not been identified.

Only tetrol I-1 was detected in the blood of some animals collected at EFPC. This metabolite was present in muskrats, shrews, and a rat (Table 18). It was not present in 27 P. leucopus collected at this site or in any species collected at the other sites.

Table 17. Benzo[a]pyrene concentrations in soil at the East Fork Poplar Creek (EFPC), White Oak Creek (WCK 2.1), White Oak Lake (WOL), and Solid Waste Storage Area-4 (SWSA-4) sites.

Site	BaP in soil (ng/g)
EFPC	70
WCK 2.1	5
WOL	14
SWSA-4	35

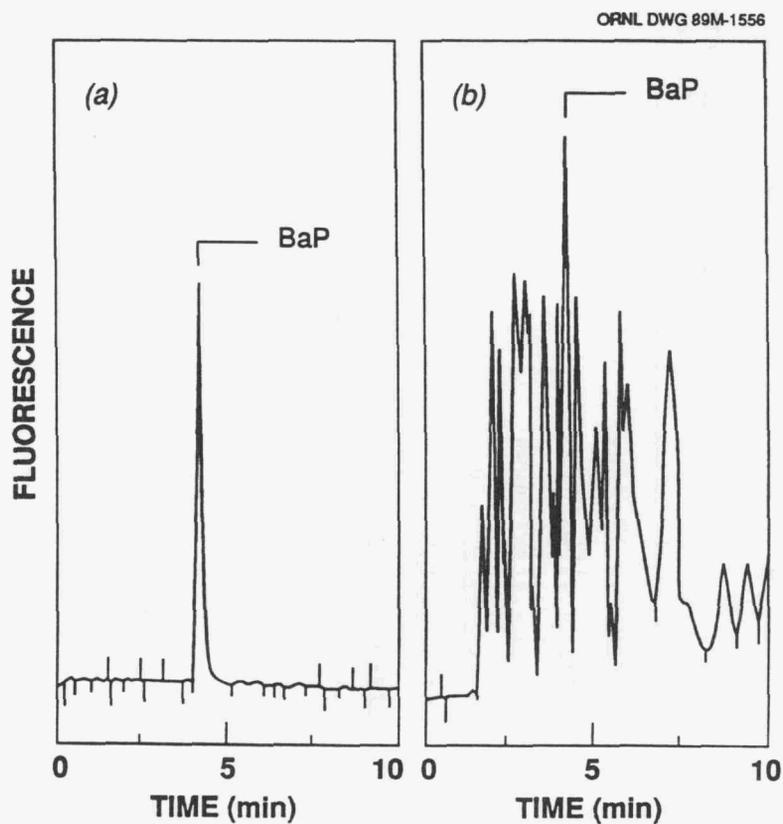


Figure 4. High performance liquid chromatography/fluorescence profiles of (a) standard solution of benzo[a]pyrene and (b) organic compounds extracted from the soil at East Fork Poplar Creek.

Table 18. Species of mammals trapped at East Fork Poplar Creek and White Oak Lake and tested for the presence of BaP metabolites. Metabolites are designated as present or not present. A dash indicates the species was not collected at that site.

Species	Number of Animals			
	<u>East Fork Poplar Creek</u>		<u>White Oak Lake</u>	
	Present	Not Present	Present	Not Present
<u>Peromyscus leucopus</u>	0	27	0	1
<u>Ondatra zibethica</u>	3	6	-	-
<u>Blarina brevicauda</u>	3	3	0	4
<u>Rattus norvegicus</u>	1	0	-	-
<u>Mus musculus</u>	0	1	0	1
<u>Sigmodon hispidus</u>	0	1	0	2

Chromatographic peaks for field-collected animals were not as clearly defined as those from laboratory-raised animals (Figure 5). As in the case of the laboratory-treated mice, only the Tetrol I-1 metabolite was present in the profile. Because of the complex mixture of chemicals found at EFPC, interfering chemicals may be present in the tetrol I-1 peaks. Therefore, concentrations found represent maximum values likely to be present and are reported here as either present or not present.

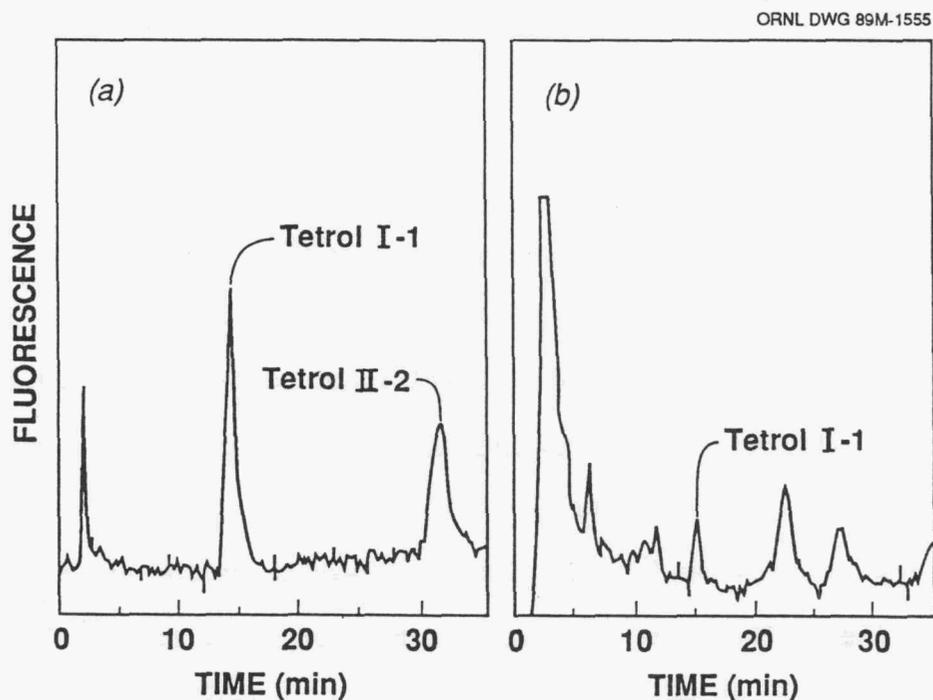


Figure 5. High performance liquid chromatography/fluorescence profiles of (a) standard solution of benzo[a]pyrene tetrols I-1 and II-2 and (b) tetrols hydrolyzed from the hemoglobin of *Blarina brevicauda* trapped at East Fork Poplar Creek.

Mercury

Mercury concentrations at EFPC averaged 348 ug/g soil in the top three cm (Table 19) and 1675 ug/g at a soil depth of 20-40 cm. Concentrations were lower along White Oak Creek; averages ranged from 0.8 ug/g at WCK 2.7 to 7.0 ug/g at WCK 3.4. Concentrations were lowest at the SWSA-4 reference site, 0.2 ug/g.

Residues of mercury were detected in kidneys of all mammals collected at the study sites (Table 19). Concentrations for the three species collected at reference sites were ≤ 3.2 ug/g, with highest concentrations in B. brevicauda. The highest mercury concentrations were found in mammals trapped at EFPC. Similarly, B. brevicauda averaged the highest Hg residues in kidney at 137 ug/g (range: 3.4 to 258 ug/g). Concentrations in P. leucopus, the only species trapped at all sites, and S. hispidus were also higher than those from WCK or SWSA-4, 4.2 ug/g and 6.7 ug/g, respectively, at EFPC vs ≤ 1.8 ug/g for both species at the other sites. Differences were significant between EFPC and the reference site (SWSA-4) for both B. brevicauda and P. leucopus. No other species were abundant enough at EFPC to make statistical comparisons. The standard deviation of the mean was large for all field-collected species. The concentration in laboratory mice fed regular laboratory chow was 0.02 ug/g (n = 8). Wet weight concentrations of mercury in kidney tissue for individual animals are listed in APPENDIX B. Concentrations for air-dried soil samples are listed in APPENDIX C.

Table 19. Mercury concentrations in soil and kidney tissue of small mammals collected on the East Fork Poplar Creek (EFPC) floodplain, White Oak Creek (WCK) floodplain, and Solid Waste Storage Area-4 (SWSA-4) site. A dash indicates the species was not collected at that site.

Site	Hg (ug\g dry weight)			Soil
	<u>Peromyscus</u> <u>leucopus</u>	<u>Blarina</u> <u>brevicauda</u>	<u>Sigmodon</u> <u>hispidus</u>	
EFPC	4.2 ± 4.2* (n=23)	137.0 ± 86.8** (n=8)	6.7 (n=1)	348 (n=2)
WCK 3.4	1.4 (n=2)	-	1.8 ± 1.1 (n=4)	7.0 (n=2)
WCK 2.7	1.4 ± 0.7 (n=5)	-	-	0.8 ± 0.5 (n=3)
WCK 2.1	0.7 ± 0.4 (n=9)	-	-	2.6 ± 0.8 (n=3)
SWSA-4	1.8 ± 1.4 (n=10)	3.2 ± 2.1 (n=3)	0.7 ± 0.4 (n=5)	0.2 ± 0.1 (n=6)

*Statistically significant difference compared to reference site (SWSA-4) at p<0.01.

**Statistically significant difference compared to reference site (SWSA-4) at p<0.005.

Strontium-90

All field-collected small mammals from sites contaminated with strontium-90 had detectable amounts of strontium-90 in bone tissue (Table 20). At SWSA-4, highest concentrations were found in R. humulis (mean: 71.5 Bq/g; range: 10.0 Bq/g to 281 Bq/g). This species was captured in the short grass immediately adjacent to the seeps. Concentrations were elevated for S. hispidus trapped at both SWSA-4 and WCK 3.4 (26.5 Bq/g and 20.7 Bq/g, respectively) and for B. brevicauda trapped at SWSA-4 (23.5 Bq/g); strontium-90 was present, but at low concentrations in P. leucopus and R. humulis trapped at WCK sites. Standard deviations of the mean values were large in all cases. Activities for animals trapped at EFPC averaged close to background. Activities in laboratory-reared mice averaged the same as background (distilled water) blanks and as blanks taken through the entire analytical procedure (12 counts/minute). Concentrations of strontium-90 in dried bone tissue of individual animals are listed in APPENDIX D.

