Sampling and Analysis Plan for the Southeast Missouri Lead Mining District Small Mammal Study

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MISSOURI DEPARTMENT OF NATURAL RESOURCES

Southeast Missouri Lead Mining District

Natural Resource Damage Assessment and Restoration

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LIST OF ACRONYMS

ALAD	Delta-Aminolevulinate Dehydratase
CERCLA	Comprehensive Environmental Response Compensation and Liability Act
CERC	Columbia Environmental Research Center
COC	Chain of Custody
DI	Deionized Water
DQO	Data Quality Objectives
ESP	Environmental Services Program
HWP	Hazardous Waste Program
ICS	Incremental Composite Sampling
IDW	Investigation Derived Waste
KCOM	Kirksville College of Osteopathic Medicine
MDHSS	Missouri Department of Health and Senior Services
MDNR	Missouri Department of Natural Resources
NRDA	Natural Resources Damage Assessment
OLB	Old Lead Belt
OPA	Oil Pollution Act
QAPP	Quality Assurance Project Plan
SEMOLMD	Southeast Missouri Lead Mining District
SOP	Standard Operating Procedure
SU	Sampling Unit
UM-SVM	University of Missouri School of Veterinary Medicine
USGS	United States Geological Survey
USFWS	United States Fish and Wildlife Service
VTLMD	Viburnum Trend Lead Mining District
XRF	X-ray Fluorescence

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1.0 Introduction

The Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), as amended, and the Oil Pollution Act (OPA) provide for the restoration of natural resources lost or injured by hazardous substance releases (§107(f)) and discharges of oil (§1006) and require the designation of certain Federal and State officials to act on behalf of the public as Trustees for natural resources. The Missouri Department of Natural Resources together with the U.S. Fish and Wildlife Service and the U.S. Forest Service, acting as Trustees, are conducting a Natural Resource Damage Assessment (NRDA) in the Southeast Missouri Lead Mining District, in both the Old Lead Belt and the Viburnum Trend. The purpose of conducting a NRDA is to identify and document the extent of injuries to natural resources, quantify the injuries and all associated losses to the public and select restoration projects.

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2.0 Site Information

2.1 Location

The work in the Southeast Missouri Lead Mining District (SEMOLMD) will take place in the Old Lead Belt (OLB), which is approximately 60 miles southwest of St. Louis, and in the Viburnum Trend area (VTLMD), also known as the New Lead Belt. The SEMOLMD lies in the geologic formation known as the Ozark Dome (see Figure 1). Table 1 lists the sampling locations.

2.2 Description

The SEMOLMD has been mined for over one hundred years and has the largest source of lead ore in the United States. Mining in SEMOLMD is conducted by the Doe Run Company, the largest producer of lead (Pb) in the nation (Wikipedia, 2012). Topography in the New Lead Belt is characterized by rolling hills dissected by narrow floodplain, creek, and river valleys. Hills

and ridges are generally steep sided with flat tops consisting of thin mantles of clayey soils. The major physical features of the area are the St. Francois Mountains in the east, and the dissected topography of the Salem Plateau in the northern and central portions. The principal drainage systems for the VTLMD are the north flowing Courtois and Huzzah Creeks and their tributaries and the east and south flowing Black River and its tributaries. The principal drainage systems for the OLB are Flat River Creek, Big River and its tributaries.

Mining in the OLB ceased in 1972 with the closing of the Federal Mine (aka St. Joe State Park). Mining in the VTLMD is ongoing, and the district remains a major producer of metals: Missouri's mines have yielded much of the United States' national production of lead, and since 1997, all the lead produced in Missouri originated in The Doe Run Company's Viburnum Trend

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	Name	Northing	Easting	Comment
Contaminated Sites	Washington State Park	4217979.013	703235.487	WSP beach on road
	St. Joe State Park	4190827.036	718728.298	MO mines by RR tracks
	Magmont Vent	4169315.283	665724.666	VT Magmont vent
	KK & 32	4168066.692	663013.162	VT County Road KK & Highway 32
	West of Buick Smelter	4166274.207	664633.762	VT west of Buick Smelter
Reference Sites	Mill Creek Recreational Area	4192153.194	594477.035	
	Council Bluff Lake	0.000	0.000	no point collected

DNR State Parks
USForest Service

Table 1. General Locations for Trapping Grids.

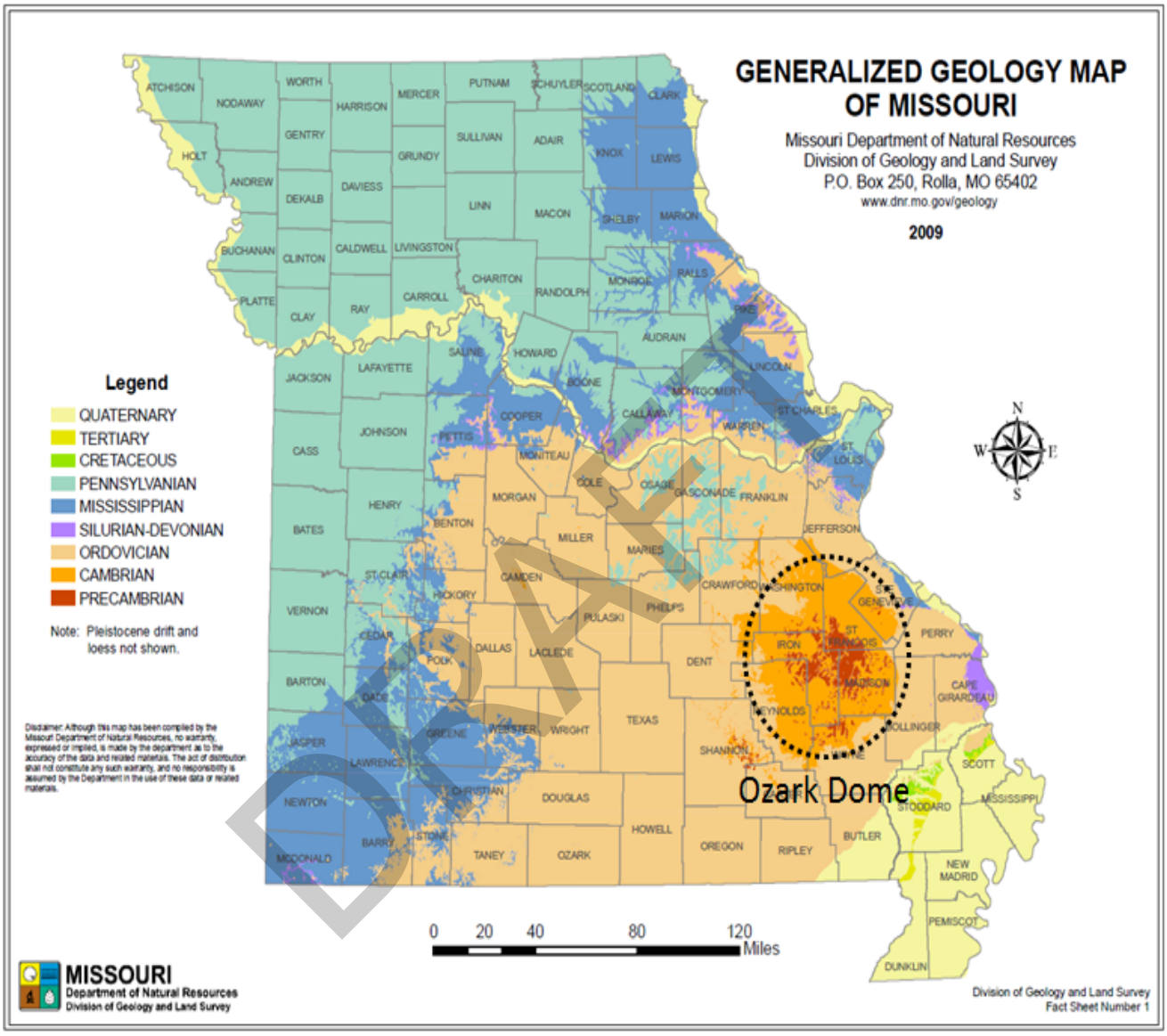

Figure 1. Cambrian and Pre-Cambrian Geologic Uplift Forming the Ozark Dome

Missouri DNR, 2009

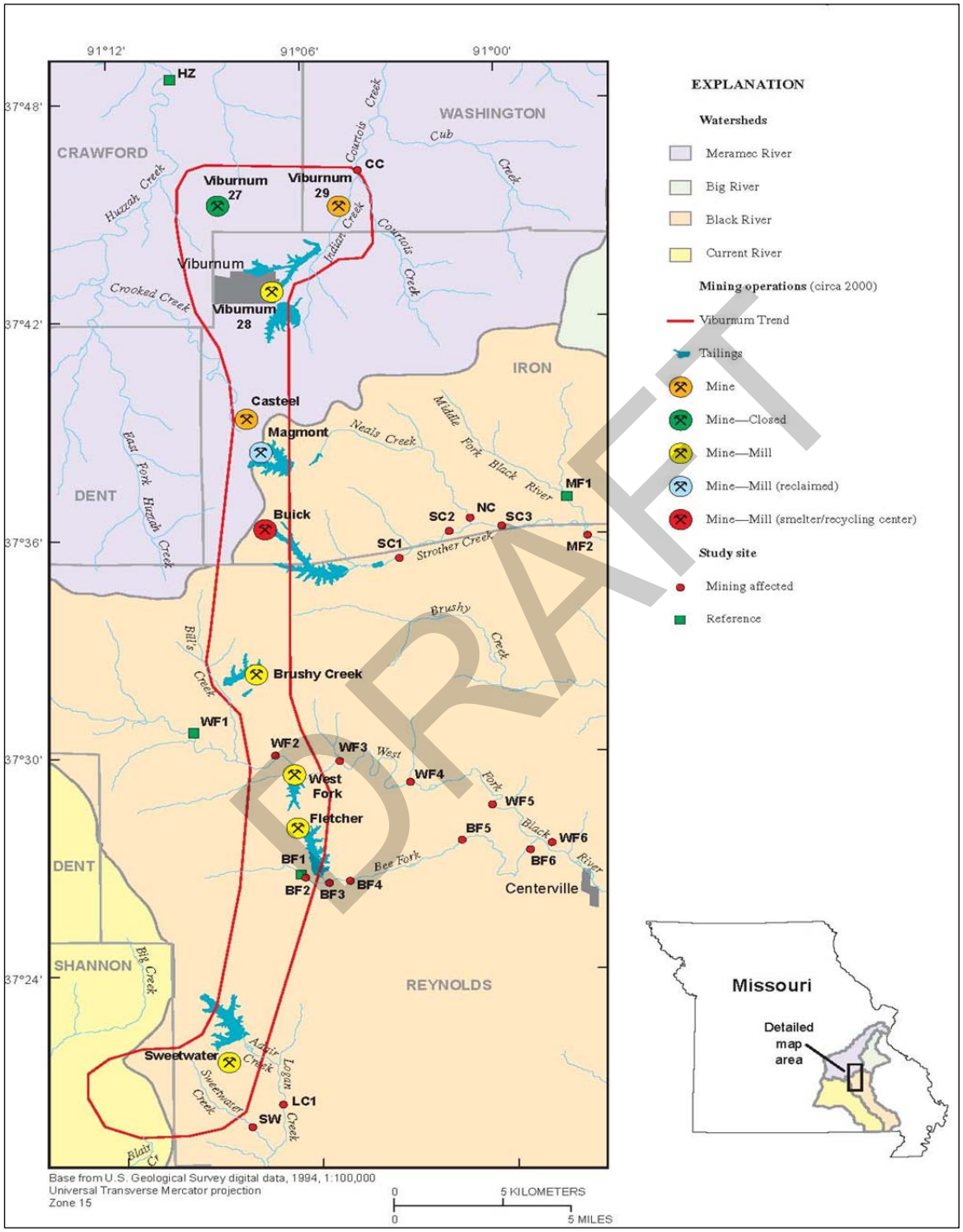

Figure 2. The Viburnum Trend Lead Mining District

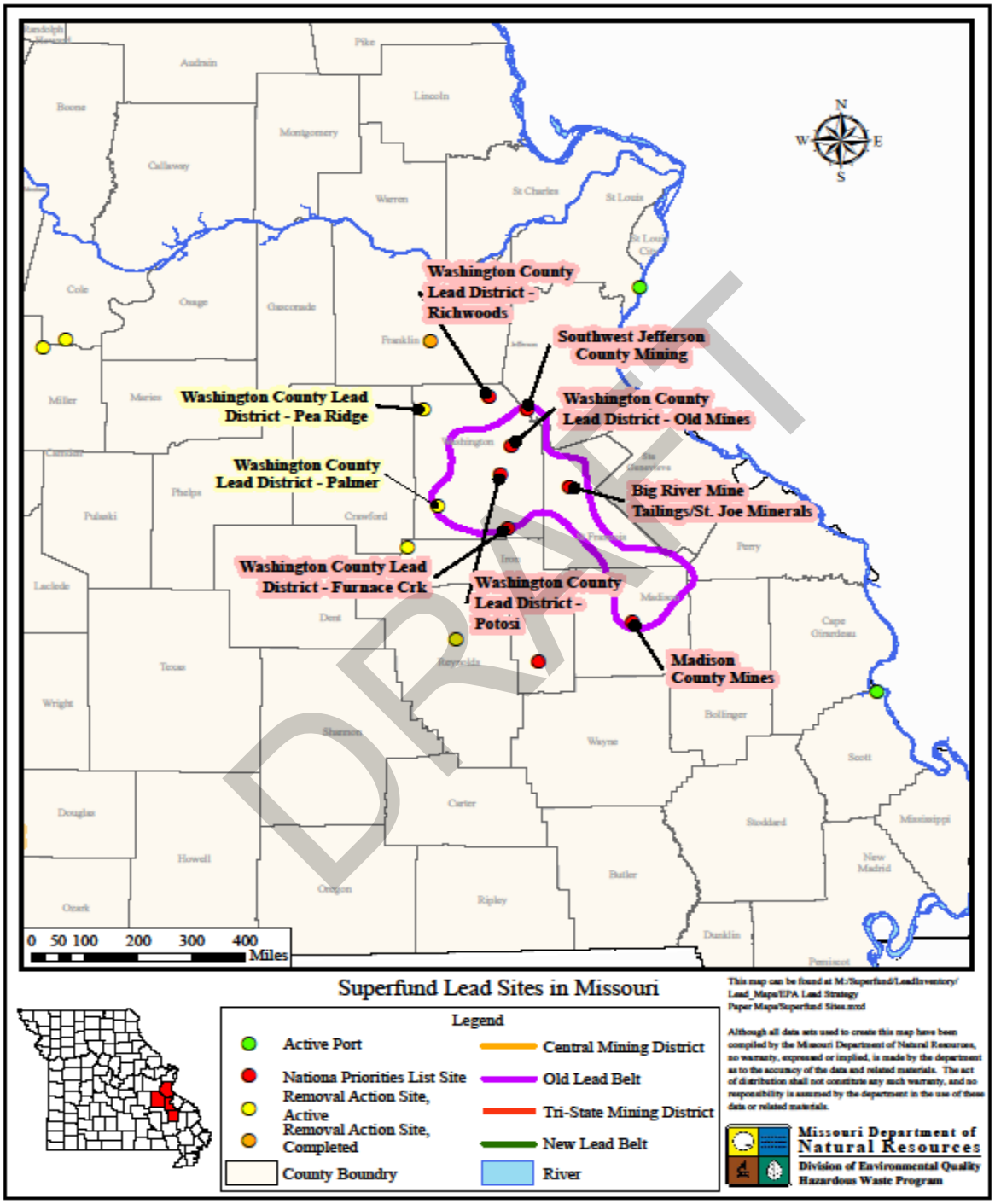

Figure 3. Old Lead Belt

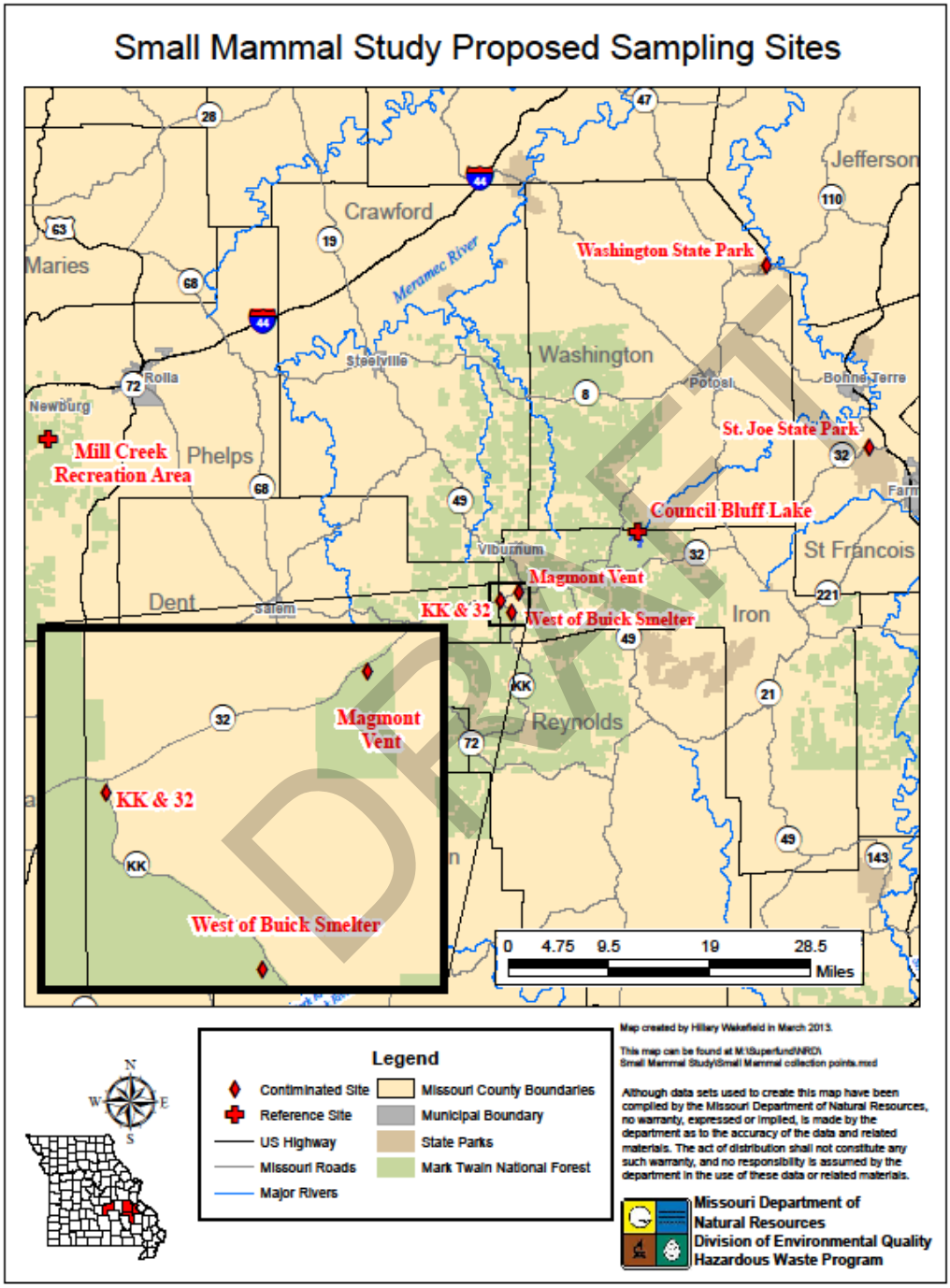

Figure 4: Proposed Small Mammal Sampling Locations

mines. In addition to lead, the mines produce substantial amounts of zinc and lesser quantities of copper and silver (Seeger 2008).

Mining operations within the VTLMD

Mining in the Viburnum Trend has consisted entirely of the room and pillar method along the ore trend (Seeger 2008). Ore is first crushed within the mine, and then removed to the surface for beneficiation, including further crushing. Following the crushing, the slurried fine material is sent to flotation cells and separated into mineral concentrates through circuits specific to each metal (lead and zinc at all mills, copper as well at all except Sweetwater and West Fork). The mineral concentrates are settled and dewatered before further action (either transport to the smelter or to a buyer). The resultant waste is collected in tailings ponds along with all process water. Unlike earlier operations in the SEMOLMD, the Viburnum Trend operations never used density separation methods, which result in chat piles (Seeger 2008).

There are seven major tailings impoundments (tailings are mill waste produced by separating ore from host rock), ten mines, and a secondary Pb smelter in the Viburnum Trend within the Black River and Meramec River watersheds.

Mining operations within the OLB

Mining in St. Francois County (aka the Old Lead Belt) was first recorded between 1742 and 1762. The introduction of the diamond drill in 1869 helped to facilitate the discovery of additional reserves and in turn increased lead production from the mines dramatically in the late 1800s. Mining was at its peak in the OLB in 1942 when 197,430 tons of lead was produced. With the closing of the Federal Mine in 1972, mining in the county ceased. There are eight mine waste areas within the OLB (either chat or tailings): Bonne Terre, Desloge, Doe Run, Elvins/Riversmines, Federal/St. Joe State Park, Hayden Creek, Leadwood, and National.

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2.3 History/Contaminants of Concern

Releases of heavy metals from the tailings impoundments, mines, and smelter in the SEMOLMD have been documented. Elevated levels of cadmium, copper, lead, nickel, and zinc have been measured in surface and shallow subsurface soil at facilities in the VT and OLB. Considering the concentrations measured relative to common screening benchmarks, it is clear that the primary contaminant of concern for the site will be lead. The Missouri Trustees for Natural Resource Damage Assessment and Restoration (Trustees) believe that there may be potential injuries to natural resources in Reynolds, Iron, and St. Francois Counties, Missouri resulting from releases of hazardous substances from the SEMOLMD. Consequently, the Trustees have designed a plan to study the extent of damages on small mammals from the potential release of heavy metals from mining facilities.

3.0 Data Quality Objectives

To help ensure precise, accurate, representative, complete, and comparable data, all field work and analyses will be conducted in accordance with the Quality Assurance Project Plan (QAPP) for Natural Resource Damages, Revision 3, April, 2011 (MDNR, 2011). The QAPP describes the general data quality objectives (DQOs), lab and field duplicates, matrix spikes and standards, chain-of-custody procedures and other specifications for site assessment investigations conducted by MDNR. Those DQOs specific to this project are described below.

3.1 Problem Statement

Levels of lead exceeding 1,000 mg/kg have been measured in surface soil at various facilities and locations within the SEMOLMD. These levels far exceed most ecological screening benchmarks for lead in soils which are in the range of 120-400 mg/kg. Beyer et al (2013) found that soil lead levels of 1,000-3,200 mg Pb/kg in the SEMOLMD would likely be toxic to songbirds. Therefore, there is concern that lead released from the tailings impoundments, haul roads, and wind transfer have resulted in injuries to terrestrial natural resources (small mammals) in the SEMOLMD area. MDNR has chosen to look specifically for mice (Peromyscus species)

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and shrews (Sorex, Blarina, and Cryptotis species) due to the nature of their habitat and eating habits. The shrew is of particular importance because of its primarily insectivorous diet (earthworms).

3.2 Planning Team

The planning team includes staff from the DNR Hazardous Waste Program (HWP), DNR Environmental Services Program (ESP), United States Geological Survey (USGS) Patuxent Wildlife Research Center, University of Missouri School of Veterinary Medicine (UM-SVM), Kirksville College of Osteopathic Medicine (KCOM), and the Missouri Department of Health and Senior Services (MDHSS).