Discussion

Small mammals were trapped at several contaminated sites to determine the practicality of biomonitoring at ORNL and to determine appropriate sentinel species for three specific contaminants. The types of species present, the number of individuals of a species, and evidence of uptake by one or more sentinel species trapped at the sites were considered important parameters of biomonitoring. This study documents species-specific uptake of three classes of contaminants by small mammals at chemically contaminated sites. It does not provide

Table 20. Strontium-90 concentrations in bone of small mammals, soil, and vegetation from the East Fork Poplar Creek (EFPC) floodplain, White Oak Creek (WCK) floodplain, and Solid Waste Storage Area-4 (SWSA-4) site. A dash indicates the species was not collected at that site.

Site	⁹⁰ Sr (Bq/g dry weight)				Soil	Vegetation
	<u>Peromyscus</u> <u>leucopus</u>	<u>Blarina</u> <u>brevicauda</u>	<u>Sigmodon</u> <u>hispidus</u>	<u>Reithrodontomys</u> <u>humulis</u>		
EFPC	<0.3 (n=20)	<0.4 (n=9)	0.7 (n=1)	-	<0.008 ^a	-
WCK 3.4	1.1 (n=2)	-	19.3 ± 17.3 (n=2)	0.9 (n=1)	-	-
WCK 2.7	1.6 ± 1.2	- (n=5)	-	-	-	-
WCK 2.1	1.6 ± 1.5	- (n=9)	-	-	-	-
SWSA-4	15.3 ± 26.7* (n=16)	23.5 ± 33.1** (n=3)	59.5 ± 41.6 (n=5)	71.5 ± 117 (n=5)	37-148 ^b	37 ^b

^aData from Hoffman et al., 1984

^bData from Garten and Lomax, 1987

*Significantly different from reference site (EFPC) at p<0.05.

**Significantly different from reference site (EFPC) at p<0.01.

evidence for the utility of using hemoglobin adducts as a measure of BaP exposure.

Benzo[a]pyrene

More than 50 different chemicals covalently bind to macromolecules in vivo (Calleman 1984, Farmer et al. 1987). These adducts are potential indicators of exposure to the chemicals. In mammalian systems, BaP is rapidly metabolized to more than twenty metabolites (Selkirk 1986). The diol-epoxide metabolites are highly reactive, attaching to macromolecules such as DNA and hemoglobin (Sims et al. 1974; Koreeda et al. 1978). Following administration of acute doses, the formation of adducts with DNA and hemoglobin is dose-dependent; the adduct with hemoglobin is stable and disappears at the same rate at which red blood cells are naturally destroyed (Shugart 1985). Chronic feeding of BaP might be expected to result in an increase of adducts over time until all available attachment sites on the macromolecules are filled (Osterman-Golker et al. 1976, Calleman 1984). In this study, however, the number of adducts as measured by picograms tetrol I-1/gram of hemoglobin decreased with time, reaching a plateau, in the case of the 2 and 10 ug treatments, by the middle of the fifth week.

The reason for the decrease of BaP adducts with time under this subchronic exposure is unknown, but is of considerable consequence for the use of adducts to monitor for BaP exposure of natural populations of small mammals. The mixed function oxidase system, which metabolizes many xenobiotic chemicals, is inducible in several tissues of C3H mice, including intestinal tissue (Nebert and Gelboin 1969, Dunn 1981, Griffin et al. 1986). Initially, concentrations of hemoglobin-DNA

adducts are high following exposure. But, following induction of the enzyme system, BaP is rapidly metabolized in the intestinal mucosa, resulting in formation of reactive metabolites that covalently bind to intestinal cell DNA. Only small quantities of the parent compound are released to the general circulation, resulting in a decrease of the hemoglobin adducts with time. If all mammalian species act similarly, then species at contaminated field sites and in equilibrium with the contaminant in their environment would have similar low, close to undetectable, levels of BaP tetrol I-1 adducts in their blood.

Although BaP is nearly ubiquitous in terrestrial systems, plant uptake is usually low. In a literature review by Edwards (1983) the concentration ratios for BaP in vegetation\BaP in soil were found to range from 0.0001 to 0.33. Preliminary studies for this report revealed that the concentrations of BaP in roots, stems, and leaves of vegetation collected at the EFPC site were an order of magnitude less than those in the soil in which the plants were growing (unpublished data).

The present study shows that BaP is present in the soil at all three study sites. The highest level was found at EFPC (70 ng/g), but this level was low compared to highly contaminated areas cited by Edwards (up to 191 ug/g). Because plant concentrations of BaP are at least an order of magnitude lower than soil concentrations, uptake from sediment or soil would be the most likely route of food chain transfer to small mammals. Results of blood analyses showed site and species differences in exposure to BaP. Low concentrations of a BaP metabolite were found in the blood of several species of mammals that have close

contact with the sediment or soil including B. brevicauda, which burrows into the ground and eats earthworms and insects contaminated with soil, and muskrats (O. zibethica), which feed primarily on sediment-contaminated vegetation along the creek bank and sometimes feed on invertebrates found on the stream bed. BaP adducts were not detectable in omnivores such as P. leucopus which ingest little soil.

A relationship between BaP in soil and tetrol metabolites in blood of mammals could not be established. Preliminary studies had shown that BaP was present in the soil at EFPC at concentrations up to 2.8 ug/g (Hoffman et al. 1984); however, the present study showed that BaP concentrations at EFPC during 1986 were considerably lower. Subchronic feeding studies with mice demonstrate that after a period of several weeks, BaP tetrol I-1 is present in blood at low concentrations compared to concentrations reported following acute exposures and compared to concentrations found in this study during the first three weeks after administration of BaP. The metabolite was present at similar low concentrations in several species having close contact with the soil at the contaminated site. Whether these low levels were due to the same mechanism(s) as in the laboratory study or whether BaP exposure to 70 ng/g soil in the field was too low to result in adduct buildup over time is unknown. Additional studies at sites contaminated with other genotoxic chemicals are needed in order to further clarify the validity of using hemoglobin adducts as bioindicators of contaminant exposure in the field.

Mercury

Mercury burdens in terrestrial mammals are related to diet and are lower in herbivores than in carnivores (National Research Council 1978). According to Wren (1986a) mercury concentrations are biomagnified within terrestrial food chains and highest levels of mercury (up to 40 ug/g wet weight of tissue) have been found in mammalian predators in areas where mercury-containing fungicides were widely used as seed disinfectants. Mercury does not appear to be concentrated in plants; levels are usually less than 0.5 ug/g fresh weight in reference areas and up to 3.5 ug/g over cinnebar deposits (Shacklette 1970).

Releases of mercury into EFPC have heavily contaminated the creek and its floodplain. Concentrations in the floodplain as high as 2,100 ug/g of soil have been measured (Gist 1985). Total mercury has also been measured in vegetables grown along EFPC. Concentrations ranged from 0.00058 ug/g to 0.31 ug/g with an average value of 0.05 ug/g (Bashor and Turri 1986).

Although mercury was not previously documented to be present in WOC, this study shows that it is present on the WOC floodplain. Mercury may have been released into WOC from the ORNL plant and/or from burial grounds used for nonradioactive waste (Boyle et al. 1982). Concentrations on the floodplain ranged from 0.8 ug/g dry weight of soil at a site where there is little flooding to 7.0 ug/g dry weight of soil at a site where the floodplain is low and wet.

The natural trace metal content of soils varies depending on the rocks from which the soil was formed and weathering conditions; for soils in general, the mercury content averages 0.3 ug/g (Lisk 1972).

The Hg concentration at SWSA-4, the contaminated reference site for mercury, averaged 0.2 ug/g dry weight.

In the present study, highest concentrations of mercury were found in B. brevicauda at EFPC. The shorttail shrew is primarily insectivorous with a diet of earthworms, insects, and occasionally mice and voles. Its position in the food chain and its food habits, particularly the eating of soil-containing earthworms, make it vulnerable to the accumulation of mercury. Peromyscus leucopus, a seed eater, and S. hispidus, an herbivore, had much lower burdens of mercury in kidney tissue. Mercury concentrations in kidney tissue of species collected along White Oak Creek (WCK) were not elevated above reference values, indicating no accumulation at soil concentrations of ≤ 7 ug/g of soil. No shrews were caught along this stream. Mercury concentrations in kidneys of all species trapped at the SWSA-4 reference site averaged less than the 3.9 ug/g indicative of environmental contamination according to Eisler (1987), but exceeded the 0.7 ug/g limit established for all tissues (National Research Council 1978). Mercury concentrations in kidney usually average higher than that of other tissues which may be the basis for the cited differences in Hg concentrations that are indicative of contaminated animals. The Hg concentration of 0.2 ug/g in kidney of mice raised in the laboratory did not vary and was lower than the published background value of 0.7 ug/g (National Research Council 1978). No information was located concerning toxic levels of inorganic mercury in tissues.

In the only other study that analyzed mercury in several environmental compartments, mercury was accumulated by two species of voles

(Beardsley et al. 1978). However, the mercury from the chlor-alkali plant was deposited on vegetation (4 ug/g), the primary food for voles. Concentrations in tissues were very low, 0.35 and 0.5 ug/g in kidney tissue, compared to concentrations of up to 281 ug/g in kidney tissue of animals from EFPC.

Strontium-90

The bathtub effect at the southwestern corner of SWSA-4 appears to have contributed to contamination of the area. Soil concentrations of strontium-90 ranged from 44 to 150 Bq/g dry weight (1,000 to 4,000 pCi/g) (Melroy et al. 1986) and fesque, the dominant vegetation at the site, averaged 37 Bq/g (1,000 pCi/g) dry weight (Garten and Lomax 1987). No previous studies reporting strontium-90 in soil and vegetation along WOC were found. Strontium-90 in floodplain soil at two sites along the reference area (EFPC), one above and one below the present trapping area, averaged less than 0.008 Bq/g of soil (Hoffman et al. 1984).

Small mammals accumulate strontium-90, a bone-seeking radionuclide, through dietary intake (Klusek 1987). At SWSA-4 highest levels of accumulation were present in bone tissue of R. humulis. Eastern harvest mice feed mainly on seeds, living and eating in the strontium-90 contaminated fesque grass; four of the five individuals were collected in the grass immediately adjacent to the seeps. Strontium-90 concentrations were high in some individuals of all species caught at this site, but there was great intraspecies variation, probably reflecting the degree of overlap of the home ranges of the animals with the small seep area, which is less than 200 m².

Peromyscus leucopus was the most common species caught along WOC, but Strontium-90 accumulation was low. Of the three S. hispidus which were caught within a few meters of each other adjacent to the creek (WCK 3.4), two showed bioaccumulation in bone (14.5 and 44 Bq/g), whereas the other had a much lower residue (3.6 Bq/g). The only P. leucopus caught at this site was trapped about 50 m from the creek and had a very low concentration (0.6 Bq/g). At EFPC, values for the six P. leucopus and the three shrews averaged the same as the background count (12 counts/minute). Arthur et al. (1987) studied uptake of radionuclides at a waste site, but studied only one species and only one tissue (lung) in addition to the carcass and pelt. Kaye and Dunaway (1962) trapped two species, but measured cesium-137, cobalt-60, and strontium-90 in carcass only.

Sentinel Species

The species of small mammals trapped in the ORNL area showed differences in their suitability as monitors of BaP, Hg, and Strontium-90 and these differences may be due to disparate food habits (Table 21). The white-footed mouse, P. leucopus, was the most abundant species at all sites, but was not the most suitable monitor of all three contaminants. Because of its opportunistic use of local habitats and its wide distribution, occurring from Canada south through the east and central U. S. to southern Mexico, it would make an ideal sentinel organism. Closely related species are found throughout the United States. McBee (1985) and Tice et al. (1987) have demonstrated the potential usefulness of this species as a monitor of genotoxic damage from environmental contaminants. They found evidence of alterations in

Table 21. Evaluation of three small mammal species as biomonitors of benzo[a]pyrene, mercury, and strontium-90 at the ORNL site.

Trophic Level/ Species	Relative Value as Sentinel Species ^a		
	BaP	Mercury	Strontium-90
Insectivore			
<u>Blarina brevicauda</u>	+/-	++	+
Omnivore			
<u>Peromyscus leucopus</u>	-	+	+
Herbivore/Omnivore			
<u>Sigmodon hispidus</u>	-	-	+

^aKey: ++ (excellent), + (good), - (unsatisfactory or inadequate data)

the frequency of micronucleated erythrocyte and proliferating cells and chromosomal aberrations in P. leucopus populations inhabiting hazardous waste sites containing complex mixtures of chemicals. Specific chemicals at the waste sites were not defined.

In the present study, however, P. leucopus was not a good monitor of the potentially genotoxic chemical, BaP, nor was it the best bioaccumulator of the heavy metal, mercury. BaP adducts to hemoglobin were not present in any of the 27 P. leucopus caught at EFPC or at the reference site. The mean mercury concentration in kidney tissue of animals from this site (4.2 ug/g) was above the 3.9 ug/g (1.1 ug/g fresh weight) level considered by Eisler (1987) as evidence of an environmental mercury problem; the difference was significant at the $P < 0.01$ level. However, 16 of the 27 P. leucopus from EFPC analyzed had mercury concentrations below 3.9 ug/g (APPENDIX B). Lack of uptake compared to Blarina brevicauda was probably due to the above ground habits of this species and the abundant vegetation (shown to be low in

mercury) which serves as the major food source. Minimal contact with the soil through habitat or eating habits restricts the intake of these soil-associated contaminants by P. leucopus. These data suggest that P. leucopus, which is primarily a granivore and lives above ground, may not be suitable for biomonitoring of polycyclic aromatic hydrocarbons and is a questionable monitor for mercury.

Peromyscus leucopus was a good monitor of strontium-90 contamination at all sites, showing a graded response to the range of soil contamination (Table 20). The mean concentration of strontium-90 was higher at the heavily contaminated SWSA-4 seepage area and lower at the less contaminated WCK sites. At SWSA-4 the range of values probably reflected home range overlap with the contaminated area. From this study, a concentration of 1.6 Bq/g of bone (dry weight) can be considered evidence of soil contamination.

Earlier studies (Dunaway and Kaye 1961, Kaye and Dunaway 1963) reported cobalt-60 and ruthenium-106 in small mammals captured on the drained WOL bed. Since that time, seepage of strontium-90 from burial sites and migration into WCK and WOL has occurred. Results of the present study indicate that active areas of leakage from the burial sites can be pinpointed using resident wildlife populations with small home ranges.

The data indicate that shrews are the most useful indicator species for monitoring a variety of pollutants. Blarina brevicauda was the second most abundant species captured at the sites and was the only small mammalian species to show evidence of BaP exposure. All short-tail shrews captured at SWSA-4 had elevated concentrations of stron-

tium-90 in bone tissue and all shrews captured at EFPC had higher levels of mercury than any other species captured. With the exception of one shrew, the concentration of mercury in kidney tissue was ≥ 15 ug/g (APPENDIX B). This concentration can be considered evidence of a highly contaminated site and the species can be considered an excellent monitor. With a mean bone concentration of 23.5 Bg/g of strontium-90, Blarina brevicauda can be considered a good monitor of this radionuclide. None of the three species listed in Table 21 was considered an excellent monitor of strontium-90 because of the high standard deviations of the mean values.

Very few biomonitoring studies report contaminant concentrations in shrews. Most studies focus on larger, more economically important species. A few studies report on heavy metal concentrations in mammals (Wren 1986b), but only one (Smith and Rongstad 1982) reported mercury in shrews. Yet the shrew family (Soricidae) is distributed throughout the United States and Canada (Burt 1976) and all species consume ground-dwelling invertebrates and sometimes vertebrates as part of their diets. Their wide distribution, soil burrowing habits, and insectivorous to carnivorous eating habits make them potentially excellent sentinel species for a variety of contaminants. Drawbacks to using shrews as sentinel animals include difficulty in trapping compared to mouse species and discontinuous distribution due to habitat requirements.

In the present study S. hispidus was not abundant enough at the EFPC site to determine contaminant uptake. It was present at SWSA-4 and WOC where it was a good monitor of strontium-90 (19.3 Bq/g at WCK

and 59.5 Bq/g at SWSA-4). A distribution limited to the southeastern United States, a habitat of grass-dominated areas, and a diet of primarily grass limit the usefulness of this species as a monitor of environmental contamination.

Other species were not trapped in large enough numbers to make meaningful comparisons concerning monitoring suitability. In addition to the shrew, only the Norway rat (R. norvegicus) and the muskrat (O. zibethica) showed traces of BaP tetrol I-1. All species at SWSA-4 showed uptake of strontium-90; thus uptake of this contaminant does not appear to be limited by food habits of the resident species because equally high concentrations of Strontium-90 occurred in both herbivores and insectivores.

General Considerations

Biological monitoring of contaminants is necessary to evaluate the movement of chemicals through food chains. There are important limitations to the use of small mammals for monitoring. Some of the contaminated study sites, such as SWSA-4, were very small in area (the most highly contaminated surface plume measured only 8 by 24 m), thus limiting the population sizes at these sites. More intensive trapping would have resulted in capture of recent immigrants.

Additional limitations to biological monitoring follow. Not all species were present at all sites; P. leucopus, an omnivore, dominated at most sites. There was extremely high variability of endpoints, probably reflecting the extent of overlap of home range of individuals with the contaminated area. However, since home ranges of most of the

animals were small, one to one and one-half acres, contaminated areas could be closely pinpointed. Not all species were good sentinels. Insectivores in general, as exemplified by the shrew, were better biomonitors than herbivores. However, if the contaminant was taken up by vegetation, as in the case of strontium-90, then herbivores could be used. The sentinel also must be compatible with the type of habitat in the contaminated zone. The eastern harvest mouse lived in the grassy area surrounding the radioactive seeps; no other species were captured in the grass. However, since the contamination had migrated via leaching or run off into a wooded area and down an adjacent bank, other species were exposed.

Despite the above limitations, biological availability of the three contaminants can be inferred from this study and if cleanup is being considered, a rationale can be provided for prioritizing sites.

IV. CONCLUSIONS

A review of terrestrial biomonitoring studies using small mammals as indicator species provided information on the presence and bioavailability of several types of contaminants at mine sites, roadside sites, industrial areas, hazardous and radioactive waste disposal sites, and agricultural and forested land. Each species was evaluated for suitability as a monitor for specific contaminants. There was a positive relationship between biomonitoring capacity and trophic level. Insectivores were the best monitors of most contaminants, followed by omnivores and herbivores. For most contaminants there were one or two specific target tissues, but many studies did not monitor the best target tissue, analyzing on a whole body basis instead. In areas where a complex mixture of unidentified chemicals was present, several types of genotoxic and cytotoxic analyses were applied. Information was sparse for many contaminants.

In order to fill in some gaps in information and to test the hypothesis on trophic levels developed in the first part of the study, a variety of small mammals were trapped in the vicinity of the ORNL reservation in areas contaminated with BaP, mercury, and strontium-90. Formation of hemoglobin adducts with BaP was used as an indicator of BaP exposure. Mercury in kidney tissue and strontium-90 in bone tissue were determined by residue analyses. Contaminant levels in soil and vegetation were either determined experimentally or obtained from field studies by others.

Peromyscus leucopus, the white-footed mouse, was the most abundant species at all sites. Blarina brevicauda, the shorttail shrew, was the

second most abundant species. Less common were S. hispidus, the cotton rat; Microtus sp., voles; and several other species.

Results of blood and tissue analyses showed site and species differences in exposure to the contaminants. Uptake was related to habitat and food preferences. Peromyscus leucopus, because of its abundance and wide distribution is the most useful species for monitoring sites where radionuclides are present; however, this species is not well suited as a biomonitor of polycyclic aromatic hydrocarbons such as BaP or heavy metals such as mercury. Blarina brevicauda is not as abundant or as widely distributed as P. leucopus, but its trophic position makes it an ideal sentinel for a variety of contaminants. This species was the only one to show evidence of exposure to all three contaminants. Mercury concentrations in kidney tissue were more than an order of magnitude greater than those of other species and were related to the soil-associated habitat and feeding habits of this species. Elevated concentrations of strontium-90 were present in the bone of all species trapped at the contaminated (SWSA-4) site. Strontium-90 was present in the vegetation at this site whereas mercury and BaP were associated with the soil only.