3.3 Field/Analytical Team

Staff from HWP and ESP will be in the field baiting/setting/checking traps and transporting small mammals caught within these traps. Staff from USGS, UM-SVM, and KCOM will be on hand to assist with the anesthesia, exsanguination, blood collection, tissue dissection and collection, and general handling of the animals.

3.4 Conceptual Site Model

Mining-related metals can be released from the tailings impoundments and other mining/milling activities in a number of ways. The primary transport mechanism for metals to the terrestrial environment surrounding the tailings impoundments is assumed to be via wind and surface runoff. Therefore a higher soil concentrations and a further extent of influence is expected in the predominant downwind and downhill directions at each impoundments or tailings/chat pile. Landscape features, surface water runoff, soil type and vegetative cover will also affect the deposition and fate of mine waste released from the tailings impoundments and smelters. The trapping will be conducted at areas which have already been tested for soil lead and have values >1000 mg/kg (on average) (Beyer 2013).

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Activity	**Person(s) Involved**	**Associated Agency**	**On Site (Y/N)**
1. Animal Trapping and Transport 2. Earthworm Collection	Kathy Rangen, Hillary Wakefield, Johns Nichols, Eric Gramlich (MDNR/HWP); Ken Hannon, Sean Counihan (ESP); Nelson Beyer (USGS); Dave Mosby, Scott Hamilton, John Weber (USFWS)	MDNR/ HWP, USFWS, USGS and ESP	Y (all)
Animal Anesthesia	Stan Casteel (UM-SVM)	UM-SVM	Y
Animal Blood Draw	Stan Casteel, Orin Mock (KCOM)	UM-SVM, USGS, and KCOM,	Y
Animal Necropsies and tissue collection	Stan Casteel	UM-SVM	Y
Blood Sample Prep	Stan Casteel	UM-SVM	Y
Femur Prep	Stan Casteel	UM-SVM	Y
Field Hematocrit	Stan Casteel	UM-SVM	Y
ALAD analysis	Barnett Rattner (USGS)	USGS	N
Blood Lead/ Tissue Metals	Lab Technician	MDHSS	N
Blood Histopathology	Lab Technician	UW-Madison	N
Organ Histopathology	Lab Technician	UM-SVM	N
Femur Analysis	Lab Technician	MDHSS	N
Oxidative Stress/DNA damage assay	Natalie Karouna (USGS)	USGS	N

Table 2. Field/Lab Activities and Roles of the Agencies

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3.5 Study Questions

As a result of the mining and smelting that has gone on in the study area for the last 100 years, the land has become contaminated with hazardous substances related to mining. Lead is the contaminant that is most likely to be toxic to small mammals such as shrews, voles, and mice; although zinc, cadmium and other metals associated with the ores are possibly toxic. The principle study questions are:

1. Determination the degree of blood ALAD (delta-aminolevulinate dehydratase) inhibition found in all small mammals captured.
2. What is the average concentration of Pb, Zn and Cd in the blood, kidneys and livers found in all small mammal species captured? What is the average concentration found within each species captured?
3. What is the average concentration of lead found in the femurs of each species captured?
4. What are the concentrations of Pb, Fe, Cu, Ni, Zn, Cd and Al in undepurated earthworms and soil samples associated with the reference and study sites?
5. What histopathologic manifestations of heavy metal exposure are identified in kidney, spleen or liver tissues?
6. Determine the relationships between soil Pb, earthworm Pb, blood Pb, liver Pb, kidney Pb, ALAD inhibition and tissue pathology.

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3.6 Inputs to Study Questions

The following lists the inputs required to address the principal study questions:

- Results of ALAD/blood tests will be used to determine if lead has caused a reduction in enzyme activity.
- The lead concentrations found in the organs of the mammals will be compared with toxicity values from other studies. Metrics will be used to determine if there is a positive correlation between lead values in the soil and/or earthworms and the level of lead found in the animals.

- The lead levels in the femurs of the animals will increase with greater exposure and increased age of the animal.
- Histopathology of organs (lesions, cysts, etcetera).
- Soil metals concentrations
- Earthworm metals concentrations

3.7 Study Boundary

The study will take place primarily in the VTLMD and in the OLB. The reference sites will be located at the Mill Creek Recreational Area, Council Bluff Lake, and/or Cedar Creek Recreational Area. The sampling locations will be located at Washington State Park, St. Joe State Park, and in the Viburnum Trend at three sub-sites: the Magmont Vent Pipe Site, at Highway 32 and State Route KK, and West of Buick Smelter. See Figure 4 for a map of the study locations.

Small mammal trapping will take place in 2014 over the course of two to three potentially non-consecutive weeks in May.

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3.8 Tolerable Limits on Decision Error

The hypothesis is that the high lead levels in the soil will be ingested by soil dwelling invertebrates, such as earthworms, and be taken up by certain plants. Small mammals that feed on these invertebrates and plants will uptake lead directly through ingestion of food or incidentally by eating food with soil particles attached.

3.9 Sampling Design

A combination of Sherman live traps, Longworth live traps and pitfall traps will be used to collect the small mammals in a sampling grid of 150 meters by 150 meters. Traps are to be placed 10 meters apart. A trial run will be scheduled to determine the ease of which the pitfall traps can be installed, since much of the Viburnum area is glade. The numbers of traps used will depend on preliminary sampling - possible numbers are 25 pitfall traps, 100 Sherman live traps

and 100 Longworth traps per site. Earthworms and soil samples will also be collected at the time of trapping.

3.10 Sampling Time Frame

A sample test run will take place one week in April, 2014. The purpose of this test run is to be certain that our trapping, processing and analytical methods are effective and that we can capture and process the necessary number of small mammals needed for a robust study outcome.

The actual timeframe for the study is currently scheduled for May 2014. The capture rate of mammals may be higher in the spring as opposed to autumn sampling. Also, the logistics determined from the test run will help to determine if we are fully prepared to do a full blown study this spring or if more preparation time is needed to obtain the best possible data.

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	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
1	S	L	S	S	L	S	S	L	S	S	L	S	S	L	S	1
2	L	P	L	L	P	L	L	P	L	L	P	L	L	P	L	2
3	S	L	S	S	L	S	S	L	S	S	L	S	S	L	S	3
4	S	L	S	S	L	S	S	L	S	S	L	S	S	L	S	4
5	L	P	L	L	P	L	L	P	L	L	P	L	L	P	L	5
6	S	L	S	S	L	S	S	L	S	S	L	S	S	L	S	6
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9	S	L	S	S	L	S	S	L	S	S	L	S	S	L	S	9
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11	L	P	L	L	P	L	L	P	L	L	P	L	L	P	L	11
12	S	L	S	S	L	S	S	L	S	S	L	S	S	L	S	12
13	S	L	S	S	L	S	S	L	S	S	L	S	S	L	S	13
14	L	P	L	L	P	L	L	P	L	L	P	L	L	P	L	14
15	S	L	S	S	L	S	S	L	S	S	L	S	S	L	S	15
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	

Figure 5. Sampling Grid (P=Pitfall, S=Sherman, L=Longworth)

4.0 Small Mammal Collection

Sampling will be conducted in two teams of two personnel to bait, set, and check the traps. Staff from the UM-SVM will be present to process the animals. Processing of the animals will include drawing blood, euthanizing the animals, and removing the organs to be analyzed. The blood will be placed on ice and transported to the MDHSS lab for lead analysis. Animal tissues and bone will be placed on ice and transported to UM-SVM for femur lead analysis and tissue pathology. Blood samples for the ALAD analysis will be frozen with liquid nitrogen and placed on dry ice to be delivered to USGS Patuxent Wildlife Research Center.

4.1 Sampling Methods

4.1.1 Mammal Trapping

All traps will be provided with Dacron bedding to prevent hypothermia in trapped animals overnight. The bait used in the traps will likely consist of a peanut butter and rolled oats combination along with freeze-dried mealworms to ensure the a variety of small mammals will be caught. The pitfall traps will also have Dacron bedding placed in them but will have live mealworms as bait, since we are primarily looking for shrews with this type of trap. All types of traps will also be provided with a source of hydration such as a gelatin cube.

Two teams of two people will be needed to check the traps twice daily when traps are opened in the morning. Traps will either be checked every few hours or be closed. In the event of warm weather, over 90 degrees, the traps would be checked every two hours. This method of checking traps will be used for the next three to four consecutive days, depending upon how many animals are captured. This will be determined in the field.

4.1.2 Mammal Handling

If an animal is found in a trap, the animal will be removed and placed into a 5 gallon bucket to be transported to the processing station. A China Marker (aka grease pencil) will be used to mark the location of the trap on the lid of the 5 gallon bucket. The trap will then be cleaned out using

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gloved fingers, re-baited, and re-set. The handling and processing station should be centralized between the sites being trapped, if possible. This will increase efficiency in the handling of the animals and decrease drive time, eliminating additional stress on the animals.

4.1.3 Mammal Processing

Trapped animals will be placed into "shrew boxes" with food and water until they can be processed. Food should be removed one hour prior to anesthetizing the animal. Live animals should be anesthetized with Carbon Dioxide (CO_2). The animals will be euthanized via exsanguination and CO_2, if necessary. Animal handling and care will comply with guidelines of the American Society of Mammalogists (Sikes 2007) or site specific methods developed as part of the study.

All animals will be deeply anesthetized to Plane 3 of anesthesia (animal has reduced respiration and surgery can be performed) to exsanguination after blood samples are collected. Animals will be anesthetized using CO_2 gas in a chamber. Chamber Method: This method employs a top opening chamber that is charged with CO_2 after introducing the animals. To operate the unit, turn only the valve on the tank (not the regulator valve). After the animals are placed in the chamber, a slow flow of CO_2 should be continued for a few minutes to maintain a high concentration at the bottom of the chamber. (Casteel, email)

Blood samples will be obtained from anesthetized animals by axial cut with surgical scissors and collected using heparinized capillary tubes or other appropriate sample containers. One hundred microliters (µL) of blood is necessary for the ALAD analyses. The samples will be placed in small plastic vials, frozen in liquid nitrogen and shipped in containers with dry ice. The amount of blood needed for field hematocrit is dependent upon the size of the collection tubes and the centrifuge used. No preservation is needed as this analysis is conducted in the field. For blood lead analyses, between 50 and 100 µL is needed (with the latter preferred). The sample will be in a small plastic heparinized vial and will be hand carried on ice. Holding times for samples are

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different for ALAD and lead analyses and are laboratory dependent. Samples will all be properly labeled for easy identification by both the laboratories and the Trustees for sample analytical reporting.

Tissue samples used for histopathological analysis should be fixed in 10% neutral buffered formalin (37% formaldehyde) and will be taken back with staff from UM-SVM to be analyzed. Samples will be properly identified with labels for ease in analyzing and reporting results.

4.2 Sampling Order

We plan to start sampling at the mining sites and finish with the reference sites. The Viburnum Trend sites will be done first, with the state parks being second, and finally the reference locations. We plan to sample two to three sites at a time with a week for trapping. Therefore, the total sampling time will likely be two to three weeks.

4.3 Sample Quantity

We plan to collect about 100 small mammals for the main study and 10 animals in preliminary trapping. Blood from those 10 animals will be used adjust the ALAD method for use with small samples of blood. It is likely that the majority of animals will be Peromyscus species (mice) but ideally we are targeting shrews, since they are insectivorous and their primary diet consists of earthworms.

4.4 Analyses Requested

All small mammals will be analyzed for blood ALAD; oxidative stress; DNA damage assay; kidney, spleen and liver tissue histopathology; blood histopathology; organs metals analysis; hematocrit; blood lead; and femur lead concentrations. Non-depurated worms will be analyzed for whole body metals.

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4.5 Chain-of-Custody

All blood samples and other tissue samples will have a chain-of-custody (COC) from the laboratory that is conducting the analyses; this will be sent with the samples to the lab.

5.0 Earthworm/Soil Collection

Earthworm and soil samples will be collected across a range of soil Pb concentrations to estimate the potential exposure of small mammals to Pb. Small mammals feeding on earthworms will incidentally ingest internal and external soil associated with earthworms.

5.1 Earthworm collection

Earthworm tissue contains some Pb, but the majority of Pb in tissue is generally much less than the soil contained in their intestines. During the May sampling of small mammals, earthworms and associated soils will be collected from holes dug at the locations of the mammal sampling. The earthworms will be rinsed with distilled water and frozen prior to depuration of the ingested soil. Earthworm samples will be sent to USGS Columbia Environmental Research Center (CERC) to be analyzed for Pb, iron (Fe), copper (Cu), nickel (Ni), Zn, Cd and aluminum (Al). Aluminum is used as a marker/indicator of soil. The samples will be analyzed using inductively coupled plasma-mass spectrometry (ICP-MS). Lab duplicates will be analyzed at a rate of 10% of total samples collected.

5.2 Soil Collection

Prior to placing the small mammal trapping grids, the area will be checked for Pb concentrations by using an X-ray fluorescence (XRF). If the area has an average Pb concentration of greater than 1000 ppm, then the area is suitable for trap placement.

Once the grids are placed, an incremental composite sampling (ICS) approach will be used to collect soil samples within the sampling units (SUs) (aka trapping grids). ICS is designed to obtain a single soil sample that contains contaminants in the same proportion in the sample as

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they are present in the volume of soil of interest (i.e. representative samples). This is achieved through the collection of many increments of adequate-mass systematically across each SU from the same depth (0-2 inches). A total of 30 increments will be collected from each SU (each sampling grid) resulting in one composite sample. These samples will be analyzed in-house using Olympus brand XRFs. Confirmatory samples (at a rate of 10% of total samples collected) will be sent to the USGS-CERC for analyses using the same method as the earthworms (ICP-MS). Soil samples will be analyzed for Pb, Zn, Cd, Cu, Al, Fe and Ni.

6.0 Quality Control

6.1 Field Decontamination

Clean disposable latex gloves will be worn over leather gloves by staff handling traps and/or the animals directly. Traps will be decontaminated after an animal has been trapped only if the trap is too dirty for re-use. All traps will be decontaminated when trapping is completed at that site. The traps will be cleaned with Simple Green Cleaner and rinsed with deionized (DI) water and allowed to air dry.

6.2 Quality Assurance/Quality Control (QA/QC) Samples

The following samples will be collected as part of the quality control/quality assurance procedures for the investigation: hematocrit; ALAD analysis; blood lead/blood tissue; blood/tissue histopathology; femur analysis; oxidative stress/DNA damage assay.

6.2.1 Laboratory QC

Laboratory precision and accuracy will be assessed as described in the Quality Assurance Project Plan (QAPP) for Natural Resource Damages, Revision 3, April, 2011. See Appendix F.

7.0 Investigation Derived Wastes (IDW) Plan

Efforts will be made to minimize IDW generation. IDW may include animal carcasses, body fluids and disposable personal protective equipment (PPE).

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Disposable PPE and disposable sampling equipment will generally be handled as solid waste, containerized, and properly disposed. Wash and rinse waters generated during the processing of the animals will generally be discharged to the ground on-site or, if warranted, containerized and returned to the UM-SVM for proper disposal. Animal carcasses will be handled at the UM-SVM as defined in their Standard Operating Procedures (SOPs).

8.0 Site Safety

A safety briefing will be held on-site prior to initiating field activities and field personnel will be required to read and sign the site-specific health and safety plan. The site safety plan is attached as Appendix A.

9.0 Reporting

Samples results will be given in reports from the respective laboratories after samples have been analyzed and results have been quality checked.

All ALAD activity values will be reported as nmol/ALAD used/min/mL RBC (red blood cells). Hematocrit will be reported as a percentage or fraction. Blood lead will be reported as µg/dL. All tissue lead values (including kidney, liver, spleen, and femur) will be reported as µg/g dry weight. Some samples may be reported as wet weight and converted to dry weight numbers.

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SIGNATURES

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Hazardous Waste Program
Missouri Department of Natural Resources

Date: _____

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APPENDIX A

Health & Safety Plan

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MISSOURI DEPARTMENT OF NATURAL RESOURCES

DIVISION OF ENVIRONMENTAL QUALITY

HAZARDOUS WASTE PROGRAM

SITE HEALTH AND SAFETY PLAN
for the
SOUTHEAST MISSOURI SMALL MAMMAL STUDY

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1.0 INTRODUCTION

This plan has been prepared for implementation by DNR employees, using operating procedures for which they are specifically trained. Any use of the plan by other agencies, organizations, or private individuals is at their own risk.

2.0 KEY PERSONNEL

STUDY CO-LEADER: Kathy Rangen

STUDY CO-LEADER: Hillary Wakefield

SAFETY OFFICER:

OTHER PERSONNEL/TITLE:

John Nichols, Environmental Specialist

Ramona Huckstep, Environmental Specialist

Eric Gramlich, Unit Chief

Sean Counihan, Environmental Specialist

John Weber, USFWS

Dave Mosby, USFWS

3.0 SITE INFORMATION

CONTAMINATED SITE NAMES: a.) Washington State Park b.) St. Joe State Park c.) Viburnum Trend (includes 3 subsites on USFS properties)

REFERENCE SITE NAMES: d.) Council Bluff Lake (USFS) e.) Mill Creek Recreation Area (USFS)

COUNTIES: Washington (a), St. Francois (b), Iron (c & d), and Phelps (e)

SAMPLING DATES: April 29th through but not limited to May 31, 2013

Site Description:

Investigation of mining chat piles in the Viburnum area.

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3.1 Overall Incident Risk/Hazard Analysis

Chemical: _____Serious _____Moderate XX Low _____Unknown

Physical: _____Serious _____Moderate XX Low _____Unknown

3.2 Contaminant(s) of Concern: Lead (Pb), Cadmium (Cd), Copper (Cu), Nickel (Ni), Zinc (Zn), and Arsenic (As).

3.2.1 Physical State: _____Liquid XX Solid _____Sludge _____Gas/Vapor

Chemical Characteristics: (check all that apply)

a. _____carcinogen _____b. biological _____c. corrosive _____d. combustible

e. _____explosive _____f. flammable _____g. volatile XX h. poison

i. _____radioactive _____j. reactive k. other: _____

3.2.2 Physical Hazards: (check all that apply)

a. _____overhead _____b. below grade _____c. confined space* _____d. noise

e. _____splash _____f. fire/burn _____g. puncture _____h. heat stress

i. _____cut XX j. slip/trip/fall XX k. cold stress _____l. electrical

m. _____mechanical/heavy equipment XX n. other: animals, insects, plants, hunters.

* The need for confined space entry by ESP personnel shall be evaluated on a site-by-site basis. A confined space entry permit must be signed by the appropriate Unit or Section Chief prior to ESP employees entering a confined space (29 CFR 1910.146). Confined space entry shall be screened in at least Level B prior to downgrade. **Adequate resources must be available and specific planning and tasks determined before confined space entry is initiated.**

3.2.3 Biological Hazards:

3.3 Task-Specific Risk Analysis (attach additional sheets as necessary)

Task Description	Chemical Hazards	Physical Hazards	Level of Protection
Sample Collection	h	J,k,n	D

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4.0 MEDICAL SURVEILLANCE AND PERSONNEL TRAINING REQUIREMENTS

All ESP field personnel participate in a medical monitoring program and are trained at least to the level of "Hazardous Substance Emergency Response-Technician" as required and specified in the department's written health and safety program located in Section 2 of the MDNR-Hazardous Substances Emergency Response Plan (HSERP). The written policy satisfies requirements set out in 29 CFR 1910.120. MDNR ESP's respiratory protection program meets the requirements of 29 CFR 1910.134.

ESP personnel will ascertain as much information as possible regarding health and safety issues associated with the site prior to initial entry. Information shall include chemical and physical hazards as listed above, types and amounts of materials involved, and citizens/areas threatened by the incident.

5.0 PERSONAL PROTECTIVE EQUIPMENT

ESP shall utilize the Protection Level categories defined in 29 CFR 1910.120, Appendix B, and known as Levels A, B, C, and D. Refer to Section 2 of the MDNR-HSERP for definitions of Protection Levels. ESP personnel shall inspect APRs and SCBAs at least monthly and maintain a record of such to ensure equipment is functional.

Levels of protection shall be reassessed and upgraded as conditions change and information is updated to comply with worker safety while performing site activities.

Action Levels for evacuation of work zone pending reassessment of conditions:
Level D: O_2 < 19.5% or > 25%; explosive atmosphere > 10% LEL; organic vapors > background levels; other_____.
Level C: O_2 < 19.5% or > 25%; explosive atmosphere > 20% LEL; organic vapors (in breathing zone) > 5 m.u.; other___.
Level B: O_2 Explosive atmosphere > 20% LEL; unknown organic vapors (in breathing zone) > 500 m.u.; other_____.
Level A: ESP personnel shall evaluate the need for entry on a site-specific basis and may utilize its emergency response contractor for Level A situations which may arise.

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6.0 FREQUENCY AND TYPE OF AIR MONITORING/SAMPLING

Instrument	Contaminant of Concern	Sample Location (Area/Source)	Frequency	Odor Threshold/ Description
NA				

7.0 SITE CONTROL MEASURES

7.1 The "Buddy-System": ESP personnel performing any work activities within the exclusion zone shall employ the "buddy-system" at all times, as required and defined in Section 2 of the MDNR-HSERP. The "buddy-system" may not be required while an ESP staff member is observing or providing oversight of cleanup activities performed by a contractor or responsible party.

7.2 Safe work Practices: Refer to Section 2 of the MDNR-HSERP for written safety practices to be followed at all times by ESP personnel while on-site at an incident.

7.3 Site Communications: The use of two-way radios or establishment of hand signals for communications shall be determined prior to entering the work zone and followed by ESP personnel.

7.4 Radiation Safety: Due to the possibility of an unknown radiation hazard being present on a site, ESP personnel shall be required to wear radiation indicator badges (TLD badges) while on-site.

7.5 Work Zones: ESP personnel shall ensure work zones are established and be aware of their locations.

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8.0 DECONTAMINATION PROCEDURE/SOLUTIONS:

Personnel: Gloves and clothing will be placed in a garbage bag and returned to Jefferson City for proper disposal.

Equipment: Returned to Jefferson City for proper decontamination.

Instruments: Returned to Jefferson City for proper decontamination or disposal.

Decontamination fluids/materials may be containerized for proper disposal.

9.0 EMERGENCY INFORMATION:

In the event of an emergency, notify the MDNR Environmental Emergency Response Office at 573/634-2436. The Duty Officer will make the appropriate notifications.

10.0 ADDITIONAL EMERGENCY INFORMATION/NUMBERS:

Hospital: Salem Memorial Hospital, 35629 Highway 72, Salem, MO (573) 729-6626

Location/Specific directions from Site: See Map directions.

	Name/Location	Telephone Number
Ambulance:	Iron County Ambulance	(573) 244-5966
Police/Sheriff:	Viburnum Police Department	(573) 244-5220
Fire:	Quad County Fire Department	(573) 244-5440

Poision Control:

Cellular Telephones/Other:

1) Central Accident Reporting Office- WORK RELATED INJURY 1-800-624-2354

This number is to be called in the event of a NON LIFE THREATENING injury PROIR to seeking medical care.

11.0 SIGNATURES

ESP personnel shall certify they have read the plan and addressed any questions regarding worker health and safety by signing and dating below followed by printing their name and title.