This study indicated that the quantification of hemoglobin adducts does not appear to be a feasible method to determine BaP exposure of small mammals collected at field sites. Analysis of blood following subchronic feeding studies with mice in the laboratory and following collection of small mammals in industrially-polluted areas showed low and erratic levels of a BaP metabolite-hemoglobin adduct.

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APPENDICES

APPENDIX A

HEAVY METAL CONCENTRATIONS IN SOIL, VEGETATION, INVERTEBRATES, AND TISSUES OF SMALL MAMMALS AT CONTAMINATED SITES.

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Arsenic	<u>Blarina brevicauda</u>	Zinc-copper mine Reference site				1.9 whole body <0.3 whole body	Results based on two animals at each site.	29
	<u>Peromyscus maniculatus</u>	Zinc-copper mine Reference site				0.5 whole body <0.3 whole body	Higher concentrations at mine site.	29
	<u>Spermophilus variagatus</u>	Various sites	8-60	1-94		0.1-9.4 liver	No relationship between concentrations in soil and vegetation and concentrations in liver.	28
Cadmium	<u>Apodemus sylvaticus</u>	Lead-zinc mine Complex M	92	4	11-34	2.6 whole body 39.7 kidney 9.9 liver	Significantly higher concentrations in kidney and liver tissue of animals from mine and smelter site.	19, 24
		Complex M reference	1	<1	1-6	0.3 whole body 1.7 kidney 0.5 liver		
		Lead-zinc-mine Complex Y	11			1.0 whole body 10.3 kidney 2.5 liver		
		Complex Y reference	2			0.3 whole body 1.7 kidney 0.9 liver		
		Smelter	46			0.8 whole body 18.0 kidney 4.4 liver		
		Smelter reference	1			0.3 whole body 2.2 kidney 0.7 liver		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
cadmium (con't)		Copper-cadmium refinery	8.5	2-4	11-13	<1.0 whole body 7.4 kidney 1.5 liver 1.0 hair 0.2 muscle 0.0 brain 1.5 bone	Significantly elevated concentrations present in kidney, liver, and hair of animals from refinery site.	14
		700 m from refinery	3.1	1-2	4-7	5.5 kidney 1.4 liver 0.4 hair 0.1 muscle 0.9 bone		
		Reference site	0.8	<1	1-2	1.5 kidney 0.5 liver 0.3 hair 0.2 muscle 0.1 brain 0.9 bone		
	<u>Blarina brevicauda</u>	Zinc-copper mine				6.2 whole body	Results based on two animals trapped at each site.	29
		Reference site				0.9 whole body		
		Reference site				1.2 whole body	Reference value only	27
	<u>Clethrionomys glareolus</u>	Lead-zinc mine complex Y	11			0.9 whole body 16.8 kidney 5.1 liver	Concentrations significantly higher in whole body and kidney and liver tissues of animals from mine site.	19
		Reference Y	2			0.2 whole body 4.6 kidney 1.1 liver		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
cadmium (con't)	<u>Microtus agrestis</u>	Lead-zinc mine	11			0.6 whole body 8.9 kidney 1.1 liver	Significantly higher concentrations in whole body and kidney and liver tissue of animals from mine site	19
		Reference Y	2			0.1 whole body 0.8 kidney 0.3 liver		
		Sewage farm		0.7		8.0 kidney 5.0 liver <0.3 bone <0.1 brain 1.0 carcass	Concentrations significantly higher in liver and kidney tissue of animals from sewage farm compared to reference site or laboratory site	4
		Reference farm		0.2		5.0 kidney 1.0 liver 0.4 brain 0.1 bone 1.0 carcass		
		Laboratory stock				1.0 kidney 1.0 liver <1.0 brain <1.0 bone 1.0 carcass		
		Copper-cadmium refinery	8.5	2-4	11-14	1.7 whole body 23.3 kidney 7.7 liver 0.7 hair 0.5 muscle 0.2 brain 1.6 bone	Concentrations significantly elevated in all tissues except brain of animals from refinery site compared to reference site.	14

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
cadmium (con't)		700 m from refinery	3.1	1-2	4-7	4.1 kidney 1.4 liver 0.5 muscle 0.3 brain 1.4 bone		
		Reference site	0.8	<1	1-2	1.3 kidney 0.7 liver 0.2 hair 0.1 muscle 0.1 brain 0.6 bone		
		Mine-waste site		4.8	23.2	1.8 whole body 5.2 kidney 1.9 liver 0.7 bone 1.3 muscle	Concentrations in kidney, muscle, and bone significantly elevated at mine site.	1
		Reference site				0.9 whole body 1.8 kidney 1.1 liver 0.4 bone 0.3 muscle		
	<u>Microtus pennsylvanicus</u>	Sewage-sludge treated fields				1.4-23 kidney 0.4-3.9 liver 0.0-0.4 lungs 0.0 testes	Concentrations in kidney and liver were significantly higher at sludge-treated site in one of two years of application.	2

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
cadmium (con't)		Fertilizer-treated fields				0.3-4.6 kidney 0.0-0.4 liver 0.0-0.1 lungs 0.0 testes		
		Reference fields				0.0-5.6 kidney 0.0-1.5 liver 0.0-0.1 lungs 0.0-0.1 testes		
		Waste-water irrigated field				0.2 liver 1.1 kidney	Concentrations in liver and kidney were higher at reference site than at treated site.	3
		Reference field				0.3 liver 2.1 kidney		
		Zinc-copper mine				1.4 whole body	Significant differences between sites; no age differences.	29
		Reference site				<0.4 whole body		
<u>Peromyscus leucopus</u>		Waste-water irrigated site				0.5 liver 2.3 kidney	Concentrations in kidney were significantly higher at the irrigated site.	3
		Reference site				0.4 liver 0.6 kidney		
<u>Peromyscus maniculatus</u>		Zinc-copper mine				1.4 whole body	Significant differences between sites; accumulation with age.	29
		Reference site				<0.4 whole body		
		Reference site				1.4 whole body	Reference concentration only.	27

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
cadmium (con't)	<u>Rattus norvegicus</u>	Urban area				0.3 liver 0.9 kidney 0.1 muscle	Concentration significantly higher in liver of urban rats compared to rural rats.	30
		Rural area				0.2 liver 0.6 kidney 0.1 muscle		
	<u>Sorex araneus</u>	Lead-zinc mine complex M	92	4	19	40 whole body	No statistical comparisons; raw data not given. Accumulation through food chain.	19, 24
		100 m from mine		2	9	17 whole body		
		Reference M	1	<1	2	4 whole body		
		Copper refinery	8.5	2-4	11-13	27.5 whole body 280 liver 193 kidney 4.8 muscle 3.0 bone 2.4 brain	Concentrations in liver, kidney, and muscle significantly elevated at refinery site compared to reference site.	14
		700 m from refinery	3	1	4-7	237 liver 139 kidney 3.2 muscle 3.3 bone 0.8 brain		
		Reference site	1	<1	1-2	25.4 liver 25.7 kidney 1.7 muscle 3.7 bone 1.4 brain		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
cadmium (con't)		Mine waste site	4.8		23.2	52.7 whole body 158 kidney 236 liver 8.0 muscle 1.9 bone	Concentrations in whole body and tissues were significantly higher at mine site.	1
		Reference site	1.2		2.1	1.2 whole body 4.1 kidney 2.9 liver 0.8 muscle 1.0 bone		
	<u>Sorex cinereus</u>	Reference site				0.7 whole body	Collected only at reference site.	29
	<u>Spermophilus variegatus</u>	Reference site	2-10	1-5		2-27 liver 3-8 bone	Significant correlation between tissue concentration and both vegetation and soil.	28
	<u>Talpa europea</u>	Metal smelter 1	2	2		112 kidney 94 liver	Accumulation reflected bioavailability as influenced by soil concentration, soil type, and uptake by invertebrates (earthworms).	20
		Metal smelter 2	6	2	79	224 kidney 227 liver		
		Metal smelter 3	9	2	114	221 kidney 172 liver		
		Metal smelter 4	<1	2		186 kidney 145 liver		
		Reference site	<1	2	19	59 kidney 30 liver		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Chromium	<u>Microtus agrestis</u>	Sewage farm	0.8			0.3 liver	Differences among sites were small.	4
						0.5 kidney		
						0.3 brain		
						<0.8 bone		
						5.0 carcass		
						Reference site		
		1.1 kidney						
		1.0 brain						
		<0.6 bone						
		4.0 carcass						
		Laboratory colony				0.1 liver		
		0.2 kidney						
0.2 brain								
<0.3 bone								
3.0 carcass								
	Waste-water irrigated site							4.7 liver
			18.3 kidney					
	Reference site				11.2 liver			
					50.9 kidney			
Peromyscus leucopus		Waste-water irrigated site				No differences between sites.	3	
								7.8 liver
								28.0 kidney
								Reference site
					28.1 kidney			

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Cobalt	<u>Microtus pennsylvanicus</u>	Waste-water irrigated site Reference site				1.4 liver 5.3 kidney 2.4 liver 4.5 kidney	No differences between sites.	3
	<u>Peromyscus leucopus</u>	Waste-water irrigated site Reference site				2.3 liver 4.5 kidney 2.6 liver 5.0 kidney		
Copper	<u>Apodemus sylvaticus</u>	Copper refinery	2,480	153-375	343-568	11.9 kidney 23.7 liver 14.7 hair 6.9 muscle 4.2 bone 9.9 brain	Mean copper concentration significantly elevated in liver tissue of animals from refinery site.	14
		700 m from refinery	246	26-49	63-86	11.7 kidney 12.6 liver 6.8 hair 7.1 muscle 4.4 bone 12.2 brain		
		Reference site	9.3	7-8	17-23	13.5 kidney 12.9 liver 11.1 hair 5.9 muscle 4.0 bone 14.4 brain		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
copper (con't)	<u>Blarina brevicauda</u>	Zinc-copper mine				13.4 whole body	Results based on two animals at each site.	29
		Reference site				10.6 whole body		
		Reference site				9.0 whole body	Reference value only.	27
	<u>Microtus agrestis</u>	Sewage farm		22.7		33 kidney 50 liver 12 bone 20 brain 10 carcass	Concentrations in liver and kidney higher at sewage farm than at reference farm or laboratory stock; no statistics.	4
		Reference farm		8.5		21 kidney 16 liver 2 bone 11 brain 11 carcass		
		Laboratory stock				19 kidney 17 liver 13 bone 15 brain 7 carcass		
		Copper refinery	2,480	153-375	343-568	22.6 kidney 13.5 liver 24.2 hair 9.3 muscle 14.7 brain 5.7 bone		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
copper (con't)		700 m from refinery	246	26-49	63-86	13.0 kidney 13.7 liver 8.3 hair 9.8 muscle 13.9 brain 5.6 bone		
		Reference site	9.3	6-8	17-23	10.8 kidney 13.4 liver 6.5 hair 8.5 muscle 14.4 brain 4.9 bone		
	<u>Microtus pennsylvanicus</u>	Sewage-sludge treated fields				18.7 liver 17.2 kidney	Liver and kidney concentrations elevated in some age/sex groups.	2
Fertilized fields					19.5 liver 16.3 kidney			
Reference field					17.3 liver 15.6 kidney			
Waste-water irrigated field					76.1 liver 83.5 kidney	Concentrations higher at reference site than waste-water treated site.	3	
Reference field					83.3 liver 122.4 kidney			
		Zinc-copper mine Reference site				24.3 whole body 11.9 whole body	Significant difference between sites.	29

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
copper (con't)	<u>Peromyscus leucopus</u>	Waste-water irrigated field				34.1 liver 25.2 kidney	Tissue concentrations higher at reference site.	3
		Reference field				41.5 liver 26.8 kidney		
	<u>Peromyscus maniculatus</u>	Zinc-copper mine				44.6 whole body	Significant interactions among site, sex, and age.	29
		Reference site				13.4 whole body		
		Reference site				10.0 whole body	Reference concentration only.	27
	<u>Sorex araneus</u>	Copper refinery	2,480	153-375	343-568	56.1 liver 38.5 kidney 17.4 muscle 7.7 bone 11.7 brain	Concentrations significantly elevated at refinery and intermediate sites compared to reference site.	14
		700 m from refinery	246	26-49	63-68	56.2 liver 30.8 kidney 14.4 muscle 7.5 bone 14.1 brain		
		Reference site	9.3	6-8	17-23	31.1 liver 22.8 kidney 10.9 muscle 4.4 brain 10.0 brain		
		Reference site						
	<u>Sorex cinereus</u>	Reference site				12.8 whole body	Collected at reference site only.	29

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference	
			Soil	Vegetation	Invertebrates	Tissue			
copper (con't)	<u>Talpa europea</u>	Metal smelter 1	12	18		27 kidney 23 liver	Sites not considered contaminated.	20	
		Metal smelter 2	25	12	28	28 kidney 27 liver			
		Metal smelter 3	40	11	28	32 kidney 27 liver			
		Metal smelter 4	27	25		27 kidney 26 liver			
		Reference site	7	13	20	25 kidney 23 liver			
Lead	<u>Apodemus sylvaticus</u>	Roadside verge	130-170	33-130	14.5-380	12 liver	Lead concentrations in tissues decreased with distance from the road.	33	
		60 m from verge	120	10-20	21-48	6.5 kidney 9.5 liver			
		0.8 km from road		10		5.0 kidney 9.0 liver			
		Major road verges		307			5.3 whole body 9.2 liver	Concentrations were significantly higher in animals trapped on verges than in fields.	15
		Minor road verges		43			5.1 whole body 8.5 liver		
		Reference fields		33			3.5 whole body 6.1 liver		
		Roadside: 35,000 vehicles/day			0.7-146	32-560	8.5 liver 9.8 kidney 67 bone	Significant difference between sites for some tissues.	6
Reference site					3.5 liver <0.4 kidney 25 bone				

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Lead (con't)		Lead-zinc mine complex M	8,430	120	62	26.7 whole body 46.6 kidney 11.7 liver 352 bone 13 brain 10 muscle	Significant differences between mine and reference site for all tissues except muscle.	19, 24, 25
		Reference M	96	21	18	2.9 whole body 12.7 kidney 7.9 liver 11.5 bone 3.3 brain 5.3 muscle		
		Lead-zinc mine complex Y	14,010	249	82	43.1 whole body 39.2 kidney 13.0 liver 189 bone 5.7 brain 6.6 muscle		
		Reference Y	78	29	22	3.8 whole body 9.4 kidney 5.4 liver 21.1 bone 3.5 brain 5.8 muscle		
		Smelter waste	4,030			65.2 kidney 12.1 liver 672 bone		

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Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
lead (con't)	<u>Blarina brevicauda</u>	Roadside: 19,600 vehicles/day				18.4 whole body 12.4 kidney 4.6 liver 67.1 bone 9.7 muscle	Concentrations varied with traffic levels; highest concentrations in bone.	11
		Roadside: 1,360 vehicles/day				6.7 whole body 5.8 kidney 2.0 liver 19.9 bone 5.7 muscle		
		Roadside: 340 vehicles/day				5.7 whole body 3.9 kidney 1.0 liver 12.2 bone 5.4 muscle		
		Reference field				3.3 whole body		
		Roadside: 21,010 vehicles/day		32-51		34.8 whole body	Concentrations related to traffic density.	12, 26
		Roadside: 8,120 vehicles/day				15.8 whole body		
		Roadside: 1,085 vehicles/day		9-11		11.6 whole body		
		Reference area				13.9 whole body		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
lead (con't)	<u>Clethrionomys glareolus</u>	Roadside: 12,345 vehicles/day	47-543	84-158		22.7 whole body	Concentrations decreased with distance from highway.	23
		25-345 m from road		78		5.2 whole body		
		Reference site	17.7	6.6		5.4 whole body		
		0-20 m from highway				76.2 whole body	Concentration significantly higher at highway compared to reference site.	7
		Reference site				5.8 whole body		
		Zinc-copper mine				2.9 whole body	Results based on two animals trapped at each site.	29
		Reference site				18.6 whole body		
		Road verge	130-170	32-130	14.5-380	13.0 kidney 13.5 liver	Concentrations decreased with distance from road.	33
		60 m from road	120	10-20	21-47.5	5.0 kidney 10.0 liver		
		0.8 km from road		10		5.0 kidney 7.5 liver		
Major road verges		307		5.7 whole body 14.4 liver	Concentrations were significantly higher in animals trapped on verges compared to those trapped in fields.	15		
Minor road verges		43		6.2 whole body 15.5 liver				
Reference fields		33		4.7 whole body 12.0 liver				
Roadside: 35,000 vehicles/day		0.7 -146	32-560	16.5 liver 12.1 kidney 38.2 bone	No animals trapped at reference site.	6		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Lead (con't)		Lead-zinc mine complex M	8,430	120	62	16.3 whole body	Mean concentration at mine site Y significantly higher than reference.	19, 24, 25
		Reference M	96	21	8	2.4 whole body		
		Lead-zinc mine complex Y	14,010	249	82	20.7 whole body		
		Reference Y	78	29	22	2.6 whole body		
	<u>Cryptotis parva</u>	Roadside: 8,120 vehicles/day				10.4 whole body	No animals trapped at reference site.	12, 26
		Roadside: 1,085 vehicles/day			8-11	6.5 whole body		
	<u>Microtus agrestis</u>	Road verge	130-170	32-130	14.5-380	9.5 kidney 10.5 liver	Number of animals trapped not given.	33
		60 m from road	120	10-20	21-47.5	5.0 kidney 5.0 liver		
		0.8 km from road		10		9.0 kidney 5.0 liver		
		Major road verges		307		10.0 whole body 13.6 liver		
		Minor road verges		43		6.8 whole body 9.0 liver		
		Lead-zinc mine complex M	8,430	120	62	140.4 whole body		
	Reference M	96	21	18	8.6 whole body			

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Lead (con't)		Lead-zinc mine complex	14,010	249	82	128.4 whole body 448 bone 60.4 kidney 13.7 liver		
		Reference Y	78	29	22	8.4 whole body 10.2 bone 8.3 kidney 4.7 liver		
		Sewage farm		21		7 kidney 3 liver 13 bone 4 brain 12 carcass	Concentrations in bone and carcass higher in animals from sewage farm than in reference or laboratory animals.	4
		Reference site		0.07		6 kidney 4 liver 6 bone 6 brain 3 carcass		
		Laboratory stock				4 kidney 12 liver 5 bone 2 brain 6 carcass		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
lead (con't)	<u>Microtus ochrogaster</u>	Roadside: 19,600 vehicles/day				8.1 whole body 8.1 kidney 1.6 liver 16.6 bone 8.2 muscle	Concentrations varied with traffic density; highest concentration in bone.	11
		Roadside: 1,360 vehicles/day				4.3 whole body 7.6 kidney 1.2 kidney 23.2 bone 3.0 muscle		
		Roadside: 340 vehicles/day				2.6 whole body 2.8 kidney 1.0 liver 4.6 bone 2.0 muscle		
		Reference site				3.3 whole body		
		<u>Microtus pennsylvanicus</u>	Roadside: 8,120 vehicles/day		8.5-11.7	12.1 whole body	No animals collected at reference site; mean concentration related to traffic volume.	12, 26
	Roadside: 1,085 vehicles/day				6.9 whole body			
	Roadside: 35,000 vehicles/day					6.1 whole body	Concentrations not significantly higher in animals trapped at roadside.	7
	Reference site					3.6 whole body		
	Roadside: 12,470 vehicles/day		41-543	50-150		16.3 whole body	Concentrations decreased with distance from highway.	23
	25-45 m from road		40	40		5.8 whole body		
135-185 m from road	20-30	30-40		3.8 whole body				
Reference site				4.9 whole body				