Signature	Printed Name/Title	Date	TLD Badge

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APPENDIX B

Field Sheets:

Sampling Area Field Sheet

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Small Mammal Study Field Sheet

Date:_____

Study Area/Site:_____ Reference Site (Y/N):_____

Time of Trap Check:_____ Team Members:_____

Time	Location (ex:C3):	Genus	Comments (ex: new trap,dead animal, etc)

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APPENDIX C
Study Area Map

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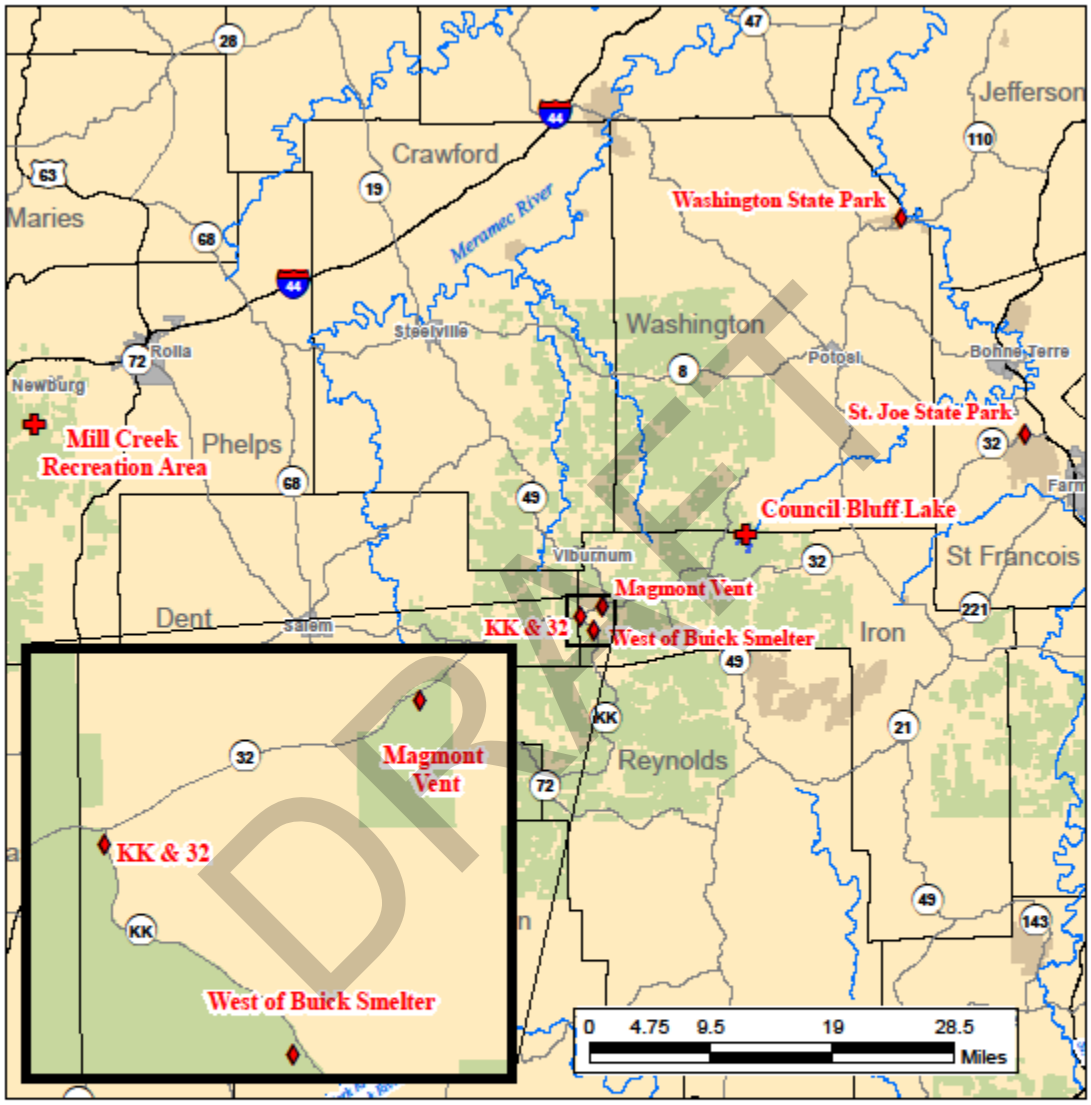
Small Mammal Study Proposed Sampling Sites



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Legend

- Contiminated Site
- Reference Site
- US Highway
- Missouri Roads
- Major Rivers
- Missouri County Boundaries
- Municipal Boundary
- State Parks
- Mark Twain National Forest

Map created by Hillary Wakefield in March 2013.

This map can be found at M:\Superfund\NRD\
Small Mammal Study\Small Mammal collection points.mxd

Although data sets used to create this map have been compiled by the Missouri Department of Natural Resources, no warranty, expressed or implied, is made by the department as to the accuracy of the data and related materials. The act of distribution shall not constitute any such warranty, and no responsibility is assumed by the department in the use of these data or related materials.

Missouri Department of
Natural Resources
Division of Environmental Quality
Hazardous Waste Program

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APPENDIX D

Standard Operating Procedures

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Missouri State Public Health Laboratory
101 N. Chestnut St.
Jefferson City, MO 65101

Title: Blood Pb Determination by Graphite Furnace Atomic Absorption Spectroscopy

Author(s): Alan Schaffer

Authorized By: Alan Schaffer

OU Name: Chemistry Unit

Date/Time of Last Update: 05-Feb-2014 13:14

Version: 22.1

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	Quality System Procedure **Missouri State Public Health Laboratory** **Chemistry Unit**	**Author: Chemistry Unit** **Issue Date:** 07-01-1983
Blood Pb Determination by Graphite Furnace Atomic Absorption Spectroscopy (AAS)		

Revision Approved By: Alan Schaffer Date: 2-4-14

Laboratory Director Approval: Date: 01/30/14

CLIA Director Approval: Ella M Swierkosz Date: 01/28/14

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Missouri Department of Health and Senior Services
Missouri State Public Health Laboratory
101 N. Chestnut Street
Jefferson City, MO 65101

Blood Pb Determination by Graphite Furnace Atomic Absorption Spectroscopy (AAS)

Blood Pb Determination by Graphite Furnace Atomic Absorption Spectroscopy - Version: 22.1. Index: Chemistry Unit 59. Printed: 05-Feb-2014 13:17

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I. Summary and Explanation of the Test

A. Intended Use

This method has been developed for use in the Missouri State Public Health Laboratory (MSPHL), Missouri Department of Health and Senior Services. It is intended for routine screening (i.e., fingerstick via capillary tubes, known as capillary analysis) and confirmation (using lead-free venous tubes, known as venous analysis) of samples received from childhood lead screening programs.

B. Summary and Explanation

Lead is determined in blood by electrothermal atomization atomic absorption spectrometry using a method based on that described by Miller, et al (1). Measurement is based on the amount of light absorbed at 283.3 nm by ground state atoms of lead from a lead lamp. Blood samples, blood-based quality control materials and water standards are diluted 1:10 with a matrix modifier solution containing nitric acid, Triton X-100 and ammonium dihydrogen phosphate. The lead content is determined using an atomic absorption spectrometer equipped with Zeeman background correction. Lead contamination must be carefully avoided throughout all procedures. All materials used for collecting and processing samples should be checked for possible lead contamination. All work must be performed under clean conditions and protected from dust.

C. Interferences

This method is free from interferences when using matrix modifiers. Matrix modifiers permit the removal of interferences during charring by retarding the analyte atomization. Use of ammonium dihydrogen phosphate ($NH_4H_2PO_4$) matrix modifier controls volatility of both the analyte and the contaminants.

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II. Sample Requirements and Processing

A. Sample Collection

Instructions for collection are provided to clients through our website at http://health.mo.gov/lab/bloodleadanalysis.php

B. Sample Transport and Storage

1. Federal regulations (42CFR Part 72) require that blood specimens be packaged according to guidelines requiring the use of tertiary containment and sufficient absorbent material in the event that the specimen leaks during transit.

2. Venous blood specimens preserved with EDTA or heparin are stable for determining lead levels in blood for up to ten (10) weeks if refrigerated at 4° C. Refrigerated temperatures are not necessary for mailing blood lead specimens. When

significant delays are expected, as might occur over a holiday weekend, it is prudent to store the sample locally refrigerated at 4° C, and ship them the next business day.

C. Processing (acceptability and rejection of specimens)

In the case of a problematic sample, attach a post-it note to the Blood Lead Form stating the problem. Log in sample last. Flagged Blood Lead Forms requiring calls need the following information documented in red on the form:

a. Initials of person making the call, date and time.
b. First and last name of person contacted.
c. Information received.
d. QA Event description statement in "For Lab Use Only" box.

The copy of a faxed information sheet with documentation and written authorization is kept with the results. The following are possible reasons for unacceptable blood lead samples:

1. Sample submitted in collection device other than MSPHL device.

   a. Venous tubes not provided by MSPHL must be certified lead free by the manufacturer. If the provider cannot provide manufacturer lead free certification, then the submitter is contacted and given the option of having the MSPHL 1) analyze the sample as a screen (i.e., a capillary sample), 2) return the sample unanalyzed, or 3) destroy the sample. If the submitter chooses to have the sample analyzed, then the blood Pb form is marked to indicate the sample is analyzed and reported as a capillary.

   b. A sample received in a capillary tube not provided by MSPHL is analyzed normally (as a screen), provided that everything else is acceptable. Call the submitter the first time they use alternate tubes, in order to inform them that MSPHL collection devices are sent to them free-of-charge.

2. Sample ID does not match ID on test request form.

   a. Under "For Lab Use Only" write "Specimen ID does not match".

   b. If this situation occurs the contact person for that facility is called and the discrepancy is discussed. A fax is sent to the submitter requesting proper written documentation of the problem. A return fax with the correct information must be received before the sample is tested and reported.

   c. The following form is faxed to the submitter:

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Patient Information and Verification Form

The Missouri State Public Health Laboratory received a blood lead testing specimen for which the form or tube was incomplete or mislabeled.

Name on tube was: _____

Name on form was: _____

Please fill out the **required** information below and fax this form to the chemistry department at (573) 522-4032.

Your Facility Information

Facility Name: _____

Address: _____

Patient Information

Name: _____

Date of Birth: _____

Sample Collection Date: _____

Reason the form and tube did not match: (Example; used mother's last name)

_____

Verification Statement

I am able to identify this specimen positively as originating from the patient named above. I accept responsibility for the sample identification and any resulting liabilities.

Signed _____ Date _____

Print Your Name _____

Upon receipt of proper documentation, attach it to the Blood Lead Form. Correct the name on the Blood Lead form, if needed. Initial and date any changes.

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Recognizing that submitting facilities have individual turnover and personnel problems, the enforcement of this policy will be on an individual basis to be handled by the QA coordinator, laboratory supervisor or their designee.

3. No return name/address on the information sheet.
   a. The information is taken from the mailer, if provided, or call the attending physician if no information is provided. In "For Lab Use Only" box write appropriate QA event description statement.

4. Sample(s) submitted that have clots or no identifying information on sample are unsatisfactory and will not be analyzed.
   a. In the "For Lab Use Only" box mark "Unable to analyze" and the appropriate box, "Insufficient Quantity", "Specimen clotted , "Specimen Leaked in Transit", "No identifying information on specimen", or "Incorrect Collection Device".

5. When a facility has been contacted about samples with identifier problems, samples are held for two weeks. If a completed patient information and verification form is not received, the specimen will be rejected for testing and the facility/requesting entity will be notified.

D. Sample Log-in

1. All blood samples are to be confined to a designated area. This includes but not limited to:

   a. Sample log-in bench and biological safety cabinet (BSC)
   b. Refrigerator for storage
   c. Racks for samples
   d. Eppendorf pipettes and other equipment that could be contaminated with blood must be sanitized before using in another area.

2. Blood sample containers are opened in BSC only. Before opening or transporting any sample put on (this is the minimum protection required):

   a. Protective gloves
   b. Safety glasses
   c. Liquid repellant lab coat

3. Open mailers, remove samples and information sheets.

   a. Match sample with information sheet.

   b. If the form is not marked for capillary or venous, do so with red ink and include QC

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Event description statement in "For Lab Use Only" box.

c. If there is blood on the information sheet:
   i. If one or two of the three copies, remove those copies.
   ii. If all the copies, transcribe the information to a new sheet. Put all contaminated sheets in biohazard bag.
   iii. Include appropriate QC Event description statement in the "For Lab Use Only" box.

d. Put centrifuge tubes and cotton into a biohazard bag and the inner plastic screw top shipping tubes in a clear unmarked bag.

e. Check for problematic samples and follow the Processing guidelines (acceptability and rejection of specimens) on page 4. All information sheets are checked for the CLIA Standard 493.1241 required information. After opening the mailer, check the form to see if the facility name and address are on the form. If it is not present the mailer may be used to get that information. If form does not have the required information, log in the sample last and try to acquire the required information. Place the sample in refrigerator and put unknown with the sample number on the board as a reminder that there is an unknown that is in the process of determining the unknown information. If the information cannot be acquired the sample will be reported as "**Unable to Analyze**" due to whatever is missing. The following required information must be checked before giving a laboratory log number:
   1. Name and address of the entity submitting the specimen.
   2. The patient's name.
   3. The sex and date of birth of patient.
   4. Specimen collection date.
   5. Capillary or venous sample check box checked.

f. Venous samples are logged in first followed by capillary samples (i.e. given a number).

4. Place number tape on sample tube and using the Rapidprint machine, stamp the Blood Lead Form in the space provided for the state lab serial number, date and time.

5. Write the lab sample number for the first sample in the next run, or any skipped samples, on the dry erase board.

6. Wipe down all surfaces daily with germicidal wipes.

6. Properly dispose of the biohazard bag in the biohazard receptacle for autoclaving and disposal by the Laboratory Sterilization room personnel.

7. Mailers are taken to Central Services. Plastic shipping tubes are bagged and placed in the biohazard receptacle for autoclaving by the Laboratory Sterilization room personnel.

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8. Mailers and plastic shipping tubes must be free of blood contamination. Contaminated mailers and plastic shipping tubes should be disposed of in the biohazard bag waste container.

9. Information sheets are given to clerical for data entry.

10. After data entry the information sheets are put into the blood lead logbook.

E. Sample Retention and Disposal

1. All QC must be in control and verified before any written or verbal result is released.

2. Samples can be disposed of after release of results. See **Sample and Waste Retention and Disposal SOP**.
   a. Capillary samples are disposed in a biohazard bag.
   b. Venous samples are disposed in a sharps biohazard container.

3. Samples with identifier problems see **II. C. 5. Processing (acceptability and rejection of specimens) on page 4.**

III. Material Requirements

A. Equipment and Apparatus

1. Perkin-Elmer Model 600 Atomic Absorption instrument.- Graphite furnace AA using transverse heated graphite atomizer with longitudinal Zeeman background correction and STPF conditions
2. Vortex Mixer
3. RapidPrint Date/Time/Log Number Stamper
4. Rotamixer
5. Eppendorf Digital Pipette 10-100 µL with Eppendorf yellow tips
6. Eppendorf Digital Pipette 100-1000 µL with Eppendorf blue tips
7. Eppendorf Digital Repeater plus Pipette with reusable 5 mL tips for matrix modifier
8. THGA Graphite Tubes with Integral Platform (P.E.#BB3000-655)
9. Blood collection tubes (lead free, heparinized or EDTA)
10. Capillary Blood Collection devices
11. Disposable autosampler cups, 2-mL
12. Plexiglas 2-mL autosampler cup holder
13. Laboratory tissues, e.g., Kimwipes™
14. Germicidal disposable wipes
15. Sharps biohazard container for venous tubes and pipette tips
16. Biohazard bags for non-sharps biohazard materials
17. Clear unmarked bags for plastic shipping tubes

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B. Reagents, Standards, and Controls

1. Lead stock solution, traceable to National Institute of Standards and Technology standards, ~10 mg/g, or alternatively 10,000 mg/L. Sources include NIST SRM 3128 and SPEX Cat# PLPB2-3Y.
2. Concentrated nitric acid (HNO_3), trace metal grade (TMG)
3. Deionized water
4. Triton X-100, non-ionic detergent
5. Ammonium dihydrogen phosphate, $NH_4H_2PO_4$. Use a grade that is lead free.
6. Argon gas, ultra high pure grade 5, equipped with an approved gas regulator.
7. Blood-based quality control materials, including a low level (< 20 µg/dL) and a high level (30-60 µg/dL). Sources include NIST SMR 955c, Kaulson Laboratory (Contox), and Bio- Rad. Freeze dried material is stored in Refrigerator 5310-2 at 2-8 ºC. Reconstituted controls are stored in Freezer 5310-1 at or less than -20ºC. See the Certificate of Analysis binder for the current standard CoA's.
8. Rinse Solution, comprised of TMG Nitric Acid, 10% Triton X and deionized water.

IV. Preparatory Procedure

Blood Lead Water Standards: See QA Manual for labeling requirements.

Standard Intermediate: 10.0 mg/L intermediate Pb stock in 1% HNO_3

Add 1.0 mL stock to 1000 mL volumetric flask. Add 10.0 mL of conc. HNO_3 and fill to mark with deionized water. Stable for three months stored at room temperature in Room 5310.

Working Standard Solutions: Prepare the 30 ug/dL and 60 ug/dL solutions monthly. Store at room temperature in bio-safety cabinet. New standards are compared to old standards before they are put into use. The 30 ug/dL standard must fall within its QC limits and the 60 ug/dL must be within 2.3 ug/dL of the original standard result. Run the A and B tubes of both the 30 and 60 ug/dL solutions along with the currently used standards for comparison. If any tested venous samples are above 60 ug/dL then prepare the 100 ug/dL standard solution. Recalibrate, including the 100 ug/L standard, when rerunning a sample that is greater than 60 ug/L. Capillary samples are screening samples and are not rerun with the 100 ug/dL standard.

1. **30 µg/dL** – Add 3.0 mL of 10.0 mg/L intermediate Pb standard, plus 1 mL of conc. HNO_3, into100 mL volumetric, then fill to mark with deionized water.

2. **60 µg/dL** – Add 6.0 mL of 10.0 mg/L intermediate Pb standard, plus 1 mL of conc. HNO_3, into 100 mL volumetric, then fill to mark with deionized water.

3. **100 µg/dL**[a] – Add 10.0 mL of 10.0 mg/L intermediate Pb standard, plus 1 mL of conc. HNO_3, into 100 mL volumetric, then fill to mark with deionized water.

[a] This standard is only prepared when a sample result requires a calibration range greater than 0 - 60 µg/dL.

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Standards: 50 µL of the working standard solution is added to 450 µL of Triton-X matrix modifier working solution.

A. Matrix Modifier: (0.2% v/v HNO_3, 0.5% v/v Triton X-100, 0.2% w/v (17.4 mM) $NH_4H_2PO_4$)

1. **Stock 10% (v/v) Triton X-100:** Add ~80 mL of DI to a 100 mL flask. Warm DI slightly. Add 10 mL of Triton X-100. Swirl to mix and add DI to mark. Mix thoroughly on a magnetic stirrer for at least one hour. Prepare monthly. Store at room temperature in Room 5310.

2. **Stock 20% (w/v) $NH_4H_2PO_4$**: Add ~75 mL of DI to a 100 mL flask. Dissolve 20 g of $NH_4H_2PO_4$, and add DI to mark. Prepare at least every six months. Store in Refrigerator 5310-2 at 2-8ºC.

3. **Working solution of matrix modifier:** Add ~300 mL of deionized water to a 500 mL flask. Add 1 mL of HNO_3 and swirl to mix. Add 25 mL of 10% (v/v) Triton X-100, 5 mL of 20% (w/v) $NH_4H_2PO_4$, fill to mark with DI, and mix thoroughly[b]. Prepare every 14 days. Store at room temperature in Room 5310.

B. Reagent:

Rinse Solution: In 2000 mL volumetric flask add 20 mL of Trace Metal Nitric Acid, 100 uL 10% Triton X, then fill to mark with deionized water. Store at room temperature in Room 5310 with no expiration date.

C. Safety Precautions

A plastic apron worn over the regular lab coat (a properly closed liquid repellent lab coat may be substituted), protective gloves, and safety glasses must be worn when handling human samples. Opened sample containers must be kept in the BSC. Gloves and gowns must be removed before leaving the work area. Dispose of used items, such as gloves and gowns, in the biohazard bag. At the end of the run, the autosampler cups containing the analyzed samples and all other possibly contaminated disposable materials are put into the biohazard bag. Pipette tips and other sharps are put into the biohazard sharps container. The bench tops, equipment, and pipettes are wiped down with germicidal disposable cloth. The biohazard bag and biohazard sharps container are then properly disposed of. See the *MSPHL Chemistry Unit Chemical Hygiene Plan* for further information.

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[b] Given a modifier concentration in diluted blood of 0.18% w/v, total mass of NH4H2PO4 deposited on platform using a 12 µL injection volume is 21.6 µg.

D. Instrument Batch Preparation:

Sample Information File Sample Locations for P.E. Model 600 AA:

LOCATION	SAMPLE ID	LOCATION	SAMPLE ID
38	blank	5-24, 26-48	samples
1	control low	25, 49	duplicates for QC
2	control high	39	30 µg/dL water cal std
3	control near reporting level[e]	40	60 µg/dL water cal std
4	control mid range		

E. Pipetting Procedure:

1. Place sample cups onto the square Plexiglas sample cup holder. Pipette 450 µL of the matrix modifier into each cup with Repeater plus pipette, taking care to not contaminate the tip.
   Replace the tip if it becomes contaminated.

2. All blood specimens and controls should be swirled gently and at room temperature prior to preparation (using vortex mixer for capillaries and hematology mixer for venous specimens and controls).

3. Pipette 50 µL of the standards, controls, and samples into each cup. Refer to the printed Sample Information file for the sample cup positions. Use a new tip on the Eppendorf Digital pipette (100-1000 uL) to mix solution in each cup [g].

V. Test and shutdown procedure:

A. Instrument Batch Requirements:

1. Standards, controls, and samples must be prepared for each instrument batch at the same time.

2. The calibration correlation coefficient must be 0.997 or greater.

3. Analysis of a sample batch requires analysis of 6 checks after calibration (a low-level control, a high-level control, a mid-range check standard, a mid-range control, a reporting-level control, and a blank), 2 checks after every 10 samples (a mid-range check standard and blank) and at the end of the batch.

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[e] A control at 2.0 µg/dL reporting level is analyzed to verify the lowest reporting value.

[g] 12 µL of this solution is pipetted on the platform by the AS-71 auto sampler.

4. Venous samples are analyzed in duplicate. Any venous sample that is marked as "Confirmatory "or "Follow Up" will be analyzed the day it is received. If the venous sample is marked as routine, or if nothing is marked, it will be analyzed when >30 samples have been received.

5. Capillary samples with a reading of ≥9.5 µg/dL must be analyzed in duplicate if the sample volume permits, if not rerun the original cup. **See Duplication Guidelines.**

6. The duplicate for QC is selected by the following criteria:

   a. Choose the first sample for a duplicate, and every 21st sample after, for a duplicate provided there is enough sample available for analysis. If there is insufficient sample volume, go to the next sample with enough volume.

7. The sample dust cover must be used during analysis.

B. Instrument Settings:

Furnace parameters are located under Method file name BLDLEAD.

C. Instrument Setup:

1. Turn on the argon and verify that the tank pressure is >100 psi and outlet pressure should be ~46 psi.

2. Turn on computer and the PerkinElmer 600 AA. The logon information window will appear on the computer. Enter "chemistry" as the password. Double-click on the WINLAB 32 AA icon. The System Status window will appear, and the instrument software will take approximately 2 minutes to load. Click on the Lamps button after software has loaded. Turn on the Lamp2 (Pb) button. The lamp is warmed up for 45 minutes before analysis begins. Before analysis check the energy of the lamp to make sure it is within those recorded in the BLOODPB.xls QC book and record the lamp energy.