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
lead (con't)		Lead-arsenate treated orchards	1,342-6,236	0.4-7.1		25.1 kidney 5.7 liver 158.8 bone	Concentrations in kidney, liver and bone tissue of animals trapped in treated orchard higher than in reference animals; no statistics.	8, 13
		Reference orchards	14.2	0.9-1.51		4.1 kidney 1.7 liver 18.4 bone		
		Sewage-sludge treated fields				0.7-2.3 liver 1.7-5.5 kidney	No treatment related trends.	2
		Fertilized fields				0.5-1.7 liver 0.9-3.5 kidney		
		Reference fields				0.5-1.3 liver 1.1-3.4 kidney		
		Waste-water irrigated field				0.7 liver 8.0 kidney	No treatment related trends.	3
		Reference field				1.0 liver 14.0 kidney		
	Zinc-copper mine Reference site				2.1 whole body 1.5 whole body	No significant difference between sites.	29	
	<u>Microtus pinetorum</u>	Lead-arsenate treated orchard	210	0.4-7.1		28.5 kidney 6.9 liver 354 bone	No animals trapped at reference site.	8, 13

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
lead (con't)	<u>Mus musculus</u>	Roadside: 19,600 vehicles/day				6.9 whole body 8.1 kidney 2.9 liver 19.2 bone 5.9 muscle	Differences among sites for whole body concentrations not significant.	11
		Roadside: 1,360 vehicles/day				3.4 whole body 6.6 kidney 1.6 liver 21.0 bone 3.9 muscle		
		Roadside: 300 vehicles/day				4.0 whole body 3.1 kidney 1.6 liver 23.5 bone 3.4 muscle		
		Reference site				4.6 whole body 3.4 kidney 1.9 liver 9.3 bone 2.8 muscle		
	<u>Peromyscus leucopus</u>	Roadside: 21,040 vehicles/day	20-110	32-51		15.6 whole body	Concentrations varied significantly with traffic density.	12, 26
		Roadside: 8,120 vehicles/day				9.7 whole body		
		Reference site	7.8			6.4 whole body		
		Roadside: 35,000 vehicles/day				22.3 whole body	Difference between sites was not significant.	7
		Reference site				5.3 whole body		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Lead (con't)		Roadside: 12,470 vehicles/day	47-543	84-158		6.8 whole body	Concentrations decreased significantly with distance from road.	23
		30-55 m from road		33-78		3.9 whole body		
		115-195 m from road	20-30	10-30		2.6 whole body		
		Reference site				2.6 whole body		
		Lead-arsenate treated orchards	218	0.4-7.1		12.2 kidney 3.9 liver 54.5 bone	No animals trapped at reference site.	8, 13
		Waste-water irrigated site				7.0 liver 24.9 kidney	Concentrations in both tissues were significantly higher in mice from the irrigated site.	3
		Reference site				2.8 liver 13.0 kidney		
	<u>Peromyscus maniculatus</u>	Roadside: 19,800 vehicles/day	73			8.5 kidney 3.3 liver 52.1 bone 0.8 brain	Concentrations in all tissues were significantly higher in mice from the roadside site than in mice from the reference site.	21
		Reference site	26.6			3.3 kidney 1.1 liver 4.8 bone 0.1 brain		
		Roadside: 38,000 vehicles/day	110-1500			4.6 liver 23.0 kidney 2.7 brain 2.7 muscle 106.0 bone	Median concentrations were signifi- cantly different for all sites.	31

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Lead (con't)		Roadside: 18,500 vehicles/day				1.3 liver 3.9 kidney 0.6 brain 0.5 muscle 27.0 bone		
		Roadside: 9,500 vehicles/day	30-550			0.8 liver 1.7 kidney 0.5 brain 0.4 muscle 8.6 bone		
		Roadside: 4,200 vehicles/day				0.7 liver 2.6 kidney 0.4 brain 0.4 muscle 5.1 bone		
		Roadside: 0-14 m				1.5 liver 5.4 kidney 1.7 brain 0.9 muscle 29.0 bone	Concentrations related to distance from highway.	31
		96 m from roadside				1.3 liver 1.6 kidney 0.9 brain 0.6 muscle 17.0 bone		
		176 m from roadside				0.4 liver 0.2 kidney 0.3 brain 0.1 muscle 7.2 bone		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Lead ($\mu\text{g}/\text{m}^3$)		Roadside: 19,600 vehicles/day				5.5 whole body	Some tissue concentrations significantly related to traffic density.	11
						3.5 liver		
					7.9 kidney			
					24.6 bone			
					6.8 muscle			
		Roadside: 1,360 vehicles/day			3.7 whole body			
					1.7 liver			
					9.0 kidney			
					8.0 bone			
		Roadside: 340 vehicles/day			7.4 muscle			
			2.4 whole body					
			1.8 liver					
			3.0 kidney					
			6.4 bone					
			1.8 muscle					
		Reference site			2.8 whole body			
					1.1 liver			
					1.8 kidney			
					5.7 bone			
					2.1 muscle			
		Zinc-copper mine			13.4 whole body	Significant difference between sites.	29	
		Reference site			1.9 whole body			
	<u>Rattus norvegicus</u>	Urban area				6.8 liver	Mean concentrations from four urban and four rural areas were significantly different.	30
					17.9 kidney			
					139.6 bone			
Rural area				3.0 liver				
				5.8 kidney				
				10.0 bone				

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
lead (con't)		Urban area				8.9 liver 47.2 kidney 266.0 bone	Significantly elevated concentrations in rats from urban areas.	22
		Rural area				1.1 liver 2.3 kidney 13.7 bone		
	<u>Reithrodontomys megalotis</u>	Roadside: 19,600 vehicles/day				10.8 whole body 4.7 liver 109.5 bone 27.5 muscle	Significant differences between high density traffic area and reference area for some tissues.	11
		Roadside: 1,360 vehicles/day				3.8 whole body 1.1 liver 2.1 kidney		
		Roadside: 340 vehicles/day				3.1 whole body 2.3 liver 4.8 kidney 18.4 bone 4.4 muscle		
		Reference site				3.1 whole body		
	<u>Sorex araneus</u>	Roadside verge	130-170	32-130	14.5-380	14.0 liver 27.0 kidney	Concentrations decreased with distance from road.	33
		60 m from road	120	10-20	21-47.5	11.0 liver 17.5 kidney		
	<u>Sorex araneus</u>	Roadside: 35,000 vehicles/day		0.7-146	32-560	17.2 liver 45.7 kidney 193.0 bone	Significant difference between sites for liver tissue.	6
		Reference site		<0.4-4.3	<0.4-89	<0.4 liver 8.6 kidney 41.0 bone		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Lead (con't)		Lead-zinc mine complex M	8,430			37.4 whole body	Concentrations decreased significantly with distance from mine.	19, 24, 25
		100 m downwind				10.7 whole body		
		500 m downwind				4.9 whole body		
		Reference site				2.7 whole body		
	<u>Sorex cinereus</u>	Roadside				13.7 whole body	No significant difference between sites.	12, 26
		Reference site				16.6 whole body		
		Reference site				2.8 whole body	Collected at reference site only.	29
	<u>Spermophilus variegatus</u>	Reference site	17-1,399	5-283		1.4-5.6 liver 45.5-195 bone	Collected at reference site only.	28
	<u>Talpa europea</u>	Metal smelter 1	38	11		10 liver 31 kidney	Bioavailability depended on soil concentrations, soil type, and uptake by invertebrates (earthworms).	20
		Metal smelter 2	115	6	25	11 liver 29 kidney		
		Metal smelter 3	135	5	25	8 liver 18 kidney		
		Metal smelter 4	149	161		34 liver 338 kidney		
		Reference site	24	27	12	9 liver 22 kidney		
	<u>Zapus hudsonius</u>	Zinc-copper mine				4.1 whole body	Concentrations higher at mine site; no statistical analysis because of small sample size.	29
		Reference site				1.6 whole body		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
Manganese	<u>Microtus agrestis</u>	Sewage farm		183		7 kidney 10 liver 4 bone 6 brain	Differences among sites were small and erratic.	4
		Reference site		38		4 carcass 6 kidney 8 liver 3 bone 2 brain		
		Laboratory stock				5 carcass 7 kidney 14 liver 5 bone 3 brain 3 carcass		
Mercury	<u>Apodemus sylvaticus</u>	Chlor-alkali plant (within 0.5 km)	3.8	4.0	1.3 ^a	0.8 hair 1.8 kidney 0.8 liver 1.9 brain 3.4 muscle	Concentrations were significantly higher within 0.5 km of plant compared to 10 to 30 km away.	5
		10-30 km from plant	0.1	0.1	0.0 ^a	0.1 hair 0.4 kidney 0.1 liver 0.2 brain 0.2 muscle		

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
mercury (con't)		Fields sowed with Hg-dressed wheat		16 ^a		1.3-2.7 whole body 11.2 kidney 7.5 liver	Body concentrations increased by a factor of 11 following sowing of seed dressed with organomercury fungicide.	16, 17
		Presowed fields				0.1 whole body		
		Fields sowed with Hg-dressed wheat		3-9		0.1-2.6 whole body	Higher concentrations persisted for 30-day observation period.	32
	<u>Blarina brevicauda</u>	Zinc-copper mine Reference site				<0.3 whole body <0.3 whole body	No difference between sites; results based on two animals.	29
	<u>Clethrionomys glareolus</u>	Chlor-alkali plant (within 0.5 km)	3.8	4.0	1.3 ^a	0.9 hair 1.2 kidney 0.5 liver 0.5 brain 1.0 muscle	Concentrations significantly higher in hair and brain of animals from contaminated site.	5
		10-30 km from plant	0.1	0.1	0.0 ^a	0.2 hair 0.3 kidney 0.2 liver 0.2 brain 0.2 muscle		
	<u>Peromyscus</u> spp.	Reference site				0.2 liver	No mice were trapped in the contam- inated area.	9
	<u>Rattus norvegicus</u>	Chlor-alkali plant	0.2-1.7	0.1-1.5		7.4 muscle 15.0 liver	Results based on one animal; no reference animals.	10

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference	
			Soil	Vegetation	Invertebrates	Tissue			
mercury (con't)	<u>Sciurus carolinensis</u>	Land use: park residential school cemetery				1.0 hair	No association between concentrations and land use or socio-economic stratum of area.	18	
						1.1 hair			
						0.9 hair			
					1.4 hair				
	<u>Sigmodon hispidus</u>	Chlor-alkali plant	0.2-1.7	0.1-1.5		0.1 muscle 3.8 liver	Results based on one animal; no reference animals.	10	
	<u>Spermophilus richardsoni</u>	Field: Hg-dressed seeds Reference site				1.1 liver 0.1 liver	Concentration higher in area where Hg-dressed seeds used.	9	
Nickel	<u>Blarina brevicauda</u>	Zinc-copper mine Reference site				1.2 whole body 3.7 whole body	Results based on two animals trapped at each site.	29	
	<u>Microtus pennsylvanicus</u>	Waste-water irrigated site Reference site				1.4 liver 4.9 kidney	Concentrations higher at reference site.	3	
						2.1 liver 9.0 kidney			
			Zinc-copper mine Reference site				3.3 whole body 3.9 whole body	No significant difference between sites.	29
	<u>Peromyscus leucopus</u>	waste-water irrigated forest Reference forest					2.8 liver 9.1 kidney 4.2 liver 15.4 kidney	Concentrations in both tissues significantly higher at reference site.	3
	<u>Peromyscus maniculatus</u>	Zinc-copper mine Reference site					6.2 whole body 5.0 whole body	No difference between sites.	29

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
nickel (con't)	<u>Sorex cinereus</u>	Reference site				4.7 whole body	Collected at reference site only.	29
	<u>Zapus hudsonius</u>	Zinc-copper mine Reference site				1.9 whole body 2.2 whole body	Small sample size; no statistics	29
Zinc	<u>Apodemus sylvaticus</u>	Lead-zinc mine complex M	21,000	340	220-270	107 whole body 101 kidney 84.8 liver 282 bone 84.7 brain 63.9 muscle	Significantly elevated concentrations in bone tissue only.	19, 24
		Reference site M	131	20	60-210	95.8 whole body 158 kidney 133 liver 200 bone 82.0 brain 71.4 muscle		
		Lead-zinc mine complex Y	1,766			114 whole body		
			Reference site Y	125			112 whole body	
	<u>Blarina brevicauda</u>	Zinc-copper mine Reference site				189 whole body 96 whole body	Results based on two animals trapped at each site.	29
	<u>Clethrionomys glareolus</u>	Lead-zinc mine complex M	21,000	340	220-270	123.4 whole body	No difference between sites.	19, 24
		Reference site M	131	20	60-210	102.6 whole body		
Lead-zinc mine complex Y		1,766			142.9 whole body			
Reference site Y		125			142.7 whole body			

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference
			Soil	Vegetation	Invertebrates	Tissue		
zinc (con't)	<u>Microtus agrestis</u>	Lead-zinc mine complex M	21,000	340	220-270	191.6 whole body	Significantly higher mean concentration at mine site M compared to reference site.	19, 24
		Reference site M	131	20	60-210	121.2 whole body		
		Lead-zinc mine complex Y	1,766			169.3 whole body		
		Reference site Y	125			121.2 whole body		
	Sewage farm		220		108 kidney 149 liver 193 bone 76 brain 174 carcass	No differences in tissue concentrations among contaminated, reference, and laboratory stock.	4	
	Reference site		47		104 kidney 120 liver 187 bone 85 brain 117 carcass			
	Laboratory stock				77 kidney 90 liver 210 bone 67 brain 101 carcass			
		<u>Microtus pennsylvanicus</u>	Sewage-sludge treated fields			151.3-195.9 liver 100.6-182.7 kidney	No treatment-related trends.	3
			Fertilizer-treated fields			160.4-197.3 liver 117.6-190.0 kidney		
		Reference fields			138.2-198.7 liver 114.5-192.4 kidney			

Contaminant	Species	Location	Contaminant Concentration (ug/g dry weight)				Observation	Reference	
			Soil	Vegetation	Invertebrates	Tissue			
zinc (con't)		Waste-water irrigated fields				86.6 liver 81.4 kidney	Mean concentrations higher at reference site.	3	
		Reference fields				134.3 liver 121.4 kidney			
		Zinc-copper mine				175.0 whole body	Significant difference between sites.	29	
		Reference site				106.8 whole body			
		<u>Peromyscus leucopus</u>	Waste-water irrigated field				109.4 liver 75.8 kidney	Concentrations higher at reference site.	3
			Reference field				161.0 liver 139.5 kidney		
		<u>Peromyscus maniculatus</u>	Zinc-copper mine				216.9 whole body	Significant interaction among site, sex, and age.	29
			Reference site				105.7 whole body		
		<u>Sorex araneus</u>	Lead-zinc mine M	21,000	210		141.1 whole body	Concentrations decreased significantly with distance from mine.	19, 24
			100 m downwind				110.9 whole body		
		500 m downwind				101.0 whole body			
		Reference site	131	90		96.4 whole body			
		Reference site				110.8 whole body	Collected at reference site only.	29	
	<u>Zapus hudsonius</u>	Zinc-copper mine				131.4 whole body	Small sample size.	29	
		Reference site				107.3 whole body			

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^aWet weight

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APPENDIX B

CONCENTRATIONS OF MERCURY IN KIDNEY
TISSUE OF SMALL MAMMALS AT ORNL

Mercury concentrations (ug/g wet wt) in kidney tissue of individual animals identified by site, species, and code.

Site	Species	Mercury (ug/g wet wt)
Laboratory control mouse (n=6)		0.02
WCK 2.1		
	<u>Peromyscus leucopus</u> (7-16A)	0.38
	<u>Peromyscus leucopus</u> (7-16C)	0.15
	<u>Peromyscus leucopus</u> (7-16E)	0.27
	<u>Peromyscus leucopus</u> (7-16I)	0.25
	<u>Peromyscus leucopus</u> (7-16J)	0.17
	<u>Peromyscus leucopus</u> (7-21D)	0.18
	<u>Peromyscus leucopus</u> (7-22H)	0.13
	<u>Peromyscus leucopus</u> (7-22I)	0.08
	<u>Peromyscus leucopus</u> (7-23B)	0.26
WCK 3.4		
	<u>Sigmodon hispidus</u> (7-21B)	0.63
	<u>Reithrodontomys humilis</u> (7-21A)	0.42
	<u>Peromyscus leucopus</u> (7-21D)	0.27
	<u>Sigmodon hispidus</u> (7-22B)	0.10
	<u>Sigmodon hispidus</u> (7-22A)	0.74
	<u>Peromyscus leucopus</u> (5-13A)	0.46
	<u>Sigmodon hispidus</u> (5-19C)	0.52
WCK 2.7		
	<u>Peromyscus leucopus</u> (7-21A)	0.35
	<u>Peromyscus leucopus</u> (7-21C)	0.62
	<u>Peromyscus leucopus</u> (7-21F)	0.20
	<u>Peromyscus leucopus</u> (7-22A)	0.26
	<u>Peromyscus leucopus</u> (7-22F)	0.41
SWSA-4		
	<u>Peromyscus leucopus</u> (S ₄ 5-27S, O, S ₄ 5-28Q S ₄ 5-290)	0.39
	<u>Peromyscus leucopus</u> (S ₄ 5-29P, S ₄ 6-4V, I, T)	1.33
	<u>Microtus pinetorum</u> (S ₄ 5-29E, S ₄ 6-10J)	0.06
	<u>Peromyscus leucopus</u> (S ₄ 7-14U)	0.60

Site	Species	Mercury (ug/g wet wt)
SWSA-4 (con't)		
	<u>Peromyscus leucopus</u> (S ₄ 7-14W)	0.22
	<u>Peromyscus leucopus</u> (S ₄ 7-17R)	0.48
	<u>Peromyscus leucopus</u> (S ₄ 7-17U)	0.15
	<u>Peromyscus leucopus</u> (S ₄ 7-16V)	0.24
	<u>Reithrodontomys humulis</u> (S ₄ 7-22D,H)	0.32
	<u>Blarina brevicauda</u> (S ₄ 7-22F)	0.36
	<u>Mus musculus</u> (S ₄ 7-21K)	0.04
	<u>Tamias striatus</u> (S ₄ 7-28T)	0.36
	<u>Tamias striatus</u> (S ₄ 7-29S)	0.05
	<u>Peromyscus leucopus</u> (S ₄ 7-21BB)	0.55
	<u>Sigmodon hispidus</u> (S ₄ 7-22BB)	0.36
	<u>Reithrodontomys humilis</u> (S ₄ 9-23E)	0.27
	<u>Blarina brevicauda</u> (S ₄ 9-23F)	0.96
	<u>Sigmodon hispidus</u> (S ₄ 9-24K)	0.10
	<u>Blarina brevicauda</u> (S ₄ 9-24N)	1.52
	<u>Sigmodon hispidus</u> (S ₄ 11-17B)	0.26
	<u>Sigmodon hispidus</u> (S ₄ 11-17C)	0.04
	<u>Sigmodon hispidus</u> (S ₄ 11-17D)	0.02
	<u>Microtus pinetorum</u> (S ₄ -7T)	0.06
	<u>Peromyscus leucopus</u> (S ₄ 5-17V)	0.20
	<u>Microtus pinetorum</u> (S ₄ 5-19S)	0.01
	<u>Peromyscus leucopus</u> (S ₄ 5-19CC)	0.82
EFPC		
	<u>Peromyscus leucopus</u> (E8-3A)	0.60
	<u>Peromyscus leucopus</u> (E8-3B)	0.51
	<u>Peromyscus leucopus</u> (E8-4A)	5.6
	<u>Peromyscus leucopus</u> (E8-4B)	3.1
	<u>Peromyscus leucopus</u> (E8-5B)	0.62
	<u>Blarina brevicauda</u> (E8-5A)	57.
	<u>Peromyscus leucopus</u> (E8-14A, E8-5A, E8-6A, E8-5B)	0.78
	<u>Blarina brevicauda</u> (E8-15A, E8-15C, 7/24/86, DOA, 10/16/85)	21.
	<u>Sigmodon hispidus</u> (E8-15B)	1.9
	<u>Peromyscus leucopus</u> (E8-6x, 7, 3)	1.94
	<u>Peromyscus leucopus</u> (E8-7(11), E8-13(6))	1.08
	<u>Peromyscus leucopus</u> (E8-13(2), E8-13(27))	0.97
	<u>Peromyscus leucopus</u> (E9-27(21A), (27))	0.70
	<u>Peromyscus leucopus</u> (E9-26(6), E-10-15)	0.82
	<u>Blarina brevicauda</u> (E9-14)	73.
	<u>Blarina brevicauda</u> (E9-15A)	42.
	<u>Blarina brevicauda</u> (E9-15B)	43.
	<u>Peromyscus leucopus</u> (E7-2A)	0.78
	<u>Peromyscus leucopus</u> (E7-14A)	0.76