2. To check the number of tube and contact firings, select Diagnostics under the Tools menu. Click on the Furnace tab and then the Set Cycles button. A Set Cycles box will appear. The number of graphite tube cycles and contact ring cycles will be displayed. If the tube has over 600 firings the tube should be replaced. If the contact rings have over 7000 firings the contact rings should be replaced when the tube is changed. See **600 AA Instrument Maintenance**.

3. In the Automated Analysis Control page click on File>Open workspace>Daily run view. Open the Blank Sample Information File. File>Open>Sample Information File> Blank Form> Open>Sample Information File Tab. Enter the Sample ID's in the appropriate positions. After entering all the ID's, save the Sample Information File by selecting File>Save As >Correct year Sample Info File > and enter the new

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file name as MMDDYY>Save. Click on the "Results Data Set Name" Open Button and the select Results Data Set Page will appear. Enter the same file name as the Sample Information File. Print the Sample Information File by selecting File>Print>Active window>OK. If you want to print the complete run list, which includes all the QC samples, look in the Automated Analysis box and click on the "Print list" button. Close the Sample Information window.

4. Pipette the blank, standards, controls and samples. See Pipetting Procedure.

5. Examine the tip of the autosampler. If it is bent or discolored, cut with scalpel and realign the tip. Check the proper alignment of the autosampler tip by analyzing a sample. If the alignment is not correct click on the Furnace Button. The Furnace Control page will appear, click on the Align Tip Button. The Align Autosampler Tip Wizard page will appear. Select Task as needed to perform from list. Press Next. Follow the directions listed on the page. Click finish when alignment task has been completed.

6. Check sample drying by analyzing a blood control and water standard. This can be checked visually with the mirror. Do not move the mirror until autosampler arm has reached the ready position. The sample must dry evenly, without boiling or sputtering. This is especially important when a new tube and/or contact rings have been installed. The temperatures and times are listed in the Method Editor: Blood Lead Method Page.

8. To shutdown the system, turn off the EDL lamp by clicking on the Lamps Button, then Lamp#2 ON/OFF Button, close the WINLAB 32 AA Software, turn off the power to the 600AA, turn off the Argon gas, and shut down the instrument computer.

D. Instrument Analysis:

PerkinElmer 600 AA

1. Load sample cups of Blanks, Standards, Controls and samples in the sample tray positions as indicated on the Sample Information File.

2. In the Automated Analysis Control Page click on the "Analyze All" button to start the analysis. The "Analyze All" button will calibrate first, then run sample cups as listed in the Sample Information File. The "Calibrate" button will calibrate only. The "Analyze Samples" button will analyze samples only. If the instrument stops on its own, due to QC issues, when you restart click on "Analyze All" and a box will pop up letting you choose where to start again. If you stop the instrument you need to go into the MethEd tab> QC>schedule QC> uncheck those QC you do not want to run again>close. Click on the "Reset Sequence" button and then the "Set up" tab. In the Sample Info File column select locations and then input the locations of the samples that are needed. Click on the "Analyze" tab and then "Rebuild list". Verify list is correct and then click on "Analyze Samples".

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3. The sample analysis results are printed out as the samples are analyzed. Print the Instrument Report by selecting File>Utilities>Data Manager (C:\data-AA\Administrator\Results\results.mdb.>Select Result file>Report>Use existing design>Browse>bldPbreport.rep>preview>Print.

E. Maintenance

Refer to the PerkinElmer THGA Graphite Furnace User Guide: Chapter 3 System Maintenance.

All maintenance to the instruments is recorded in the Excel QC folder: BloodPb/600 AA Maintenance Log.

Graphite Tube Change and Conditioning

1. Loosen the autosampler holding screw and swing the autosampler away from the furnace.

2. Select Diagnostics under the Tools menu. Click on the Furnace tab and the number of graphite tube cycles will be displayed. Set the tube cycles to zero (number of cycles are recorded in the maintenance file). Click on the furnace button on the toolbar and open the furnace.

3. See Perkin Elmer THGA Graphite Furnace User Guide Pg. 3-10 to 3-18, for instructions on how to change the tube.

4. The contact rings are swabbed with alcohol before installing the new tube. Wear protective gear when replacing tube.

5. Once the tube is changed, close the furnace and press the Condition Tube button. Make sure the temperature program has started. Conditioning twice may provide better results.

6. Check the autosampler alignment and droplet delivery after the conditioning program has run. See Perkin Elmer THGA Graphite Furnace User Guide, pg. 2-17 to 2-23.

Autosampler Tip Replacement

1. See “Repairing a pipet” in PerkinElmer THGA Graphite Furnace User Guide, Pg. 3-47 to 3-51.

Contact Ring Replacement

1. See “Changing the graphite contacts” in PerkinElmer THGA Graphite Furnace User Guide, Chapter 3, Pg. 3-19 to 3-31, for instructions on how to change the contact rings.

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Blood Lead Atomization Adjustment

1. As the atomization temperature is increased, the Pb absorbance of the blood controls (matrix) decreases at a faster rate than the water standards (matrix). The point at which the absorbance values are equal is selected as the atomization temperature. In general, at the temperature of 1200 the lead starts to volatilize, but the peaks are broad and inconsistent. At 1300 there are consistent and well-shaped peaks, but the blood control absorbance is low compared to that of the water standards. Setting the temperature at 1250 produces consistent, well-shaped peaks in which the blood and water absorbance match.

2. Setting the temperature at 1250 does not take into account variations among tubes during the lifetime of a tube. To solve this problem, the temperature may need to be adjusted in order to avoid running consistently high or low. The atomization temperature needs to be increased 50 degrees if the low and high controls at the beginning of the tray are running above the upper warning limit and the temperature may need to be decreased 50 degrees if the controls are running below the lower warning limit. The temperature should not be decreased to a point in which the water peaks do not come off within the 3-second atomization time. The temperature may have to be increased 50 degrees to keep peaks within the 3-second atomization time. When a change is made in the atomization temperature, it is saved in the element file. The date the change is made, the old temperature, and the new temperature is recorded in the BLOODPB.xls maintenance log for that instrument.

EPPENDORF PIPETTES

1. After use, pipettes are sanitized with Sani-Cloths®. Perform gravimetric testing of accuracy and precision for each Eppendorf and record in Pipette Maintenance and QC notebook yearly or as need indicates.

VI. Quality Control

1. Any unusual circumstance should be reported to supervisor as soon as possible.

2. Reliable QC materials should be used for this analysis. Avoid using 'spiked' blood material that may cause a variety of matrix effects. Ensure that reputable laboratories using several methods establish the QC target value. Sources for standards and QC materials should be independent of each other.

3. Freeze-dried materials must be reconstituted according to the manufacturer's instructions. It should be noted that reconstituted freeze-dried blood, even more so than fresh whole blood, is a suspension rather than a solution and must be well mixed to obtain reliable results. If freeze-dried blood is mixed too vigorously, however, bubbles will result, leading to pipetting errors. There are 3 levels of Bio-Rad or Kaulson Laboratories QC controls used in this method, Level 1 (low level), Level 2 (mid-level), and Level 3 (high-level). The Minimum Reporting Level (MRL) control is made by diluting Level 1 to

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produce the reporting limit value of 2.0 µg/dL. The amount of de-ionized water used in the dilution is totally dependent on the initial concentration value of the Level 1 QC control.

4. Reconstituted vials are mixed on the hematology mixer for at least 20 minutes.

A. Controls

1. X-bar for each blood lead control chart shall be based on control's mean value. The upper and lower limits for control are ± 3 standard deviations and must not exceed ± 4 µg/dL for controls with a value of less than 40 µg/dL or ± 10% with a value greater than 40 ug/dL. Charts are recalculated when a new lot of controls are put in service and at least 7 data points have been accumulated.

2. X-bar and standard deviation must be monitored on each control for each instrument.

3. Precision and accuracy are checked on the instrument using lead in bovine NIST SRMs[j]. NIST SRMs need only be analyzed when a new Bio-Rad or Kaulson control is reconstituted. Aliquots of the reconstituted Bio-Rad or Kaulson control and the comparable NIST Level are analyzed with the old Bio-Rad or Kaulson control and can be analyzed with samples or be analyzed on a separate tray. Results are recorded in BLOODPB.xls QC Book Monthly NIST sheet.
   a. Minimal criteria for NIST controls:

      i. Results < 40 µg/dL must be within ± 4.0 µg/dL, or for results > 40 µg/dL ± 10%, of target values.

   b. Storage of NIST controls:

      i. Controls will be stored at or below -20 degrees C in a non-frost free freezer in room 5310 in the original box and aluminized bag.

   c. When handling refrigerated NIST controls:

      i. Follow Certificate of Analysis Use and Cautions **.** Thaw at room temperature and swirl gently on the hematology mixer. Do not use if clotted or if less than 1/3 of original volume remains.
      ii. Following good laboratory practices: **do not leave controls at room temperature** in excess of time needed to measure control.
      iii. Monitor for deterioration.

   d. Log containing the following will be kept:

[j] NIST SRM - National Institute of Standards & Technology Office of Reference Material. Standard Reference Material consists of blood from lead dosed cows. Target values determined by ICP IDMS (± uncertainly at the 0.95 confidence level).

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i. Date run, analyst, date opened, NIST values obtained, Bio-Rad or Kaulson values obtained.

B. Proficiency Testing

Three times a year the laboratory receives a set of 5 CDC CLIA Regulatory proficiency samples from the Wisconsin State Laboratory of Hygiene (WSLH), the reported results are faxed to the WSLH. If the laboratory misses a PT on a CLIA regulated method (Blood Lead), a Corrective Action Report (CAR) will need to be initiated and the laboratory will need to run an unknown control for an unbiased test of the ability to identify and quantify. The QM will need to prepare the unknown (an old PT) in these cases and it will need to be listed that one was run in the CAR that is being written for the missed PT.

C. Duplicates

Duplicates samples are run every 20 samples and must be recorded in two ranges:

1. Values ≥ 20 μg/dL as +/- 2.3 μg/dL duplicate difference.

2. Values < 20 μg/dL
   If the sample and duplicate sample results are <0.4 (the current MDL), the dup difference is zero (0 minus 0). If one result is above the MDL and the other is below, report the difference as positive or negative for the results above the MDL (e.g. 1.0 – 0 = 1.0, or 0 – 1.0 = -1.0).

Duplication Guidelines

1. Capillary, and venous samples run as a screening sample, have results ≥ 9.6 make a second cup and, if they duplicate, average the two results. If there is not enough to make a second cup, rerun cup #1. If it duplicates, average the two results. If it does not match, report to the supervisor and he/she will determine to report the sample as: a numerical value, QNS, or unable to analyze.

2. If venous sample cups #1 and #2 do not duplicate, rerun both cups. If they duplicate, average the results. If the rerun does not duplicate, make a third cup, run and average the cups that match. If there is not enough to make cup #3, report to the supervisor and he/she will determine whether to report the sample as: QNS, or unable to analyze.

3. If venous sample cups #1, #2, and #3 do not match, report to the supervisor and he/she will determine whether to report the sample as QNS or unable to analyze.

4. For the duplicate QC samples: If the original sample and it's duplicate do not duplicate within the allowed limits rerun the duplicate sample. If it still does not duplicate within limits remake the duplicate or another sample near the beginning of that

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set of 20 samples if there is insufficient blood volume in the tube. Report to the supervisor if that duplication is out of limits for determination of the next course of action.

<u>NOTE:</u> Analyst is required to document all duplication reruns and outcomes.

D. QC Manual And Monthly Maintenance Schedule

1. QC and maintenance must be kept for each instrument.

2. The following information will be kept for controls and duplicates for each instrument:
    a. Date analyzed
    b. Source of control and standard
    c. True value, obtained value
    d. Duplicate difference
    e. Analyst
    f. Date new vial opened/date new standards made.

E. Quality Control for Blood Lead Collection Devices

For new lot numbers of collection devices to be used:

1. The number of devices to be ordered from vendor is reserved with the same lot number.

2. Partial shipment is made, and devices are analyzed for contamination.

    a. Randomly select twenty collection devices from newly ordered lot.
    b. Fill sample containers 1.25 mL of dilute nitric acid (2% v/v)
    c. Store twenty-four hours at room temperature.
    d. Analyze leachate for lead and calculate the total amount of lead extracted.
    e. Report any contamination to supervisor.
    f. Record run date, lot #, analyst, and results on capillary blank chart.

4. If the new lot number meets the criteria, shipment is completed and the new lot number can be shipped to submitters.

5. QC Criteria for collection device- NO result equal to or greater than 2µg/dL and 90 % of results less than or equal to 1 µg/dL.

For lot numbers of collection devices and/or repacks currently being used:

1. Collection devices being shipped to submitters must be checked for contamination when repacks are done in the mailroom.
    a. Randomly select ten collection devices from current stock.
    b. Follow 2.b. through 2.d. of "new lot numbers of collection devices to be used"

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c. Report any contamination to supervisor. If any measurable increase in lead concentration of repacks compared to same lot number results, cause must be found and corrected.
d. Record run date, lot #, repack date, analyst, and results on capillary blank chart.
e. QC Criteria for collection device- NO result equal to or greater than 2μg/dL and 90% of results less than or equal to 1 μg/dL.

F. Preparation of Capillary Kits

1. Contents of kit:
   a. 10 or 50 Capillary collection devices
   b. 10 or 50 Capillary labels
   c. 10 or 50 forms Lab 13C (1-04)
   d. 1 instruction sheet (use copy of the original).
   e. Mailers/return labels as requested.

2. Preparation of kit
   a. Before preparing kits clean work area with soap and water.
   b. Cover work area with bench protectors (obtained from Chemistry Unit).
   c. Prepare a minimum of 20 kits at a time.
   d. All collection devices **MUST** be handled with **POWDER-FREE** gloves .
   e. **DO NOT** handle the collection devices by the capillary (the straw-like part) or the cap.
   f. Label kit with:
      i. Expiration date (from original bag).
      ii. Date kit was prepared.
   g. After all kits for day are prepared take one bag selected at random to chemistry unit for QC checking.

G. Corrective Action to take when Quality Control fails Laboratory Criteria

See the Corrective Action section of the Quality Assurance Manual for the process taken when a quality control failure occurs.

VII. Sample Reporting and Results

1. Before reporting:

   a. Blood Lead Instrument Report must be printed and venous samples averaged. File>utilities>Data Manager>Select result file> Report>Use existing design> Browse>BldPb Report.rep>Preview>Print
   b. Required data is entered into the Blood Pb QC in Excel.
   c. QC must be verified to be in control in NWA.

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d. Instrument Reports/sample analysis results must have a data review (See QA Manual-Data Reduction, validation and Reporting) by a peer analyst.

2. Print the Blood Lead Log sheet from Access. Transcribe results from the Instrument report on to the Blood Lead Log Sheet.

3. Results on the Blood Lead Log Sheet are transcribed onto the Blood Lead Lab form 13C.

4. The completed Instrument report with sample analysis results, log sheet and Blood Lead Lab forms are taken to a peer analyst where the transcription check is completed as follows:

a. The Blood Pb instrument report is checked against the Blood Lead Log Sheet. Initial and date the Blood Pb instrument report and the Log Sheet.
b. The Blood Lead Log Sheet is checked against the Blood Lead Lab Forms. Initial and date all Blood Lead Lab Forms.

5. All Blood Lead Lab Forms are date/time stamped.

6. Blood Lead results are released for distribution.

7. The Blood Lead Log Sheet and Blood Lead Lab Forms are taken to clerical for data entry.

8. Clerical does data entry and generates a Blood Lead Log Sheet with the computer-entered results. This is transcription checked and initialed by clerical.

9. The Blood Pb instrument report with the sample analysis results is filed.

10. The Blood Lead Log Sheet with the computer-entered results is placed in the Blood Lead Log Sheet file folder.

A. Reporting Results

1. All QC must be in control and verified before any written or verbal result is released.

2. The top copy of the Blood Lead Lab Form is sent to the submitter, one copy is sent to the Lead Poisoning Prevention Program, and one copy is filed at MSPHL.

3. Results are expressed as micrograms (μ) lead (Pb) /deciliter (dL) blood.

a. Capillary and venous are reported to the nearest whole number on form Lab 13C. The Reporting limit is <2 μg/dL blood

4. See I:\Chemistry\QC\Excel QC Books\BLOODPB for the Method Detection Limit.

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B. Verbal Release of Results

1. Replying to telephone requests for results:

   a. Blood lead results are NEVER given to a person calling the lab.

   b. Information is taken from the caller.

   c. A return call is made if the original caller is authorized to receive the result.

3. Calling of high results:

   a. All QC must be in control and verified before a result is released.

   b. Any result of $\geq$ 20 µg/dL blood is called to the submitting facility. An analyst or clerical may make the call.

   c. Document on form the first and last name of person receiving results, the date and time of the call, and initials of person placing call.

   d. If the submitter cannot be reached by the end of the day make a copy of the form and send the batch of samples to clerical so that they can be mailed out. After the submitter has been called bring the copy to clerical to be filed.

C. Interpretation of Results and Normal Values

Information to interpret results is found on back of the blood lead sample form. This information and the contact information for the Missouri Department of Health and Senior Service blood lead program can be found on our website at http://health.mo.gov/lab/bloodleadanalysis.php

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VIII. Continuation of Operations

The blood lead plan for continuation of operations in the event the test is unable to be performed at this laboratory is in the MSPHL COOP plan.

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APPENDIX:
REFERENCE SHEET FOR BLOOD LEAD ANALYSIS SOLUTIONS

STANDARD INTERMEDIATE: 10.0 mg/L intermediate Pb stock in 1% HNO_3
Stable for three months stored at room temperature in Room 5310.

1.0 mL 10,000 mg/L stock to 1000 mL in a volumetric flask.
10.0 mL of conc. HNO_3
Fill to mark with deionized water.

WORKING STANDARD SOLUTIONS:
Prepared in an Acid rinsed 100 mL volumetric.

1. **30 μg/dL** – 3.0 mL of 10.0 mg/L intermediate Pb standard
   1 mL of conc. HNO_3, into
   Fill to mark with deionized water.
2. **60 μg/dL** – 6.0 mL of 10.0 mg/L intermediate Pb standard
   1 mL of conc. HNO_3
   Fill to mark with deionized water.
3. **100 μg/dL** –10.0 mL of 10.0 mg/L intermediate Pb standard
   1 mL of conc. HNO_3,
   Fill to mark with deionized water.

MATRIX MODIFIER: (0.2% v/v HNO_3, 0.5% v/v Triton X-100, 0.2% w/v (17.4 mM) $NH_4H_2PO_4$)

1. **Stock 10% (v/v) Triton X-100:**
   Add ~80 mL of slightly warm DI to a 100 mL flask.
   10 mL of Triton X-100. Swirl to mix.
   Fill to mark with deionized water.
   Mix thoroughly on a magnetic stirrer for at least one hour.
   Prepare monthly. Store at room temperature in Room 5310.

2. **Stock 20% (w/v) $NH_4H_2PO_4$:**
   Add ~75 mL of DI to a 100 mL flask.
   Add 20 g of $NH_4H_2PO_4$, dissolve before proceeding.
   Fill to mark with deionized water.
   Prepare at least every six months. Store in Refrigerator 5310-2 at 2-8ºC.

3. **Working solution of matrix modifier:**
   Add ~300 mL of deionized water to a 500 mL flask.
   Add 1 mL of HNO_3 and swirl to mix.
   Add 25 mL of 10% (v/v) Triton X-100.
   Add 5 mL of 20% (w/v) $NH_4H_2PO_4$.
   Fill to mark with deionized water and mix thoroughly.
   Prepare every 14 days. Store at room temperature in Room 5310.

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REAGENT - Rinse Solution:

In 2000 mL volumetric flask
Add 20 mL of Trace Metal Nitric Acid to a 2000 mL flask.
Add 100 uL 10% Triton X.
Fill to mark with deionized water.
Store at room temperature in Room 5310 with no expiration date.

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Date/Time of Last Update: 19-Mar-2013 09:37

Version: 1.0

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	Quality System Procedure **Missouri State Public Health Laboratory** **Chemistry Unit**	**Author: Sondra Kekec** **Issue Date:** 3/19/13
Metals in Food by ICPMS		

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Metals in Food by ICPMS

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Scope

This method provides procedures for determination of metal contamination in food samples. It is applicable to the following elements:

Arsenic (As)	Lead (Pb)
Cadmium (Cd)	Mercury (Hg)
Chromium (Cr)	Vanadium (V)

Summary of Method

The method describes the multi-element determination of trace elements by ICPMS. Food samples will be homogenized, acid digested, and diluted. Then, sample material in solution is introduced by pneumatic nebulization into a radiofrequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass to-charge ratio by a quadrupole mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. The ions transmitted through the quadrupole are detected by an electron multiplier or Faraday detector and the ion information processed by a data handling system. Interferences relating to the technique must be recognized and corrected for. Such corrections must include compensation for isobaric elemental interferences and interferences from polyatomic ions derived from the plasma gas, reagents or sample matrix. Instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix must be corrected for by the use of internal standards.

Interferences

Isobaric elemental interferences - Are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. Of the analytical isotopes recommended for use with this method, only molybdenum-98 (ruthenium) and selenium-82 (krypton) have isobaric elemental interferences. If alternative analytical isotopes having higher natural abundance are selected in order to achieve greater sensitivity, an isobaric interference may occur. All data obtained under such conditions must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. A record of this correction process should be included with the report of the data. It should be noted that such corrections will only be as accurate as the accuracy of the isotope ratio used in the elemental equation for data calculations. Relevant isotope ratios should be established prior to the application of any corrections.

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Abundance sensitivity - Is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Wing overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.

Isobaric polyatomic ion interferences - Are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. Most of the common interferences have been identified. Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions. In particular, the common ^{82}Kr interference that affects the determination of arsenic can be greatly reduced with the use of high purity krypton free argon.

Physical interferences - Are associated with the physical processes which govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute deposits of material on the extraction and/or skimmer cones reducing the effective diameter of the orifices and therefore ion transmission. Dissolved solids levels not exceeding 0.2% (w/v) have been recommended to reduce such effects. Internal standardization may be effectively used to compensate for many physical interference effects. Internal standards ideally should have similar analytical behavior to the elements being determined.

Memory interferences - Result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to 10 times the upper end of the linear range for a normal sample

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analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of 10 of the method detection limit, should be noted. Memory interferences may also be assessed within an analytical run by using a minimum of three replicate integrations for data acquisition. If the integrated signal values drop consecutively, the analyst should be alerted to the possibility of a memory effect, and should examine the analyte concentration in the previous sample to identify if this was high. If a memory interference is suspected, the sample should be reanalyzed after a long rinse period. In the determination of mercury, which suffers from severe memory effects, the addition of 200 µg/L gold will effectively rinse 5 µg/L mercury in approximately two minutes. Higher concentrations will require a longer rinse time.

Safety

The toxicity or carcinogenicity of reagents used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be available to all personnel involved in the chemical analysis. Specifically, concentrated nitric acid presents various hazards and is moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.

The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.

It is the responsibility of the user of this method to comply with relevant disposal and waste regulations.

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Equipment and Supplies

Inductively coupled plasma mass spectrometer:

Instrument capable of scanning the mass range 5-250 amu with a minimum resolution capability of 1 amu peak width at 5% peak height. Instrument may be fitted with a conventional or extended dynamic range detection system. **Note:** If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result.

Radio-frequency generator compliant with FCC regulations.

Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders.

Ammonia gas supply – High purity grade (99.999%). A lecture bottle is used to supply this reaction cell gas.

A variable-speed peristaltic pump is required for solution delivery to the nebulizer.

A mass-flow controller on the nebulizer gas supply is required. A water-cooled spray chamber may be of benefit in reducing some types of interferences (e.g., from polyatomic oxide species).

If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result. Samples having high concentrations of elements beyond the linear range of the instrument and with isotopes falling within scanning windows should be diluted prior to analysis.

MARS Express microwave accelerated reaction system.

Mechanical pipettes.

Centrifuge tubes (15 and 50 mL) and centrifuge tube racks.

Volumetric flasks (polypropylene).

Analytical balance.

250 mL polypropylene reagent bottles.

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Instrument Parameters

Parameter	Setting
RF power	1.45 KW
Ar nebulizer gas flow	0.90-1.05 LPM
Detector mode	Dual
Measurement units	Cps
Replicates	3
Readings/replicate	1
Autolens	On
Blank subtraction	After internal standard
Curve type	Linear through zero
Sweeps/reading	8
Dwell time	75 ms
DRC Channel	A
DRC Gas	Ammonia
DRC – Flow (analysis)	0.5 mL/min
DRC-RPq	0.25
DRC-RPa	0

Settings for Uptake and Rinse Times

	Pump Speed	Duration
Sample flush	-34 rpm	60 seconds
Read delay and analysis	-20 rpm	15 seconds
Wash	-24 rpm	45 seconds

Terminology

Calibration Blank - A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP instrument.

Calibration Standard - A solution of known concentration prepared from the dilution of stock standard solutions. The calibration solutions are used to calibrate the instrument response with respect to analyte concentration.

Daily Performance Check (DPC) - This solution is used to determine acceptable instrument performance prior to calibration and sample analyses by checking the sensitivity, oxides, doubly-charged ions and background of the instrument.

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Digestion Control – A food sample with known concentration of method analytes. The Digestion Control is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance.

Fortified Analytical Portion (FAP) – A portion of a food sample to which known quantities of the method analytes are added in the laboratory before digestion. The FAP is digested and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the FAP corrected for background concentrations that have been normalized for the portion's weight. One FAP will be run per food type (sample matrix). Matrix effects should be eliminated by the dilution of the digested sample.

Fortified Analytical Solution (FAS) – An aliquot of sample after digestion and dilution to which known quantities of the method analytes are added in the laboratory. The FAS will be prepared as needed when the FAP is unsuccessful or when the FAP concentrations are inappropriate for the background concentration of any analytes. Fortification should be roughly twice the background concentration.

Internal Standard - Pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

Laboratory Fortified Blank (LFB) - An aliquot of DI water to which known quantities of the method analytes are added in the laboratory before digestion. The LFB is digested and analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

Method Detection Limit (MDL) - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

Quality Control Sample (QCS) - A solution of method analytes of known concentrations which is used to fortify an aliquot of calibration blank. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance.

Stock Standard Solution - A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

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Reagents, Standards, and Controls

Nitric acid, concentrated, double distilled "Optima" and trace metal grade.

Nitric acid 1+1 with 10 mg/L Au:

Two liters of solution = 1000 mL of trace metal nitric acid + 1000 mL DI + 20 mL of 1000 mg/L Au. This is the same solution used for metals in water analysis for sample preparation. The solution is kept in the east-end hood of room 5310.

Internal Standard (IS):

In a 50 mL centrifuge tube add:

- DI H_2O (to total volume of 50 mL),
- 2 mL of Nitric acid 1+1 with 10 mg/L Au, and
- 500 µL of 10 mg/L Ir & Rh stock solution (Perkin Elmer Environmental Standard Mix 6).

Internal Standard should be prepared every three months or as needed. 100 µL of internal standard will be added to each 10 mL digested sample or control aliquot.

Standard Stock Solutions:

Stock standards may be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99-99.999% pure).

Elements	Source	Concentration
As, Cd, Cr, Pb, V	Perkin Elmer kit	100 mg/L
Hg	Perkin Elmer kit	10 mg/L

NOTE: Fresh multi-element calibration standards and intermediates should be prepared every three months or as needed.

Standard Intermediates:

Calibration Standard Intermediates	Concentration	Standard Source	Preparation Instructions
Multi-element (M.E.): As, Cd, Cr, Pb, V	1 mg/L	Perkin Elmer kit 100 mg/L	In a 100 mL volumetric add: • DI H_2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, and • 1 mL of stock from standard source
Hg	0.1 mg/L	Perkin Elmer kit 10 mg/L	In a 100 mL volumetric add: • DI H_2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, and • 1 mL of stock from standard source.

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Calibration Standards:

Calibration Standard	Elements and Concentration	Preparation Instructions
Calibration Blank		In a 100 mL volumetric add: • DI H_2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, and • Bring to volume, then add1 mL internal standard.
Standard 1	As, Cd, Cr, Hg, Pb, V 0.1 µg/L	In a 100 mL volumetric add: • DI H_2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, • 100 µL Hg intermediate, • 10 µL M.E. intermediate, and • Bring to volume, then add1 mL internal standard.
Standard 2	As, Cd, Cr, Hg, Pb, V 0.5 µg/L	In a 100 mL volumetric add: • DI H_2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, • 500 µL Hg intermediate, • 50 µL M.E. intermediate, and • Bring to volume, then add1 mL internal standard.
Standard 3	As, Cd, Cr, Hg, Pb, V 1.0 µg/L	In a 100 mL volumetric add: • DI H_2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, • 1000 µL Hg intermediate, • 100 µL M.E. intermediate, and • Bring to volume, then add 1 mL internal standard.
Standard 4	As, Cd, Cr, Pb, V 10 µg/L	In a 100 mL volumetric add: • DI H2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, • 1 mL M.E. intermediate, and • Bring to volume, then add 1 mL internal standard.
Standard 5	As, Cd, Cr, Pb, V 50 µg/L	In a 100 mL volumetric add: • DI H2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, • 5 mL M.E. intermediate, and • Bring to volume, then add 1 mL internal standard.

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Control Stock Solutions:

Control standards may be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99-99.999% pure).

Elements	Source	Concentration
As, Cd, Cr, Pb, V	Spex multi-element	10 mg/L
Hg	Single element stocks	1000 mg/L

NOTE: Fresh multi-element control and intermediates should be prepared every three months or as needed.

Control Intermediates:

Control Intermediate	Concentration	Standard Source	Preparation Instructions
Hg	1 mg/L	Single element 1000 mg/L	In a 100 mL volumetric add: • DI H_2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, and • 100 µL of stock from standard source.

Control	Elements and Concentration	Preparation Instructions
Control (QCS)	As, Cd, Cr, Pb, V: 5 µg/L Hg: 0.75 µg/L	In a 100 mL volumetric add: • DI H_2O, • 4 mL of Nitric acid 1+1 with 10 mg/L Au, • 75 µL Hg intermediate, • 50 µL M.E. stock, and • 1 mL internal standard.

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Daily Performance Check (DPC) Stock Solutions:

Elements	Source	Concentration
Be, U, In, Ce, Co, Pb, and Ba	Spex single element stocks	1000 mg/L

	Concentration	Preparation Instructions
DPC Intermediate	1 mg/L Be, U, In, Ce, Co, Pb, and Ba	In a 100 mL volumetric add: • DI H_2O, • 0.5 mL of Optima Nitric acid, and • 100 µL of stock from each standard source.
DPC Solution	1 µg/L Be, U, In, Ce, Co, Pb, and Ba	In a 200 mL volumetric add: • DI H_2O, • 1 mL of Optima Nitric acid, and • 200 µL of DPC Intermediate solution.

Rinse Solution

Add 20 mL of trace metal HNO_3 and 400 µL of 1000 mg/L Au stock solution to DI with a final volume of 1000 mL. This solution expires in six months.

Dual Detector Calibration Solution

In a 50 mL centrifuge tube, add:
- DI H_2O (to 50 mL total volume),
- 1 mL Nitric acid 1+1 with 10 mg/L Au,
- 100 µL Perkin Elmer multi-element standard stock (100 mg/L),
- 100 µL Perkin Elmer Th, U standard stock (100 mg/L), and
- 100 µL metals in water Li spiking intermediate (100 mg/L).

This solution expires in one year.

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Quality Control

Instrument performance - For all determinations the laboratory must check instrument performance and verify that the instrument is properly calibrated on a continuing basis. To verify calibration, run the calibration blank and calibration standard three as surrogate samples immediately following each calibration routine, after every ten samples, and at the end of the sample run. The results of the analyses of the standards will indicate whether the calibration remains valid. For the initial calibration and calibration blank verification, all analytes within the standard solutions must be within ±10% of calibration. If the calibration cannot be verified within the specified limits, the instrument must be recalibrated. For the continuing calibration and calibration blank verification, all analytes within the standard solutions must be within ±15% of calibration. If the continuing calibration check is not confirmed within ±15%, the previous 10 samples must be reanalyzed after recalibration.

Quality control sample (QCS) – Verify the calibration standards and acceptable instrument performance with the preparation and analysis of a QCS. To verify the calibration standards, the determined mean concentration from three analyses of the QCS must be within ±10% of the stated QCS value. If the QCS is not within the required limits, an immediate second analysis of the QCS is recommended to confirm unacceptable performance. If the calibration standards and/or acceptable instrument performance cannot be verified, the source of the problem should be identified and corrected before continuing with on-going analyses. If necessary, the QCS may be rerun after sample analysis is complete.

Laboratory fortified blank (LFB) - The laboratory must analyze at least one LFB with each batch of samples. The LFB will be fortified, then digested and analyzed like any sample. If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses. If necessary, the LFB may be rerun after sample analysis is complete.

Digestion Control – If available, a digestion control will be analyzed with each set of samples to be digested. This is a food matrix sample with known concentrations of method analytes that will be digested and analyzed to verify laboratory and instrument performance. The percent recovery must fall within statistically derived limits.

Duplicates – Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate analyses can in some cases assess the effect. A duplicate sample will be digested and analyzed for each sample. The relative percent difference will be calculated and must be less than or equal to 25% for any analyte whose concentration is above the reporting limit.

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Fortified Analytical Portion (FAP) - Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for fortified analyses can in some cases assess the effect. The laboratory will add a known amount of analyte to at least one sample per sample matrix. In each case the FAP aliquot must be a duplicate of the aliquot used for sample digestion and analysis and for total recoverable determinations added prior to sample preparation. The FAP's added analyte concentration will be the same as that used in the laboratory fortified blank. Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample and normalized for the amount of sample digested, and compare these values to the designated FAP recovery range of 70-130%. If the FAP recovery is unsuccessful, proceed with preparation and analysis of a fortified analytical solution (FAS) for that sample.

Fortified Analytical Solution (FAS) – When the FAP fails, an aliquot of already digested and diluted sample will be fortified with a known concentration of analyte from the standard source. The added concentration should equal around double the background concentration of the sample solution.

Upper Calibration Limit Verification – Any time a sample is analyzed with a concentration above that of the highest calibration standard, a QC standard with a concentration above the highest sample concentration must be prepared and analyzed to verify the linear dynamic range is valid up to that concentration. The determined concentrations for the QC standard must be within 10% of expected values.

Internal standards responses - The analyst is expected to monitor the responses from the internal standards throughout the sample set being analyzed. Ratios of the internal standards responses against each other should also be monitored routinely. This information may be used to detect potential problems caused by mass dependent drift, errors incurred in adding the internal standards or increases in the concentrations of individual internal standards caused by background contributions from the sample. The absolute response of any one internal standard must not deviate more than 60-125% of the original response in the calibration blank. If deviations greater than these are observed, flush the instrument with the rinse blank and monitor the responses in the calibration blank. If the responses of the internal standards are now within the limit, take a fresh aliquot of the sample, dilute by a further factor of two, add the internal standards and reanalyze. If after flushing the response of the internal standards in the calibration blank are out of limits, terminate the analysis and determine the cause of the drift. Possible causes of drift may be a partially blocked sampling cone or a change in the tuning condition of the instrument.

Method detection limits (MDL) should be established for all analytes, using reagent water (blank) fortified at a concentration of two to five times the estimated detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method.

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Element, Isotope monitored, and Correction factors

Element	Isotope Monitored	Correction Factor	Internal Standard
V	50.944		Rh
Cr	52.9407		Rh
As	74.9216		Rh
Rh	102.905		
Cd	110.904	- 1.073 * Pd108 -(0.712*Pd106)	Rh
Ir	192.963		
Hg	201.971		Ir
Pb	207.977	+1*Pb206 +1*Pb207	Ir

Sample Preparation Procedure

When solid food samples are received, they must be homogenized. Cut into manageable pieces and load into the food processor. Blend until homogenized and then store in the fridge in Ziploc bags. Liquids and powders will be measured as received.

Each digestion will consist of a blank, LFB, Control (if available), samples in duplicate, and at least one FAP per matrix.

Digestion vessels must be digestion cleaned directly before analysis. This can also be done the day before analysis and the vessels left closed in the turntable overnight. Put 10 mL of trace metal grade nitric acid into each vessel and assemble the vessel (place stopper on top of the vessel, screw on the cap, and tighten with the provided tool until it clicks). You need only to digestion clean as many vessels as you will need for your current analysis. Place vessels in the turntable arranged symmetrically. Place turntable into the microwave. From the Home screen select “Load Method” → “User Directory”→ “Cleaning – Xpress Method” → “Start”.

After the digestion cleaning method is complete, allow the vessels to cool and take them to the hood in the middle of 5310. Fill the vessel with DI, and pour out into the acid waste, then rinse the digestion vessels with DI.

Tare the vessels, then weigh samples into them to 1 g ± 0.1 g. Record the weight in the Metals in Food log book. For the blank and LFB, pipet 1 mL DI water into each vessel.

To each vessel, add:

- 8 mL of Optima HNO3 from the repipettor in the hood in the middle of 5310
- 80 µL of 1000 mg/L Au stock solution

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To each FAP and the LFB, add:

Spike Solution	Amount Added	Final Concentration
ME Standard Intermediate (As, Cd, Cr, Pb, V 1 mg/L)	100 µL	0.5 µg/L
Hg from Perkin Elmer 10 mg/L	10 uL	0.5 µg/L

Place stopper on top of the vessel. Screw on the cap and tighten with the provided tool until it clicks.

Place each vessel into the turntable arranging the vessels symmetrically. Place the turntable into the microwave system. From the Home screen select "Load Method" → "User Directory". Select the Metals in Food method. Press "Start" to begin the digestion.

Metals in Food Digestion Parameters:

Stage	Power		Ramp	°C	Hold
	Max	%			
1	1600W	100	25:00	200	15:00

When the method is complete, the instrument will proceed to post-run cool down to prevent the possibility of operator burns or acid spills. Permit the vessels to cool for approximately 30 minutes. Carefully remove the vessels from the turntable, open them in the fume hood, and transfer with DI water into a tared 250 mL bottle. Rinse the digestion vessel thoroughly with DI into the 250 mL bottle, and dilute to a final weight of 200 g for the sample solution. Mix thoroughly.

Clean Up: Wipe out the inside of the microwave and turntable with a damp towel. Send the vessels, stoppers, and caps to the kitchen to be washed. They will be acid rinsed when they come back from the kitchen and left closed and ready to be digestion cleaned for the next run.

Prior to sample analysis, pipet 10 mL of sample into a 15 mL centrifuge tube. Add 100 µL of internal standard to each 10 mL aliquot. Mix well and analyze.

If an FAS needs to be prepared, follow the formula below to determine how many uL of which standard to add to a 10 mL aliquot of the sample:

$$\frac{\text{Final Concentration Required}(ug/L)*10}{\text{Standard Concentration}(mg/L)} = \text{uL Standard added}$$

The uL added to the 10 mL aliquot should be less than 500 uL. If it is more, use a higher standard if possible or adjust final concentration accordingly.

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Instrument and Software Setup for the ICP-MS Procedure

1. Turn on the computer and re-circulator and log into the operating system. Check the waste collection vessel to see if it needs to be emptied.

2. If the mixing tee is connected, remove it with the internal standard tubing and attach the autosampler line directly to the nebulizer. Set up the peristaltic pump tubing for the sample rinse station and for the sample introduction system by positioning the tubing and closing the pump clamps. Check tubing for wear and flattening; replace if necessary.

3. Start the ELAN® ICPMS software from Windows™.

4. Perform daily maintenance checks as described in the *ELAN® ICPMS Hardware Guide* section 4-2 (i.e., Ar supply pressure and tank level, interface components cleanliness and positioning, interface pump oil condition, etc.). Note the base vacuum pressure in the INSTRUMENT window of the software. Before igniting the plasma, the vacuum is typically between 8 x 10^{-7} and 1.8 x 10^{-6} torr. Record any maintenance procedures, along with the base vacuum pressure, in the *Excel QC Book (Ernie Instrument and Performance Log).*

5. In the INSTRUMENT window of the software, press the "Start" button to ignite the ELAN® ICPMS plasma.

6. Place the autosampler rinse line in DI water during warm-up. Turn on the ammonia tank.

7. In the DEVICES window, go to the Autosampler tab → press the "Probe" button→ "Go to Rinse".

8. In the DATASET window → File → New → open a new dataset by entering Year\XX Month\XXXXXXx (i.e. 2012\06 June\062112a).

9. In the METHOD window → File → Open → Metals in Food Conditioning DRC. Then, in the SAMPLE window → "Manual" tab → "Analyze Sample". Warm up the instrument for 30 minutes with the plasma on while the DRC is being conditioned.

10. Check standards and controls to see if they have expired. Remake as needed.

11. After the DRC conditioning is complete, open the SAMPLE window → File → Open → XXXXXXx Metals in Food by ICPMS.sam template. Edit the sample template by replacing "Sample" with the sample log number. If more rows are needed, copy and paste existing rows and then edit the Sample ID and Autosampler Location. For each sample, enter the metals for which you will be analyzing in the description. Spike samples have nothing in the description. All samples including

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spikes should have the Sample Type listed as “Sample”. Save the sample file by replacing XXXXXXx with the date (MMDDYY) followed by a letter.

12. To print a copy of the run list, highlight all the batch index rows and then click on build run list. Select Printable View and then print the run list.

13. After warm-up is complete, perform a daily optimization and performance check.

- In the SMARTTUNE window → Open → MIW Daily Performance Check.swz. Verify that Default.dac and Default.tun are selected. For the dataset, select “Browse” and open the dataset you created.
- Place the autosampler rinse line in the MIF rinse solution, uncap the Daily Performance Check Solution in autosampler position 1, and press “Optimize”.
- The smarttune will automatically perform an autolens calibration and a daily performance check. If the daily performance check passes, it will stop. If it does not, it will perform a nebulizer gas flow optimization and then another daily performance check.
- If the second daily performance check still doesn’t pass, open the MIW Troubleshooting.swz file in the SMARTTUNE window, and optimize again following the same steps as before.

14. Record the lens voltage for Indium in *Ernie Instrument and Performance Log.* This can be found in the OPTIMIZE window → Autolens tab, after the autolens calibration is complete.

15. Reopen the files in the ELAN software that are going to be used to analyze the samples. Press the “R” button on the toolbar on the left hand side of the screen and verify the following files are open:

- Method: Metals in Food by ICPMS.mth
- Dataset and Sample: Check the dates.
- Report: Metals in Food.rop
- Tuning: default.tun
- Optimization: default.dac

16. Put the solutions to be analyzed in the autosampler positions listed on the printed run list. In the SAMPLE window, highlight the rows and click Build Run List. If you are prompted to save Default.dac, do so by overwriting the existing file. Check the run list to make sure all the QC standards are running after the calibration and that the CCV and blank are running after every ten samples and after all samples are completed. Analyze.

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Reprocessing

While a sample set is being analyzed, results can be checked to see if they are in control by opening a second copy of the ELAN software. The second copy is titled *ELAN Edit/Reprocess Session*.

1. Open the current method, dataset, and the Metals in Food report option.

2. From the menu, select Analysis then Clear Calibration and Blank to remove any previously stored calibrations. In the Dataset Window, highlight the blank and standards needed for the calibration curve and then click on the reprocess button.

3. View the standard five report in the Report View window. Go to the Calibration View window to check the graphs and the correlation coefficients by selecting the "Stats" button. Verify that the calibration curves have passed the correlation coefficient limit of 0.999.

4. Reprocess each QC individually and verify all quality control being checked is within limits. If results are out of limits, rerun the QC sample. If it is still out of limits, repour if appropriate. If that is out of limits, remake if appropriate. If still out of limits, remake standards and recalibrate if necessary. Digestion control and Spikes will need to be entered into the MIF calculator to calculate percent recovery. QCS and ICV/CCV percent recoveries can be easily calculated at the bench to verify they are within limits.

5. Reprocess each sample individually. Check sample concentration values to make sure they are below the highest standard concentrations (50 µg/L for As, Cd, Cr, V, Pb; 1.0 µg/L for Hg). If results are above the highest standard concentration, the upper calibration limit must be verified by running a QC standard of known concentration above the highest sample concentration. This sample must be within ±10% of the expected value to verify that the linear dynamic range extends to that concentration. For example, if an analyzed sample contains 75 µg/L Pb, a known standard solution containing 100 µg/L Pb should be prepared and run. (NOTE: If a dilution is necessary for a sample for which we cannot verify the linear range to its concentration, dilute with 4% HNO_3, not blank, to a total of 10 mL and then add internal standard.)

6. If QC samples were run with "Sample" as the Sample Type, the data file can be temporarily changed to the type of QC standard that was analyzed. Right click on the Sample Type before reprocessing and select the appropriate QC standard. This must be re-done every time you reprocess.

7. After analysis has been completed and reprocessed, proceed with instrument shutdown. Put the autosampler rinse line into DI water for 5 minutes. Then take out the line to run dry. When the sample line has dried, put the autosampler into standby

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position (Go to Device window → Autosampler tab → “Probe” → “Go to standby” → OK).

8. Once the nebulizer has dried, go to the INSTRUMENT window → “Stop” to turn off the plasma, and wait to hear the instrument turn off (there will be a loud click).

9. Turn off the ammonia tank.

10. Close the program and shutdown the computer. Turn off the recirculator and release the clamps on the pump tubing.

Reprocessing and Reporting

1. To print the Metals in Food reports, in the METHOD window open the method used to run the samples.

2. Select the report tab on the far right of the screen. Select “Send to Printer” under report view; “metals in food.rop” should be the template.

3. Select “Send to File” under Report to File; “metals in food summary for qc transfer.rop” should be the template. Enter today’s date under Report File Name. Make sure Use Delimiter and Use Separator under Report Format are selected. Append must be selected under File Write Option.

4. In the DATASET window → Analysis → “Clear Calibration and Blank”. Make sure the Read Type column has the right selection for QC samples. Select the rows and click on Reprocess to print the reports. If a point is to be taken out, be sure to do standards first, then take a point out, and then reprocess the rest of the samples.

5. To print out a Quantitative Analysis Calibration Report, in the REPORT VIEW window → Open → Quantitative Calibration.rop → right click anywhere on the screen → Print. Add this to the report right after the calibration.

6. To enter the data in the Excel QC book, open the Metals in Food Data Transfer Macro Excel book located in the I:\Chemistry\QC\Excel QC Books folder. Select Enable Macros. Click on Go!. Open the Report Output file. Close the small box. Click OK. Data will automatically be entered into the Metals in Food by ICPMS Excel QC book. Remove data that is not needed or is out of control. Only the beginning and end blank and CCV are needed. Enter the date on each row that has data entered. Do not save the Metals in Food Data Transfer Macro Excel book when closing it.