Site	Species	Mercury (ug/g wet wt)
EFPC (con't)		
	<u>Peromyscus leucopus</u> (E7-17Q)	1.58
	<u>Peromyscus leucopus</u> (E7-23P)	1.19
	<u>Peromyscus leucopus</u> (E7-25E)	0.90
	<u>Peromyscus leucopus</u> (E7-29C)	1.77
	<u>Rattus norvegicus</u> (E7-30A)	4.77
	<u>Peromyscus leucopus</u> (E7-31A)	0.42
	<u>Peromyscus leucopus</u> (E7-31B)	0.08
	<u>Peromyscus leucopus</u> (E8-7A)	0.59
	<u>Blarina brevicauda</u> (E8-7B)	58.50
	<u>Blarina brevicauda</u> (E8-15C)	0.96
	<u>Peromyscus leucopus</u> (E9-24A)	0.31
	<u>Peromyscus leucopus</u> (E9-24B)	0.62
	<u>Blarina brevicauda</u> (E9-29L)	14.80
	<u>Peromyscus leucopus</u> (E10-16A)	0.87
	<u>Rattus norvegicus</u> (E10-17A)	5.17

APPENDIX C

CONCENTRATIONS OF MERCURY IN SOIL AT ORNL

Mercury concentrations in soil samples (top 3 cm) identified by site and trap location.

Site	Mercury (ug/g dry wt)
<hr/>	
WCK 2.1	
Trap A	3.5
Trap E	2.3
Trap I	2.0
WCK 3.4	
Trap A	6.6
Trap B	7.3
WCK 2.7	
Trap A	0.34
Trap B	0.89
Trap C	1.3
SWSA-4	
Trap B	0.32
Trap H	0.28
Trap Q	0.15
Trap V	0.17
Trap V	0.09
Trap U	0.24
EFPC	
1-20 cm (2)	348
30-40 cm (2)	1665

APPENDIX D

CONCENTRATIONS OF STRONTIUM-90 IN BONES
OF SMALL MAMMALS AT ORNL

Strontium-90 concentrations in bone (Bq/g dry wt) of individual animals identified by site, species, and code.

Site	Species	Strontium-90 (Bq/g dry wt)
Laboratory control mouse (7 samples)		n.d.
SWSA-4		
	<u>Peromyscus leucopus</u> (S ₄ 5-270)	5.67
	<u>Peromyscus leucopus</u> (S ₄ 5-27S)	1.64
	<u>Peromyscus leucopus</u> (S ₄ 5-28Q)	3.97
	<u>Tamias striatus</u> (S ₄ 5-28T)	8.91
	<u>Microtus pinetorum</u> (S ₄ 5-29E)	1.45
	<u>Peromyscus leucopus</u> (S ₄ 5-290)	3.81
	<u>Tamias striatus</u> (S ₄ 5-29S)	0.95
	<u>Peromyscus leucopus</u> (S ₄ 5-29P)	4.29
	<u>Reithrodontomys humulis</u> (S ₄ 6-4D)	10.43
	<u>Peromyscus leucopus</u> (S ₄ 6-4V)	0.85
	<u>Peromyscus leucopus</u> (S ₄ 6-4I)	1.56
	<u>Peromyscus leucopus</u> (S ₄ 6-4T)	2.34
	<u>Reithrodontomys humulis</u> (S ₄ 6-10B)	0.04
	<u>Microtus pinetorum</u> (S ₄ 6-10J)	31.81
	<u>Peromyscus leucopus</u> (S ₄ 7-14U)	3.85
	<u>Peromyscus leucopus</u> (S ₄ 7-14W)	3.40
	<u>Peromyscus leucopus</u> (S ₄ 7-16V)	1.20
	<u>Peromyscus leucopus</u> (S ₄ 7-17R)	48.76
	<u>Peromyscus leucopus</u> (S ₄ 7-17U)	9.88
	<u>Mus musculus</u> (S ₄ 7-21K)	1.29
	<u>Peromyscus leucopus</u> (S ₄ 7-21BB)	86.11
	<u>Reithrodontomys humulis</u> (S ₄ 7-22D)	16.00
	<u>Blarina brevicauda</u> (S ₄ 7-22F)	3.90
	<u>Reithrodontomys humulis</u> (S ₄ 7-22H)	10.17
	<u>Sigmodon hispidus</u> (S ₄ 7-22BB)	1.33
	<u>Reithrodontomys humulis</u> (S ₄ 9-23E)	280.76
	<u>Blarina brevicauda</u> (S ₄ 9-23F)	4.99
	<u>Sigmodon hispidus</u> (S ₄ 9-24K)	51.64
	<u>Blarina brevicauda</u> (S ₄ 9-24N)	61.72
	<u>Sigmodon hispidus</u> (S ₄ 11-17B)	111.82
	<u>Sigmodon hispidus</u> (S ₄ 11-17C)	74.80
	<u>Sigmodon hispidus</u> (S ₄ 11-17D)	49.46

Site	Species	Strontium-90 (Bq/g dry wt)
SWSA-4 (con't)		
	<u>Microtus pinetorum</u> (S ₄ 4-7T)	38.03
	<u>Peromyscus leucopus</u> (S ₄ 5-17V)	66.67
	<u>Microtus pinetorum</u> (S ₄ 5-19S)	78.79
	<u>Peromyscus leucopus</u> (S ₄ 5-19CC)	0.30
WCK 2.1		
	<u>Peromyscus leucopus</u> (7-16A)	0.19
	<u>Peromyscus leucopus</u> (7-16C)	1.60
	<u>Peromyscus leucopus</u> (7-16E)	1.08
	<u>Peromyscus leucopus</u> (7-16I)	2.49
	<u>Peromyscus leucopus</u> (7-16J)	4.69
	<u>Peromyscus leucopus</u> (7-21D)	2.15
	<u>Peromyscus leucopus</u> (7-22H)	0.14
	<u>Peromyscus leucopus</u> (7-22I)	0.75
	<u>Peromyscus leucopus</u> (7-23B)	1.22
WCK 3.4		
	<u>Reithrodontomys humulis</u> (7-21A)	0.93
	<u>Sigmodon hispidus</u> (7-21B)	14.46
	<u>Peromyscus leucopus</u> (7-21D)	0.62
	<u>Sigmodon hispidus</u> (7-22A)	3.58
	<u>Sigmodon hispidus</u> (7-22B)	44.06
	<u>Peromyscus leucopus</u> (5-13A)	1.62
	<u>Sigmodon hispidus</u> (5-19C)	15.04
WCK 2.7		
	<u>Peromyscus leucopus</u> (7-21A)	1.36
	<u>Peromyscus leucopus</u> (7-21C)	1.36
	<u>Peromyscus leucopus</u> (7-21F)	1.43
	<u>Peromyscus leucopus</u> (7-22A)	0.32
	<u>Peromyscus leucopus</u> (7-22F)	3.66
EFPC		
	<u>Peromyscus leucopus</u> (E8-6[x])	n.d.
	<u>Peromyscus leucopus</u> (E8-6[11])	n.d.
	<u>Peromyscus leucopus</u> (E8-3A)	0.02
	<u>Peromyscus leucopus</u> (E8-3B)	0.05
	<u>Peromyscus leucopus</u> (E8-4A)	n.d.
	<u>Peromyscus leucopus</u> (E8-4B)	n.d.
	<u>Blarina brevicauda</u> (E8-5A)	n.d.
	<u>Peromyscus leucopus</u> (E8-5B)	n.d.
	<u>Blarina brevicauda</u> (E9-14A)	n.d.
	<u>Blarina brevicauda</u> (E9-15A)	0.44
	<u>Blarina brevicauda</u> (E9-15B)	n.d.
	<u>Peromyscus leucopus</u> (E7-2A)	0.33

Site	Species	Strontium-90 (Bq/g dry wt)
EFPC (con't)		
	<u>Rattus norvegicus</u> (E7-17A)	n.d.
	<u>Peromyscus leucopus</u> (E7-17Q)	n.d.
	<u>Peromyscus leucopus</u> (7-23P)	0.29
	<u>Peromyscus leucopus</u> (E7-25E)	n.d.
	<u>Peromyscus leucopus</u> (E7-29C)	0.16
	<u>Blarina brevicauda</u> (E7-29L)	0.76
	<u>Rattus norvegicus</u> (E7-30A)	n.d.
	<u>Peromyscus leucopus</u> (E7-31A)	0.16
	<u>Peromyscus leucopus</u> (E7-31B)	n.d.
	<u>Peromyscus leucopus</u> (E8-5B)	n.d.
	<u>Peromyscus leucopus</u> (E8-6A)	0.21
	<u>Peromyscus leucopus</u> (E8-7A)	n.d.
	<u>Blarina brevicauda</u> (E8-7B)	n.d.
	<u>Blarina brevicauda</u> (E8-15A)	0.06
	<u>Sigmodon hispidus</u> (E8-15B)	0.71
	<u>Blarina brevicauda</u> (E8-15C)	n.d.
	<u>Peromyscus leucopus</u> (E9-24A)	n.d.
	<u>Peromyscus leucopus</u> (E9-24B)	n.d.
	<u>Peromyscus leucopus</u> (E10-16A)	0.03
	<u>Blarina brevicauda</u> (E10-16B)	n.d.

n.d. = not detectable

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