7. Open the MIF Calculator in I:\Chemistry\QC\Calculators\Metals in Food. Save as XXXXXXx. Enter the weight of each digested sample in the appropriate space from

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the digestion logbook. (NOTE: If an FAS is run for a sample instead of an FAP, be certain that the weight for the spike is the same as the original sample.) Enter the µg/L values for each sample from the printed reports. When completed, enter the digestion control and spike percent recoveries and the dup difference for any samples above the reporting limit in the Metals in Food Excel QC book. Print the calculator and place it with the report.

8. The reporting limits are 0.02 µg/g for As, Cd, Hg, Pb, V, and Cr.

References

1. EPA method 200.8, Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma - Mass Spectrometry, revision 5.4, EMMC version.
2. Perkin Elmer application note, EPA Method 200.8 for the Analysis of Drinking and Wastewaters.
3. Perkin Elmer Application note, Interference Removal and Analysis of Environmental Waters using the ELAN DRC-e ICP-MS.
4. FDA document # T039, Determination of Trace Elements in Aqueous Solutions by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

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Patuxent Wildlife Research Center
Laurel, MD 20708

SOP No: TOP
Date: September 25, 1991
Replaces:

TECHNICAL OPERATING PROCEDURE

PROCEDURE TITLE: ALAD (delta-aminolevulinic acid dehydratase) determination in red blood cells.

AREA OF APPLICABILITY: This procedure may be used for determining ALAD activity in the blood of vertebrates as an initial bioindicator of recent lead exposure.

SCOPE: This procedure has been successfully used for determining red blood cell ALAD activity using the whole blood of different species of birds (adults, nestlings, and embryos), small mammals, and fish. Other candidate species include amphibians and reptiles.

PRINCIPLE: ALAD in a completely hemolysed sample of whole blood is incubated with excess substrate (ALA; delta-aminolevulinic acid). ALAD catalyses the condensation of two ALA molecules to form porphobilinogen. The reaction is allowed to proceed for a set time and then stopped by the addition of trichloroacetic acid. Porphobilinogen, when mixed with Ehrlich's reagent, forms a purple color which can be measured spectrophotometrically at 555 nm. The quantity of porphobilinogen formed is directly proportional to the ALAD activity which in many species is inversely proportional to the log of the blood lead concentration over the lowest range of lead exposure (Burch and Siegel, 1971; Pain 1987);

PROCEDURE:

1. Reagents

   a. Solution A (0.1 M Na_2HPO_4) 1.78 g of disodium hydrogenphosphate dissolved in 100 ml distilled water.
   b. Solution B (0.1 M NaH_2PO_4) 1.38 g of sodium dihydrogenphosphate dissolved in 100 ml of distilled water.
   c. Sodium phosphate buffer (0.1 M)- prepare 100 ml by mixing solution A with solution B to attain the proper pH for a given species.
   d. Substrate Solution - This is prepared fresh prior to each assay by mixing 167.6 mg of aminolevulinic acid hydrochloride (ALA) (Sigma Chemical Co., St. Louis, MO) into 100 ml of the sodium phosphate buffer.
   e. Ehrlich's reagent-prepare under a hood. Dissolve 2.5 g of p-dimethylaminobenzaldehyde (Sigma Chemical Co.) into 50 ml of glacial acetic acid. Then 24.5 ml of perchloric acid (70%, SGI 0.7) is added. Allow to cool and make volume to 100 ml with glacial acetic acid. Store in a dark bottle and refrigerate. Can be stored for 30 days, but discard if any brown color appears.
   f. Trichloroacetic acid solution (10% W/V in distilled water).

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2. Sample Collection and Storage

Venous blood is collected in heparinized tubes, hematocrits are determined and an aliquot of whole blood is snap frozen in liquid nitrogen (-176°C) (dry ice may also be used). Blood samples are then stored in an ultracold freezer (-80°C). Assays should be performed within two months. It is important that an adequate number of concurrent blood samples are collected from reference control animals of the same species, sex, and age.

3. Assay Incubation Procedure

a. Two aliquots of blood (0.1 ml) are each diluted with 1.4 ml of distilled water in clean plastic tubes (suitable for centrifugation). The tubes are vortexed for 15 seconds to insure complete hemolysis and homogeneity of suspension.
b. The tubes as well as a water blank are then placed into a water bath at 38°C for 10 min. After 10 min., 1 ml of the freshly prepared substrate solution is added to each tube and mixed. The tubes are left to incubate in the water bath in darkness for one hour (porphobilinogen is light sensitive).
c. After the one hour incubation, 1 ml of trichloroacetic acid solution is added to each tube and mixed to stop the reaction. The tubes are then centrifuged at 2000 rpm for 10 minutes.
d. One ml of the clear supernatant is then pipetted off into a clear tube or cuvette. Add 1 ml of Ehrlich's solution down the side of each tube under a hood and stir each with a rod. Five to seven minutes later, sample absorbance is read against a water blank at 555 nm in a 1 cm cell using a spectrophotometer.

4. Calculations of Enzyme Activity

$$\frac{\text{Absorbance at 555 nm} \times 1881 \times 2}{\text{hematocrit}} = \text{ALAD activity (nmol ALA used/min/ml RBC)}$$

Activities of subjects should be compared with the reference or control activities that were run concurrently.

5. Data Interpretation

Delta-aminolevulinic acid dehydratase (ALAD) is a cytosolic enzyme found in many tissues and active in the biosynthetic pathway of heme necessary for maintenance of hemoglobin content in erythrocytes and for cytochromes in various other tissues. ALAD is widely recognized as the enzyme in heme synthesis that is the most sensitive to lead exposure. Inhibition of ALAD activity by lead results in a block in the usage of delta-aminolevulinic acid (ALA) with decreased porphobilinogen formation, and subsequent decline in heme synthesis. The ALAD activity

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of peripheral red blood cells may be the most sensitive biological indicator of lead effects that is readily quantifiable. Historically, numerous human health studies examining venous blood samples from both adults and children revealed a highly significant negative correlation between the log of ALAD activity and blood lead concentration from 10 to about 90 ug/100 ml (Goyer and Mushak, 1977).

There are several reasons why erythrocyte ALAD activity is considered to be the most suitable index of exposure to lead. ALAD activity reflects immediate exposure to blood lead whereas samples of bone and other tissues are usually attainable only at postmortem and reflect chronic rather than immediate exposure. The mobility of lead in blood permits a greater degree of biological activity than stored lead in tissue such as bone, leading to more immediate physiological damage. Advantages of the ALAD assay over blood lead analysis as a first line screen for lead exposure include small sample volume, relatively inexpensive cost, ease of performance using basic and highly portable lab equipment, high reproducibility, and high sensitivity in the lowest range of blood lead concentrations. The assay has been adapted to detect lead contamination in a wide range of species including numerous bird species, small rodents, rabbits, fish, and even invertebrates.

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Utilization of ALAD for Experimental Wildlife Studies

Waterfowl

Significant negative correlations have been reported between ALAD activity and lead concentration in mallards where 0.2 ppm lead in the blood resulted in greater than 50% inhibition of erythrocyte ALAD activity following dosing with lead shot; retention of lead shot for at least 24 hours resulted in reduced activity for 4 weeks (Dieter and Finley, 1979). Inhibition of over 80% was reported after one week in ducklings with blood lead of 0.4 ppm wet weight due to experimental consumption of lead in automotive waste oil (Eastin et al 1983). Mallard embryos were also found to exhibit ALAD depression following topical egg exposure to only 5 ul of waste oil. Other studies have revealed black ducks to be equally or more sensitive than mallards to lead shot ingestion as reflected by subsequent erythrocyte ALAD depression (Rattner et al., 1989).

Raptors

Dosing of bald eagles, unfit for captive propagation, with lead shot resulted in inhibition of red blood cell ALAD by nearly 80% within 24 hours with a mean blood lead concentration of 0.8 ppm (Hoffman et al., 1981). When day-old American kestrel nestlings were orally dosed for 10 days with metallic lead, 2.5 mg/kg ingestion resulted in 50% inhibition of the enzyme (Hoffman et al., 1985). Eastern screech owls receiving lead acetate at a concentration causing 50% mortality exhibited over 90% ALAD depression (Beyer et al., 1981).

Other Avian Species

When ring doves were exposed either orally or via i.p. injection over two days, a significant negative correlation between blood lead concentration and ALAD activity occurred independent of mode of administration (Scheuhammer, 1987). Lead exposure has also been reported to cause ALAD inhibition in pheasants, red-winged black birds, cowbirds, grackles, and in northern bobwhite and their embryos (Beyer et al. 1988).

Mammals

The degree of ALAD activity in mammals has been correlated with the percentage of reticulocytes in the circulation which is species dependant, resulting in a wider activity range than seen in birds where eythrocytes are nucleated. ALAD has been used as an indicator of lead exposure in experimental studies using small rodents and rabbits (Mouw et al., 1975).

Fish

Limited studies with fish have shown a dose dependent decrease in erythrocyte ALAD activity.

Field Study Validation and Utility of ALAD Assay

Waterfowl

Dieter et al., (1976) were the first to apply the diagnostic use of the ALAD assay to wild waterfowl, demonstrating a highly significant inverse correlation between ALAD activity and blood lead concentration in canvasback ducks trapped on the Chesapeake Bay. Similarly Pain (1987) demonstrated effects in black ducks on the Chesapeake Bay; hematological screening techniques were compared for three species of wild waterfowl and it was concluded that an adapted ALAD assay proved the most accurate and useful predictor of blood lead level and acute exposure.

Other Avian Species

Red blood cell ALAD activity was lower in highway nesting barn swallows than in their rural counterparts and related to highway lead concentrations (Grue et al., 1984). Nestling european starlings along highways were more sensitive to lead exposure than adults as reflected by depression of red blood cell ALAD and depressed hemoglobin concentration (Grue et al., 1986). Others have shown a significant field relationship in feral pigeons between lead exposure and ALAD in urban locations. Near the site of a zinc smelter where soil was contaminated with lead, a variety of passerine species exhibited lower ALAD activities than did corresponding species from a control area (Beyer et al.,

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1985). Similar observations were reported in Tundra swans and in osprey near a lead mining site in Idaho.

Mammals

Shrews and mice in the vicinity of a zinc smelter with lead contaminated soil showed depression of ALAD but correspondingly higher body burdens of lead than did controls from a more pristine location (Beyer et al., 1985). A similar relationship was demonstrated for lead exposed urban rats in contrast to rural rats (Mouw et al., 1975).

Fish

Field studies have demonstrated ALAD inhibition in fish from lead polluted lakes.

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Signature, Title

DATE: 10/28/91

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Quality Assurance Representative

DATE: _____

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Determination of GSH/GSSG using the Glutathione Fluorescent Detection Kit, Arbor Assays

- Arbor Assays K006-F1. Stored at 4ºC. One kit is adequate for approximately 120 wells.
- Reagents not supplied: SSA (Sigma, cat# S2130-100G); 10X PBS (Fisher, cat# BP399-1).
- Costar half plate supplied. For extra plates use Corning* 96 Well Half Area Black Flat Bottom Polystyrene NBS* (Fisher, cat# 07201205)
- Liver concentration: 8.3 ug/ul (2011 samples) to 12.5 ug/ul (2010 samples), in 100 ul 1X PBS.

1) Bring all reagents to RT before use. Fast cool centrifuge to 4°C.
2) Turn on microplate reader and use 390 ex/520 em.
3) Thaw PCR tube containing 25 ug/ul of liver supernatant in 1X PBS. Dilute to appropriate predetermined concentration (ug/ul) in 1X PBS.
4) Prepare:
    a. Sample Diluent (for diluting standards and samples): one part 5% SSA, four parts Assay Buffer. For 1.5 mls: 300 ul 5%SSA + 1.2 mls Assay Buffer
    b. Reaction Mixture (25 ul per well): This volume of reagent is adequate for 59 wells.

	Half plate
NADPH conc	150 ul
Glutathione Reductase Conc	150 ul
Assay Buffer	1.2 mls

5) Transfer 100 ul of supernatant to a 0.5 ml tube. Add 100 ul of 5% SSA. Incubate for 10 minutes at 4°C, inverting 3 times during incubation. Centrifuge 10,000 x g for 10 minutes at 4°C.
6) Prepare standards from 250 uM Glutathione standard.

    NOTE: If a large number of samples are being processed, prepare the standards immediately before adding to the plate so as to use standards within 1 hour.

Conc	**Sample Diluent**	**Standard**
25	315 ul	35 ul Glutathione Standard
12.5	150 ul	150 ul of 25 uM
9.375	125 ul	75 ul of 25 uM
6.25	150 ul	150 ul of 12.5uM
3.125	150 ul	150 ul of 6.25 uM
1.56	150 ul	150 ul of 3.125 uM
0.781	150 ul	150 ul of 1.56 uM

7) Add 150 ul of Assay Buffer to 0.5 ml tube. Transfer 100 ul of the supernatant to the 150 ul of Assay buffer.

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8) Add 50 ul of each blank, standard and sample to a black half plate in duplicate using the dispense mode of an automatic pipette.
9) Add 25 ul of ThioStar reagent to each well with an automatic pipette. Mix on plate mixer for 5 seconds.
10) Incubate at RT for 15 minutes. Set up microplate reader.
11) Set gain on microplate reader using the highest standard, read for Free GSH.
12) Add 25 ul of Reaction Mixture to each well. Mix on plate mixer for 5 seconds.
13) Incubate at RT for 15 minutes.
14) Set gain on plate reader using highest standard, read. This will determine total GSH.
15) Fluorometric calculations are based on blank corrected values, 4-Parameter logistic curve fit.
16) GSSG = (Total GSH-Free GSH)/2

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Oxidative Stress Liver Preparation

- Process 8-10 samples at a time. This protocol will yield 4 aliquots for each sample. Three of the aliquots are 25 ug/ul, 100 ul in 1X PBS.

1) Prepare labels and label 3 0.5 ml tubes with sample ID, 25 ug/ul in 100X PBS, date.
2) Place vials containing the liver samples on ice.
3) Tare a frozen weigh boat.
4) Observe the frozen sample to estimate the total weight. If the amount approximates 100 mg, weigh the entire sample. If the weight approximates greater than 100 mg, use a frozen spatula to slice and mix the sample while it's nearly thawed. This ensures a representative sample. Weigh out 100-115 mg of tissue.
5) Record the weight. Transfer the liver to a labeled homogenization tube on ice.
6) Re-weigh the weigh boat and subtract any residual liquid from the weight.
7) To ensure even homogenization, "cut" the liver tissue in the homogenization tube with the end of a spatula.
8) Add a volume of 1X PBS for an equivalent concentration of 100 mg of tissue per ml of PBS.
9) Cool the probe of the homogenizer by "homogenizing" the probe in ice cold DI water. Wipe probe and with the tube in a beaker of ice, homogenize the liver for 5 seconds, twice. Clean the homogenizer by rinsing thoroughly, wiping with a Kimwipe and then "homogenizing" ice cold DI water. Use forceps if needed to remove any tissue imbedded in the probe.
10) Transfer homogenate to a 1.5 ml centrifuge tube.
11) Centrifuge homogenate for 10 minutes at 10,000 x g in the pre-cooled, 4°C centrifuge.
12) Transfer supernatant to 1.5 ml centrifuge tube labeled with the sample ID, date and "100 mg/ml in 1X PBS".
13) Transfer 125 ul of supernatant to a 0.5 ml centrifuge tube. Add 375 ul of 1X PBS. Aliquot into 3 pre-labeled PCR tubes at 100 ul per tube.
14) Store the 5 tubes, 3 at 100 ul/tube, 1 at > 100 ul/tube, and the 1.5 ml tube (100 ug/ul) in the -80 ºC.

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Protocol for Determination of DNA Damage, 8- OHDG, in Liver

DNA Purification: Gentra Puregene Tissue Kit (Qiagen, cat# 158622 or 158667)

- *Reagents not supplied:* Proteinase K (Qiagen cat# 158918); RNase A (Qiagen, cat# 158922); Isopropanol (Fisher cat# BP2618); Ethanol (Sigma-Aldrich, cat#r E7148)

1) Place the rocker in the incubator and set to 55ºC. Set heat block to 37 ºC. Optional: prepare a set of tubes with 300 ul of isopropanol.
2) Remove liver tissue from -80ºC and place on ice. Tissue sample can be placed in the -20ºC if they are at risk of thawing to quickly.
3) Flame sterilize forceps and scalpel. Cool.
4) Chill pre-labeled tubes and tare the first tube. With tissue still frozen, slice off a small piece of tissue and transfer to tube. Weigh out approximately 10 mg of tissue.
5) Using a pestle, grind the tissue, keeping the tissue from the base of the tube where it would be compacted.
6) Add 300 ul of cell lysis buffer and "wash" the pestle to remove any tissue stuck to it. Work quickly as DNA strands form a viscous solution.
7) Add 2 ul Proteinase K to the sample cap. Cap, invert 25 times, incubate 3 hours on rocker, at 55ºC. Cool to R.T. Place a piece of tape over the tubes to keep them in place and to facilitate turning them over every 60 minutes.
8) Add 2 ul RNase A solution. Mix by inversion, 25 times and incubate at 37ºC for 15 minutes. Incubate 1 minute on ice (*tissue* protocol, not in blood protocol). Set heat block to 65ºC for rehydration step.
9) Add 100 ul Protein Precipitate Solution, vortex vigorously, high setting, 20 seconds.
10) Centrifuge 15,800 x g 20ºC, 3 minutes. If pellet is loose, vortex, place on ice 5 minutes and recentrifuge.
11) Transfer the supernatant to a new 1.5 ml tube containing 300 ul of 100% isopropanol. Immediately invert gently 10-25 times, proceed to next sample. After all samples are in isopropanol, mix all by gentle inversion for a total of 50 times.
12) Centrifuge 15,800 x g for 1 minute (set centrifuge to 2 minutes as it takes one minute for the centrifuge to reach speed). Use a 1000 ul and 100 ul pipette to discard isopropanol.
13) Add 300 ul of 70% ethanol and invert several times (10 times).
14) Place tube in centrifuge in the opposite direction from the previous centrifugation. This will force the pellet to the other side of the tube and provide more efficient washing. Centrifuge 15,800 x g for 1 minute (set centrifuge to 2 minutes). Remove as much ethanol as possible using a pipette.
15) Air dry 12 minutes.

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16) Add 150 ul DNA hydration solution.
17) Incubate sample at 65ºC, 45 minutes. "Flick" the tube every 15 minutes. Leave at room temperature overnight to complete rehydration OR cool 10 minutes and place in 4ºC, for short term storage.
18) Determine DNA concentration and purity spectrophotometrically.
19) Normalize samples to 15 ug in 100 ul of DNA Hydration buffer. Lower concentrations can be used.

Waste Disposal: Collect discarded isopropanol and ethanol in a plastic container for disposal; neither is listed as a hazardous waste in the Chemical Waste Management Manual.

DNA Digestion for ELISA

- For digestion, 15 ug of DNA in 100 ul of DNA hydration buffer is required. Protocol can be scaled.
- *Reagents not supplied:* Nuclease P1(Sigma-Aldrich cat# N8630); 3M sodium acetate, pH 5.2 (Fisher cat# FERR1181); 1 M Tris pH 7.5 (Fisher, cat# BP 1757); Alkaline Phosphatase (NEB, cat# M02905); 0.1M Zinc Chloride (Sigma-Aldrich, cat# 39059).

1) Prepare a working solution of Nuclease P1 (5 U/ml) by diluting an aliquot of 500 U/ml, 1:100 in 40 mM NaOAc. This is done by adding 180 ul of 40 mM NaOAC to the 20 ul aliquot, transferring 100 ul of this 50 U/ml solution to 900 ul of 40 mM NaOAC. One tube is adequate for ~2 ml of 5 U/ml working solution or nearly 40 samples. Concentration of working solution is 5 U/ml. Keep on ice.
2) Remove an aliquot of Alkaline Phosphatase, 10 U/ml from the -20ºC. Keep working aliquot on ice.
3) Thaw normalized samples.
4) Denature the DNA at 95-100ºC. Cool completely on ice 5 minutes. Microfuge 5 seconds or tap, shake or flick any condensate down into tube.
5) Add 50 ul 40 mM NaOAc pH 5.0-5.4, 0.4 mM ZnCl.
6) Add 50 ul of 5 U/ml Nuclease P1. Invert tubes to mix. Microfuge 5 seconds or tap, shake or flick any condensate down into tube.
7) Incubate at 37ºC for 30 minutes.
8) Adjust pH to 7.5-8.0 by adding 20 ul 1M Tris-HCl pH 7.5 (1/10 volume).
9) Add 15 ul of 10 U/ml alkaline phosphatase. Invert to mix. Microfuge 5 seconds or tap, shake or flick any condensate down into tube.
10) Incubate at 37ºC for 30 minutes.
11) Boil samples for 10 minutes at 95ºC to inactivate alkaline phosphatase. Place samples on ice.

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12) Total volume is 235 ul. Aliquot samples, 2 ug per tube, 4 tubes. For instance, if 13 ug DNA is digested, 36.4 ul per tube will equal 2 ug per tube. The remaining volume is stored in original tube. Samples will be diluted in EIA buffer for ELISA.
13) Store samples at -20ºC

Waste Disposal:

None of the solutions for this protocol is hazardous.

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DNA/RNA Oxidative Damage EIA Kit Cayman Chemical (Cat# 589320) for 8-OHDG

- Please note that the Cayman booklet for this kit defines B_0 has maximum binding. To avoid confusion, we define "MX" as maximum binding in this protocol, and "B" as blank.
- Optimal concentration of digested DNA is 10 ng/ul or 500 ng per well.
- Reagents not supplied: Ultrapure water (e.g. Fisher, Cat# BP2819-1)

1) Bring reagents to room temperature. Keep ELISA wells in foil pouch until ready for use.
2) Define plate map. Include 6 standards, blank, NSB (non-specific binding), MX (maximum bound), reference sample(s) and the day's samples. All are run in duplicate. Also reserve one well for TA (Total Activity).
3) Dilute buffers. *Diluted buffer are stable for 2 months at 4ºC*
    a. EIA Buffer: 5 ml of 10X EIA buffer in 45 ml Ultrapure water. Approximately 5 ml is required per assay.
    b. Wash Buffer: 0.5 ml of 400X in 200 ml of Ultrapure water. Approximately 200 ml is required per plate.
4) Reconstitute Tracer and Antibody: *Tracer and Antibody are stable for 4 weeks at 4ºC*
    a. Reconstitute AChE Tracer with 6 ml of EIA buffer. Approximately 5 ml is required per plate.
    b. Reconstitute EIA Monoclonal Antibody with 6 ml of EIA buffer. Approximately 5 ml is required per plate.
5) Prepare Standards: *standard at a concentration of 30 ng/ml is stable for 6 weeks at 4ºC. All other diluted standards in EIA buffer, stored at 4ºC, are stable for 24 hours.*
    a. Equilibrate a pipette tip in ethanol.
    b. Transfer 100 ul of 8OHdG 300 ng/ml stock into a tube and add 900 ul of UltraPure water for a concentration of 30 ng/ml. All subsequent dilutions are in EIA buffer. Equilibrate pipette tip in the standard before transferring.

ul Standard	**ul EIA Buffer**	**Final Concentration**	**Volume (ul) for std curve**
100 ul of 30 ng/ml	900	3 ng/ml	Not used, for serial dilution
400 ul of 3 ng/ml	500	1.33 ng/ml	500
400 ul of 1.33ng/ml	500	592.6 pg/ml	500
400 ul of 592.6 pg/ml	500	263.4 pg/ml	500
400 ul of 263.4 pg/ml	500	117.1 pg/ml	500
400 ul of 117.1 pg/ml	500	52 pg/ml	500

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6) Thaw digested DNA samples on ice. Dilute with EIA buffer to 10 ng/ul. If an aliquot contains 2 ug of digested DNA in 36.4 ul, add 164 ul of EIA buffer. Mix PCR strips thoroughly by inversion. Individual tubes can be vortexed.

   At 50 ul of sample per well, the concentration of the sample per well will be 500 ng. Samples may require further dilution. Samples can be concentrated by pooling 2 or more 2 ug aliquots.

7) Determine the amount of Tracer required. Add Tracer dye to a concentration of 1% v/v, (e.g. 50 ul of dye per 5.0 ml of Tracer).

8) Determine the amount of Monoclonal Antibody required. Add Monoclonal Antibody dye to a concentration of 1% v/v.

9) Add 100 ul EIA buffer to non-specific binding wells.

10) Add 50 ul EIA buffer to maximum binding wells.

11) Add standards, 50 ul, in duplicate.

12) Add 50 ul of sample to wells.

13) To all wells **except Blank** and **TA**, add 50 ul of Tracer

14) To all wells **except Blank**, **TA and NSB**, add 50 ul of Monoclonal Antibody

15) Cover plate with sealer and parafilm. Incubate 18 hours at 4^0C on rocker in refrigerator.

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Plate configuration example:

	1	**2**	**3**	**4**
A	B	B	Sample	Sample
B	NSB/Negative Control	NSB/Negative Control	Sample	Sample
C	MX/Positive Control	MX/Positive Control	Sample	Sample
D	S5	S5	Sample	Sample
E	S4	S4	Sample	Sample
F	S3	S3	Reference 1	Reference 1
G	S2	S2	Reference 2	Reference 2
H	S1	S1	TA	

S1-S5 = Standards, S5 lowest (52 pg/ml) = 50 ul sample + 50 ul Tracer + 50 ul Antibody

NSB/Negative Control = Non-specific binding = 100 ul EIA buffer + 50 ul Tracer

MX/Positive Control = Maximum binding = 50 ul EIA buffer + 50 ul Tracer + 50 ul Antibody

TA = Total Activity = Tracer added at development

Well	EIA Buffer	Standard/Sample	Tracer	Antibody
Blank (Empty)	--	--	--	--
TA	--	--	5 ul at DEVELOPMENT	--
NSB	100 ul	--	50 ul	--
MX	50 ul	--	50 ul	50 ul
Std/Sample		50 ul	50 ul	50 ul

DAY 2 of ELISA

16) Bring a vial of Ellman's to room temperature. Immediately before use/before wash step, reconstitute Ellman's Reagent by adding 20 ml UltraPure water to one vial of 100 dtn or 50 ml Ultrapure water to 250 dtn. Protect from light.
17) Retrieve the plate from the refrigerator. Carefully remove the plate sealer. Number the rows and/or pinch the sides of the holder together as strips may free themselves from the holder during the wash step. Empty the wells and collect this in a waste bottle (contains trace sodium azide). Rinse the plate five times with Wash Buffer, approximately 300 ul per well with each rinse. Blot well after each wash.
18) Add 200 ul Ellman's reagent to each well with multichannel automatic, dispense mode.
19) Add 5 ul Tracer to Total Activity (TA) well.
20) Cover the plate with sealer and foil, place on rocker.
21) Rock in the dark at RT for 90 min.
22) Wipe plate with a clean tissue and carefully remove sealer. If there's a loss of Ellman's Reagent, wash the plate three times with Wash Buffer, add fresh Ellman's reagent and re-incubate. *Do not spin.*
23) Read at 410 nm. Check the absorbance at the MX wells. Read the plate when the absorbance of the MX wells are in the range of 0.3-1.0 AU (blank subtracted) or >0.3 and <2.0 (no subtraction).
24) Read the plate again at 120 minutes. Reread immediately. If the absorbance exceeds 2.0, wash the plate, add fresh Ellman's and redevelop.

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Waste Disposal:

- EIA Buffer contains 0.1% Sodium Azide as a preservative. Sodium azide is a hazardous waste, P105 in the Chemical Waste Management Manual. The concentration is below 3%, nonetheless, Ellman's reagent, samples, tracer and antibody are collected for hazardous waste disposal.
- Ellman's reagent is 5,5'-Dithio-bis-(2-nitrobenzoic acid) and is not listed in the Chemical Waste Management Manual. Since it is reconstituted in EIA buffer (0.1% Sodium

Azide), it is collected for hazardous waste disposal. The percent of reconstituted Ellman's reagent (w/v) is unknown.

- Standard stock 300ng/ml is in ethanol. Ethanol can be combined with the ethanol waste from the DNA purification. Ethanol is not listed in the Chemical Waste Management Manual.
- Wash buffer can be safely disposed down the drain.

Data Analysis and Calculations:

"MX" (not B_0) is defined as maximum bond, and "B" is defined as blank.

1) Corrected MX (Maximum Binding)= average NSB (blank corrected) minus the average MX (blank corrected); MX – NSB. The value should be approximately "1"
2) Sample and Std Bound/MX = Sample or Standard blank corrected absorbance – average NSB (blank corrected)/Corrected MX
3) Use the values from step 2 to determine the 4-parameter curve fit.

Acceptance Criteria:

- %CV for the standard curve is based on blank-corrected raw data and must be ≤20.
- %CV for samples is based on 4-parameter fit and must be ≤ 20.
- %CV for reference samples must be ≤ 20 between plates.
- Blank (raw data) between runs should be comparable.
- NSB (Non-specific binding), blank corrected should be approximately 0.
- MX (Maximum binding) blank corrected should be below 2.0. Subtraction of NSB from MX should be approximately 1. This is the Corrected MX.
- TA (Total activity) raw data should be approximately 3.5.
- R2 values must be above 0.99.

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Oxidative Stress TBARS Assay

- Bioassay Solutions, kit: DTBA-100. Range of assay: 0.1 uM to 1.5 uM MDA.
- Reagents not supplied: Trichloroacetic acid (TCA; Sigma, Cat# T9159-100G); 10X PBS (Fisher, cat# BP399-1)
- Optimal tissue concentration: 25 ug/ul in 100 ul in 1X PBS.

1) Thaw TBA reagent. Set heat block with 2 inserts for 2.0 ml tubes to 100 ºC. Fast cool centrifuge to 4 ºC. Turn on microplate reader.
2) Design plate map. Include 7 standards, blank, 2 different reference samples and the day's samples. All are run in duplicate.
3) Thaw liver aliquot 25 ug/ul in 1X PBS, 100 ul on ice.
4) Thaw a 25 ul aliquot of 150 uM MDA standard on ice.
5) If the BCA assay follows, transfer 15 ul of sample to a separate tube containing 60 ul 1X PBS. Add 170 ul of 10% TCA (1:3). If no BCA assay follows, add 200 ul of 10% TCA to 100 ul of 25 ug/ul aliquot. Immediately invert to mix. After last sample, invert all samples. Invert again at 5, 10 and 15 minutes. Incubate for a total of 15 minutes on ice.
6) Centrifuge 10K x g for 10 minutes at 4°C.
7) During TCA incubation, prepare standards:

Conc	MDA	H2O
1.5 uM	20 ul 150 uM	1980 ul
1.0 uM	600 ul 1.5 uM	300 ul
0.75 uM	500 ul 1.5 uM	500 ul
0.5 uM	500 ul 1.0 uM	500 ul
0.375 uM	500 ul 0.75 uM	500 ul
0.188 uM	500 ul 0.375 uM	500 ul
0.094 uM	500 ul 0.1875 uM	500 ul
0		200ul directly to tube

8) Transfer 200 ul of standard or sample to a 2.0 ml boiling tube. Add 200 ul TBA reagent, vortex, protect from light with foil. Place weight on top. Boil for 1 hour at 100ºC.
9) Cool samples to RT (~ 8 minutes, change slots in rack to cool). Vortex, microfuge.
10) Add 150 ul of sample and standards in duplicate to assay plate. Sample mixtures contain TCA, DMSO and are volatile. If possible, open tubes and add to the round bottom plate under a hood.
11) Set gain on plate reader using highest standard, read samples at 544 ex/590 em.
12) Fluorometric calculations are based on blank corrected values, 4-Parameter logistic curve fit. CV% must be <15. Curve will be linear.
13) Return to the hood to decant assay solution into a hazardous waste vessel.

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Calculations:

Results are in uM (nmol/ml) of product.

uM product * 150 ul (volume per well) = umol per well/1000 = nmol/well.

100 ul * 25 (25 ug/ul aliquot)/300 ul * (200 ul/400 ul) * 150 ul sample volume = 6.3×10^{-4} g of tissue per well

nmol/g= nmol per well/ gram of tissue per well

Waste Disposal:

Collect waste in a plastic container. Waste solution from this assay contains approximately 50% DMSO (Dimethylsulfonic acid), 3.5% TCA (Trichloroacetic acid), and 0.35% TBA (4,6, dihydroxy-2-mercaptopyrimidine).

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Oxidative Stress: Thiol Assay

- Measure-IT™ Thiol Assay Kit (Life Technologies, Cat# M30550.)
- Reagent not supplied: 10X PBS (Fisher, Cat# BP399-1)
- Half plate not supplied. Corning 96 Well Half Area Black Flat Bottom Polystyrene NBS (Fisher, cat# 07201205)
- Optimal tissue concentration: 83 ug/well (2011 samples) to 125 ug/well (2010 samples).

1) Allow Component A and Component B to come to room temperature. All additions and dilutions are with an auto-pipette.
2) Ideally the Thiol assay follows immediately after the Glutathione assay and uses the same 8.3 ug/ul in 1X PBS aliquot. If the sample is on ice more than 5 hours it will run low. If a fresh aliquot is used, keep it on ice for 3 hours. Samples on ice for a short time will run high.
3) Design plate map to include 7 standards, blank, 2 different reference samples and the day's samples. All are run in duplicate.
4) Prepare standards from 6.6 mM stock (5/7/12). Dilute 1:6 with water for 1.1 mM. Further dilute in water 1:10 for 110 uM.

Standard Curve:

Conc (uM)	**Standard**	**PBS**
55*	920 ul of 110 uM	920 ul of **2XPBS**
40	640 ul of 55 uM	240ul of 1XPBS
27.5	300 ul of 55 uM	300ul of 1XPBS
20	300 ul of 40 uM	300ul of 1XPBS
13.75	300 ul of 27.5 uM	300ul of 1XPBS
10	300 ul of 20 uM	300ul of 1XPBS
6.88	300 ul of 13.75 uM	300ul of 1XPBS

*55 uM is the highest standard. Above 55 uM curve is non-linear.

5) Prepare working solution of thiol quantitation reagent. Protect from light and use within 1 hour:

   100 ul/well, dilute 100X (Component A) with quantitation buffer (Component B)

   For a full plate: 100 ul (Component A) + 10 mls quantitation buffer (Component B)

3) Add 10 ul of each standard and sample in duplicate.
4) Add 100 ul of working solution to each well. Mix on plate mixer for 3 minutes. Measure fluorescence after 5 minutes total using a microplate reader (excitation/emission 494/517 nm).
5) Set gain on plate reader using highest standard, read.
6) Fluorometric calculations are based on 4-parameter fit but linear regression fit is equivalent.

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7) Immediately repeat any samples as necessary, standard curve/samples are stable for at least an hour.
8) Fluorometric calculations are based on blank corrected values, 4-Parameter logistic curve fit. CV% must be <15. Curve will be linear.

Calculations:

Results are in uM of product

uM product * 0.11 (volume per well) = nmol/well

10 ul * 8.3 (ug/ul) = 83 ug per well or 8.3×10^{-5} g per well

Umol/g = nmol per well/ g per well

Waste Disposal:

Collect waste in a plastic container. Waste solution from this assay contains approximately 1% 1,2 propanediol (= 1,2 dihydroxypropane, propylene glycol).

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APPENDIX E

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APPENDIX F

Study Species of Concern

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KEY TO THE SPECIES BY SKULLS OF ADULTS

1a. Inflated bone surrounding inner ear (see plate 7); bony arch (zygomatic arch) present at side of eye socket. **Eastern Mole** *(Scalopus aquaticus)* p. 45

1b. Ringlike bone surrounding inner ear (see plates 3–6); no bony arch (zygomatic arch) at side of eye socket. **Go to 2**

2a. (From 1b) Total teeth 30; 3 small single-pointed teeth (unicuspids) visible from side between first upper incisor and first upper cheek tooth (see plate 6). **Least Shrew** *(Cryptotis parva)* p. 41

2b. (From 1b) Total teeth 32; 4 or 5 small single-pointed teeth (unicuspids) visible from side between first upper incisor and first upper cheek tooth. **Go to 3**

3a. (From 2b) Prominent sharp projection on each side of braincase (seen in top view) (see plate 5); length of skull 3/4 inch (19 mm) or more. **Short-tailed Shrew** *(Blarina brevicauda)* p. 35

3b. (From 2b) No prominent sharp projection on each side of braincase (seen in top view) (see plates 3–4); length of skull less than 3/4 inch (19 mm). **Go to 4**

4a. (From 3b) Braincase high domed; rostrum narrow; third upper single-pointed tooth (unicuspid) slightly larger than fourth upper single-pointed tooth. **Masked Shrew** *(Sorex cinereus)* p. 30

4b. (From 3b) Braincase flattened; rostrum broad; third upper single-pointed tooth (unicuspid) slightly smaller than fourth upper single-pointed tooth. **Southeastern Shrew** *(Sorex longirostris)* p. 33

Shrews (Family Soricidae)

The family name, Soricidae, is based on the Latin word, *sorex*, for "shrew-mouse." Members of this family have the first or central incisors of both upper and lower jaws greatly enlarged and specialized into grasping pincers. The front and hind feet are about equal in size. Small external ears, or flaps, may be present. The eyes are tiny and probably capable of very limited vision. The fur has a plushlike quality.

Four species of this family occur in Missouri.

Masked Shrew (*Sorex cinereus*)

Name

The first part of the scientific name, *Sorex*, is the Latin word for "shrew-mouse." The last part, *cinereus*, is from the Latin word *cinis* for "ash-colored." The common name, "masked," describes the slightly darker (but not always apparent) coloration over the eyes, while "shrew" is from the Anglo-Saxon name *screawa*.

Description (Plate 3)

The general characteristics of the shrews in Missouri are given in the accounts of the Short-tailed and Least shrews. The Masked Shrew is distinguished as follows: from the Short-tailed and Least shrews by a longer tail (tail more than one-half the length of head and body) and from the Southeastern Shrew by the grayish brown color on the upperparts and slightly longer tail with an obvious constriction at the base.

Measurements

Total length	2 3/4–4 3/8	in.	69–111	mm
Tail	1–2	in.	25–50	mm
Hind foot	3/8–9/16	in.	9–14	mm
Ear	1/8–5/16	in.	3–7	mm
Skull length	9/16–11/16	in.	14–17	mm
Skull width	5/16	in.	7	mm
Weight	1/10–1/5	oz.	2–5	g

Teeth and skull. The dental formula of the Masked Shrew is:

$$I\ \frac{3}{1}\ C\ \frac{1}{1}\ P\ \frac{3}{1}\ M\ \frac{3}{3} = 32$$

There are 5 single-pointed teeth (unicuspids) visible from the side between the first upper incisor and the first upper cheek tooth. The third of these is slightly larger than the fourth.

The skull has a relatively narrow rostrum and high-domed braincase. The skull of the Masked Shrew is distinguished from that of the Least Shrew by having 32 teeth and 5 small single-pointed teeth (unicuspids) visible from the side between the first upper incisor and first upper cheek tooth; from that of the Short-tailed Shrew by lacking the prominent sharp projection on each side of the braincase (seen in top view) and by the smaller size; and from that of the Southeastern Shrew by the narrow rostrum and high-domed braincase and by having the third upper single-pointed tooth (unicuspid) slightly larger than the fourth.

Longevity. The average life span is short, probably up to 12 or 18 months. In captivity, one lived for 23 months.

Voice and sounds. Masked Shrews have several calls: staccato squeaks, especially when fighting or mad; faint twittering noises when searching for food; and a slow grinding of the teeth.

Glands. Small paired glands on the flanks of the body have a strong odor, particularly in males at the onset of the breeding season. The odor possibly is related to sexual attraction.

Distribution and Abundance

This little shrew has one of the widest ranges of all American mammals, occurring from Alaska and Canada south into the northern half of the United States. Its North American distribution is shown on the accompanying map. While it is relatively scarce over the entire range, in some places it may be very abundant at times.

The Masked Shrew was not reported in Missouri at the first writing of this book but since then has been

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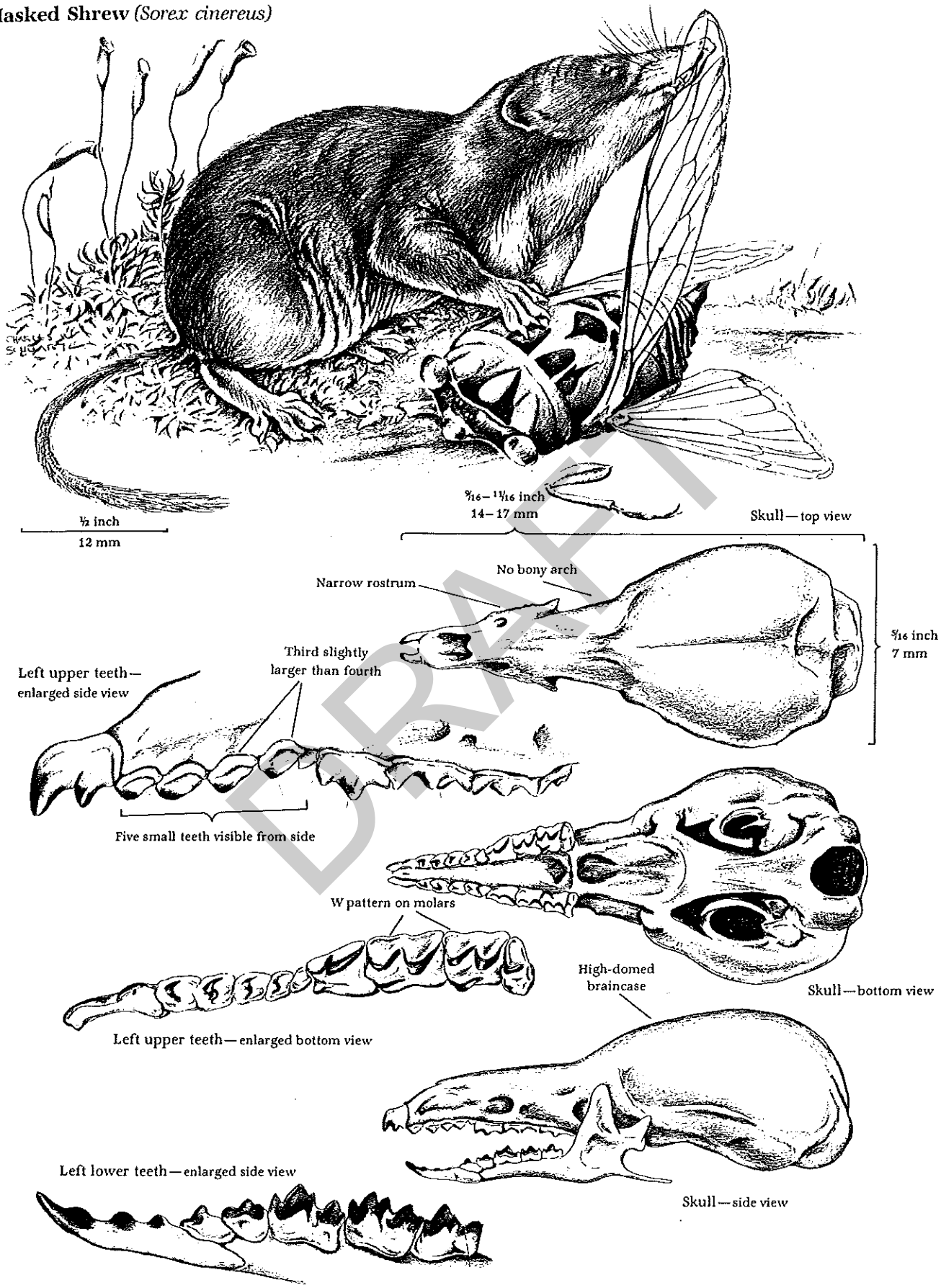
Plate 3

Masked Shrew *(Sorex cinereus)*



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taken fairly commonly in six northwestern counties (Worth, Harrison, Nodaway, Gentry, Davies, and Grundy). It is not known if this shrew has always lived in Missouri and was overlooked or if a recent southward extension of range occurred from Iowa. The latter explanation is favored because intensive collecting in the same area for many years did not find them until recently. It is now believed that this shrew occurs throughout much of northern Missouri in river-bottom habitats.

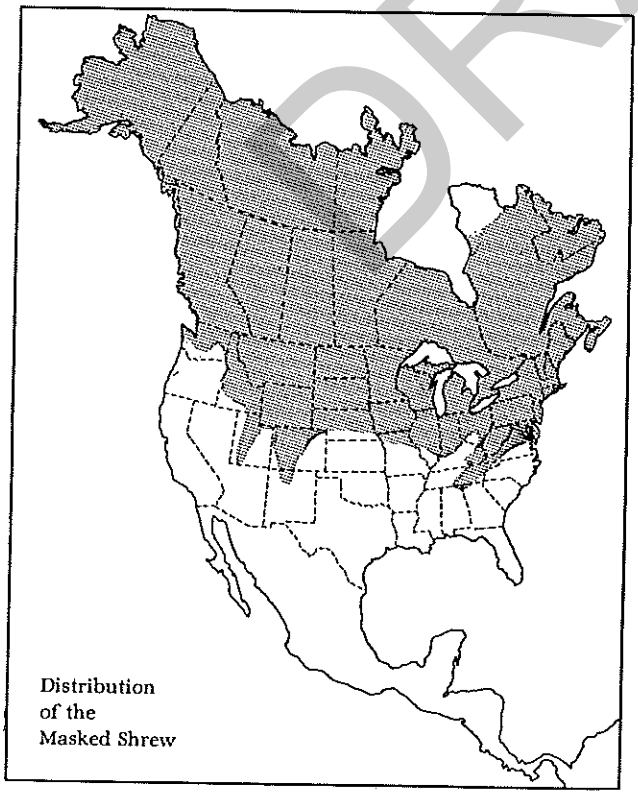
Habitat and Home

Masked Shrews prefer low, damp areas in stream valleys or floodplains. They live in some of the same general areas as Least Shrews but utilize restricted microhabitats and are not generally found in grasslands like Least Shrews.

For their home they dig burrows 3/4 inch in diameter, going down about 9 inches. In this tunnel system they have several chambers, some for food storage, some for resting, and some for a nest. The nest is a woven sphere of dry or fresh grass and leaves, possessing one or more exits.

Habits

Masked Shrews prefer a moist environment. While they are active all hours of the day and night, they are mostly nocturnal. They show increased activity during and after a rain, on cloudy nights, and in warmer weather. Their response to rain may indicate a preference for moist conditions or a change in available foods related to more moisture.



Distribution of the Masked Shrew

Many shrews often live in one general area and call to each other as if carrying on some sort of communication. Twenty shrews were seen and heard calling over 25 square yards; in another location several were seen and heard in an area of 100 square feet, while more were heard in the surrounding 10,000 square feet. When two of these shrews that had been observed calling met in a small runway, they reared up on their hind legs, struck each other in the face until one or both fell over backward, then ran in the opposite direction.

Masked Shrews have been reported to climb a sloping tree trunk, navigate a horizontal branch, and plunder a small bird's nest.

Foods

Larvae of butterflies and moths are the most fed-upon items, while larvae of beetles are second, followed by slugs and snails, spiders, and miscellaneous insects. Only small amounts of annelid worms, vertebrates, and vegetable matter occur in the diet. Masked Shrews are voracious feeders, eating three times their own weight every twenty-four hours.

Reproduction

There are several litters per season and individual females can be both nursing and pregnant. Litters are recorded from March to September, with young of the year producing their first litter in their first fall. From 4 to 10 young constitute a litter.

At birth, the tiny shrew weighs 1/100 ounce. The skin is transparent, the eyelids are sealed, and the ears are closed and fused to the head. About 8 days of age, guard hairs show for the first time and in a few more days the underfur appears. In the next week the incisors erupt through the gums, and the eyes and ears open. Weaning occurs after the 20th day. The nestlings now have nearly four times as much subcutaneous fat as a grown shrew, and it is thought this fat may help the young survive while they are learning to hunt.

Four wild young captured from the same nest at nest-leaving time exhibited an interesting behavior. They ran about their new quarters in single file—a caravan formation—each shrew maintaining contact with the rump of the one in front by burying its nose in the fur near the tail. This was continued repeatedly until the 5th day after capture.

Some Adverse Factors

Mortality is greatest during the first two months after birth. Excessive rainfall and wetness are major causes

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of death in the nest. After the young leave the nest, mortality declines gradually. In the population as a whole, losses are lower in winter than in summer.

Hawks, owls, shrikes, herons, mergansers, foxes, weasels, and fish are known predators on Masked Shrews.

Importance and Management or Control

These animals should be appreciated because of their rareness and valuable role in the wildlife community.

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Southeastern Shrew *(Sorex longirostris)*

Name

The first part of the scientific name, *Sorex*, is the Latin word for "shrew-mouse." The last part, *longirostris*, is from two Latin words, *longus* and *rostrum*, meaning "long snout." The common name, "southeastern," refers to the general area of North America where it lives, and "shrew" is from the Anglo-Saxon name *screawa*.

Description (Plate 4)

The general characteristics of the shrews in Missouri

are given in the accounts of the Short-tailed and Least shrews. The Southeastern Shrew is distinguished as follows: from the Short-tailed and Least shrews by a longer tail (tail more than one-half the length of head and body) and from the Masked Shrew by the reddish brown color on the upperparts and a slightly shorter tail without an obvious constriction at the base.

Measurements

Total length	3–4 1/4	in.	76–107	mm
Tail	1 1/16–1 5/8	in.	26–41	mm
Hind foot	3/8– 1/2	in.	9–12	mm
Ear	1/4	in.	6	mm
Skull length	9/16–11/16	in.	14–17	mm
Skull width	5/16	in.	7	mm
Weight	1/10	oz.	2	g

Teeth and skull. The dental formula of the Southeastern Shrew is:

$$1\ \frac{3}{1}\ C\ \frac{1}{1}\ P\ \frac{3}{1}\ M\ \frac{3}{3} = 32$$

There are 5 single-pointed teeth (unicuspids) visible from the side between the first upper incisor and the first upper cheek tooth. The third of these is slightly smaller than the fourth.

The skull of the Southeastern Shrew has a relatively broad rostrum and a flattened braincase. It is distinguished from that of the Least Shrew by having 32 teeth and 5 small single-pointed teeth (unicuspids) visible from the side between the first upper incisor and first upper cheek tooth; from that of the Short-tailed Shrew by lacking the prominent sharp projection on each side of the braincase (seen in top view) and by the smaller size; and from that of the Masked Shrew by the broader rostrum and flattened braincase and by having the third upper single-pointed tooth (unicuspid) slightly smaller than the fourth.

Distribution and Abundance

This species lives in the southeastern part of the United States. Its North American range is shown on the accompanying map.

When this book was first written, the Southeastern Shrew was not known from Missouri. Since then it has

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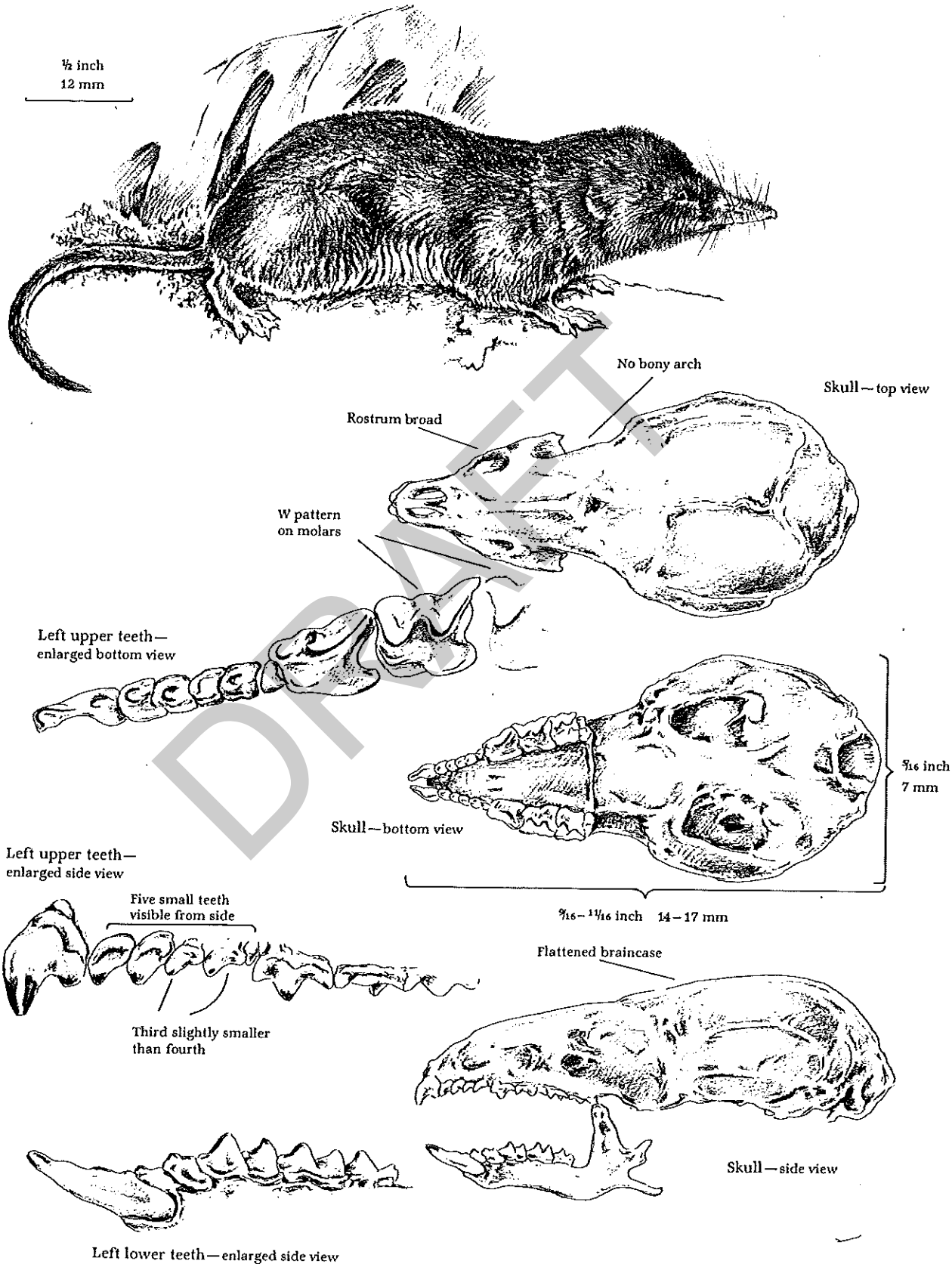
Plate 4

Southeastern Shrew *(Sorex longirostris)*



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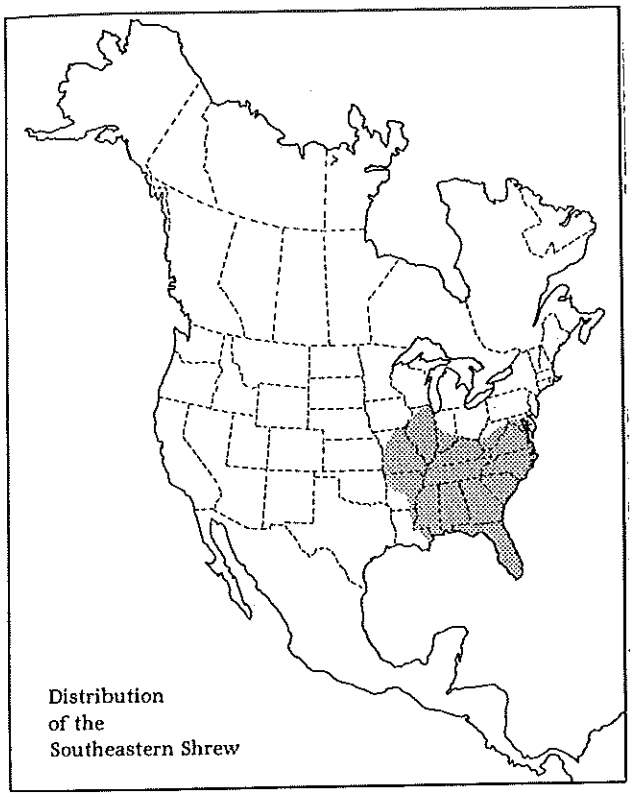

Distribution of the Southeastern Shrew

been taken commonly in Adair, Schuyler, and Macon counties, and specimens have been recorded for Barry and St. Louis counties. Possibly it occurs statewide, but it is probably rare. It seldom is taken in snaptraps but primarily in sunken-can traps.

Habitat and Home

Throughout its range, the Southeastern Shrew is found primarily in bogs, marshy or swampy areas, or in dense ground cover (briers or honeysuckle vines) in wooded areas. Only occasionally has it been taken in upland fields some distance from water. Its known Missouri range consists of generally dry upland sites with some woods.

Nests are shallow depressions lined with leaf litter. They are built under a decaying log or deep inside the log.

Habits

These shrews generally live underground, coming aboveground mostly during or after a rain or on dewy nights.

Foods

Foods of this little-known shrew consist predominantly of spiders, followed by larvae of butterflies and moths, slugs and snails, some vegetable matter, and centipedes.

Reproduction

Four or 5 young compose a litter. The young are born in April and possibly at other times during the summer months.

Some Adverse Factors

Southeastern Shrews are known to be preyed upon by opossums, barn owls, barred owls, and hooded mergansers.

Importance and Management or Control

These subjects are discussed under accounts of the other shrews in Missouri.

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See also discussion of this species in general references, page 20.

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Short-tailed Shrew *(Blarina brevicauda)*

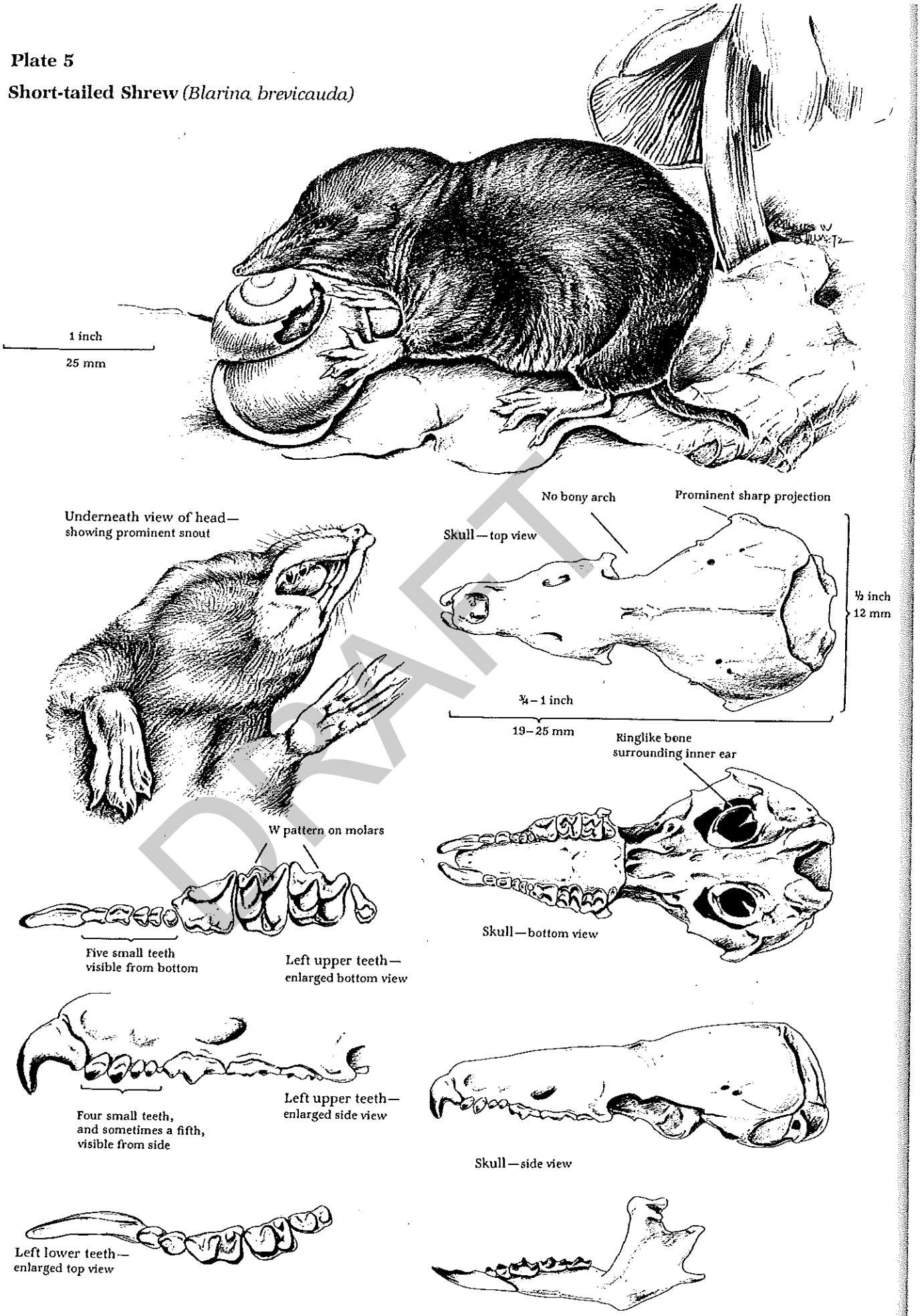
Name

The origin of *Blarina*, the first part of the scientific name, is uncertain: it is considered to be a coined name. The second part, *brevicauda*, is from two Latin words and means "short tail" (*brevis*, "short," and *cauda*, "tail"). This appropriate term is also used for the common name. "Shrew" is from the Anglo-Saxon word *screawa*, which was applied to the Common Shrew of Europe as well as to persons with a harmful influence similar to the then-supposed, and rightly so, poisonous bite of the shrew.

Some authorities believe *B. brevicauda* should be divided into two species—those larger Short-tailed Shrews in the northern part of Missouri being assigned to *B. brevicauda* and the smaller ones in the rest of Missouri to *B. carolinensis*. Because the criteria for separating these species are primarily size and are difficult to determine without a series of specimens, for

Plate 5

Short-tailed Shrew *(Blarina brevicauda)*



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the present we are retaining the name *B. brevicauda* for all Short-tailed Shrews in Missouri.

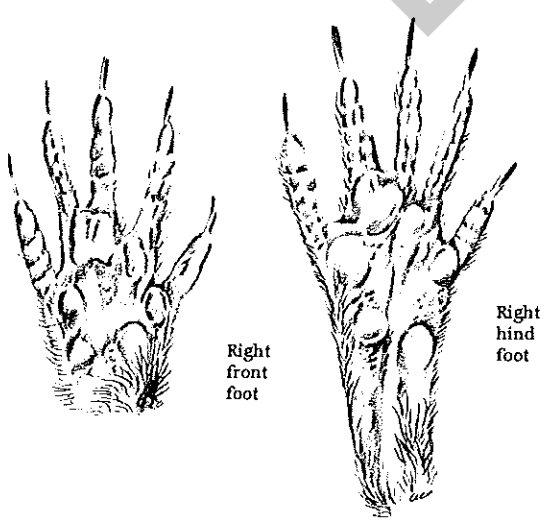
Description (Plate 5)

The Short-tailed Shrew is a very small mammal with an exceedingly pugnacious and energetic nature. It has a pointed head; distinct neck; cylindrical body; short, slender legs with 5 clawed toes on each foot; and a short, furred tail. The flexible, sensitive snout, which contains the nostrils, projects slightly over and beyond the mouth. The tiny, black eyes are capable of light perception but probably see only about 2/20 on an optician's scale compared to the 20/20 that is normal for humans. The external ear opening is large but concealed in the fur. The senses of touch and hearing are well developed; that of smell is poorly developed. The velvetlike fur, which shows no differentiation into underfur and overhair, is short and brushes equally well in any direction. A microscopical examination shows whiplike tips on the hairs, similar to the hairs of moles but unlike those of any other mammal.

This shrew is distinguished from the Masked and Southeastern shrews by its grayer color and shorter tail and from the Least Shrew by its larger size and dark gray color on the upperparts.

Color. While the Short-tailed Shrew is generally gray in color, it is darker on the back and lighter on the underparts, feet, undersurface of the tail, and around the mouth. Only rarely does a white shrew occur. Short-tailed Shrews are typically darker in winter than in summer but show no sexual difference in coloration.

Molting occurs in spring and fall. In the spring molt, females follow a regular progression of hair replacement from the head toward the tail; males, however, have an irregular pattern. In the fall molt, second-year shrews and early-born, first-year animals start to molt at the rear of the body and show a gradual forward replacement of hairs. Late-born, first-year animals start to molt in the head region and show a gradual replacement of hairs toward the rear.



Feet of Short-tailed Shrew

Measurements

Total length	3¾–5	in.	95–127	mm
Tail	¾–1	in.	19–25	mm
Hind foot	½–¾	in.	12–19	mm
Skull length	¾–1	in.	19–25	mm
Skull width	½	in.	12	mm
Weight	½–1	oz.	14–28	g

Males are slightly larger and heavier than females.

Teeth and skull. Shrews are readily recognized by their distinctive teeth, but they are not well differentiated as to kind. The dental formula of the Short-tailed Shrew is given in two ways, depending upon the name assigned to specific teeth:

$$\mathrm{I}\ \frac{3}{1}\ \mathrm{C}\ \frac{1}{1}\ \mathrm{P}\ \frac{3}{1}\ \mathrm{M}\ \frac{3}{3} \quad \text{or} \quad \mathrm{I}\ \frac{4}{2}\ \mathrm{C}\ \frac{1}{0}\ \mathrm{P}\ \frac{2}{1}\ \mathrm{M}\ \frac{3}{3} = 32$$

The first or central incisors on both upper and lower jaws are greatly enlarged and specialized into grasping pincers. Between these large incisors and the first, large cheek teeth on each half of the upper jaw are 5 small, single-pointed teeth (unicuspids); usually only 4 of these are visible from the side and the very tiny fifth one, which may be seen from the side, is best viewed from below. The 2 large upper molars have a distinct *W* pattern on their surfaces. The teeth are usually tipped with brown, being more so in younger individuals.

All shrew skulls have a ringlike bone surrounding the inner ear and lack a bony arch (zygomatic arch) at the side of the eye socket. The Short-tailed Shrew's skull possesses a prominent sharp projection on each side of the braincase when seen in top view.

The skull of the Short-tailed Shrew is distinguished from that of the Least Shrew by 32 teeth, by 4 or 5 small single-pointed teeth (unicuspids) visible from the side between the first upper incisor and first upper cheek tooth, and by its larger size. It is distinguished from Masked and Southeastern shrews by the prominent sharp projection on each side of the braincase (seen in top view) and length of ¾ inch or more.

Sex criteria and sex ratio. It is difficult to determine the sex of shrews externally. Males do not possess a scrotum, the testes being contained within the body cavity throughout life. Males are slightly larger and heavier than females and have better developed scent glands and thicker skin over the throat during the breeding season. Females have three pairs of teats in the groin region. Males tend to outnumber females in the shrew population.

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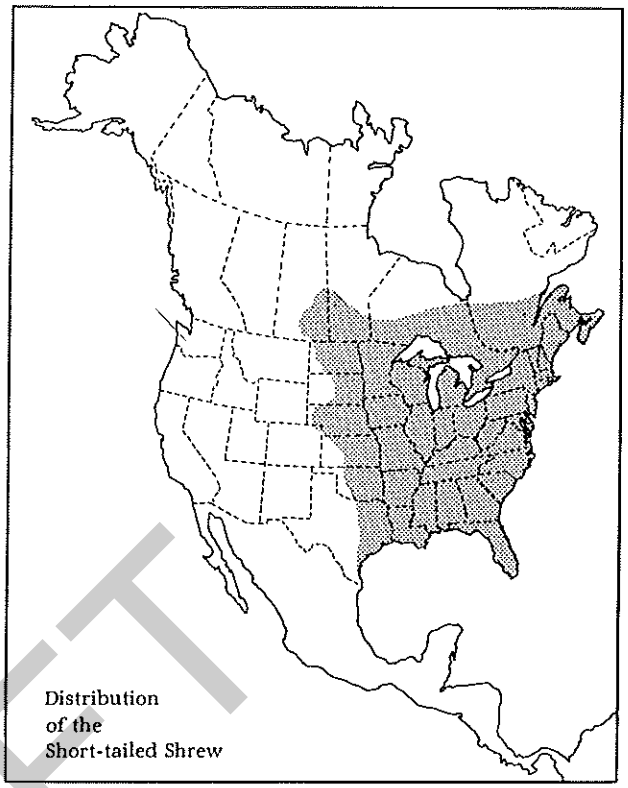
Age criteria and longevity. Young shrews are distinguished from adults by the darker color of their fur, narrower heads, and thinner snouts. As shrews age, the brown color on the teeth gradually becomes less noticeable and the teeth show wear. In very old shrews the teeth may be worn down to the gums. While most shrews probably live no longer than one winter in the wild, some survive for two. In captivity a few have lived through three breeding seasons.

Glands. Short-tailed Shrews possess a pair of small scent glands, about 1/8 inch long, on the flanks. These are active in males at all times and in females at times other than the breeding season. The rank, musky odor from these glands, when rubbed on the tunnel, serves as a sign of ownership and of the occupant's sex; during the breeding season males probably avoid tunnels with a strong scent (made by a male occupant) and look elsewhere for a female.

A single, large gland (1 1/4 inches long by 3/8 inch wide) occurs on the belly of both sexes. The odor from these glands possibly discourages would-be predators but, in spite of this, many shrews are killed and eaten.

Certain salivary glands in the mouth, opening between the bases of the large lower incisors, contain a poison that flows with the saliva and enters an animal when it is bitten by the shrew. This poison slows the heart and breathing of the victim and may cause disintegration of muscle. The submaxillary glands of one adult Short-tailed Shrew contain enough poison to kill 200 mice. This poison also immobilizes insects; acting slowly, it extends the time fresh food is available.

Voice and sounds. The vocalizations of Short-tailed Shrews extend from low to high ranges (audible by man) into the ultrasonic range inaudible by man. The sounds we hear are loud squeaks, a long shrill chatter, a low note of contentment that often accompanies feeding, a slow grinding of the teeth, and a series of clicks given by males when courting or as a warning or challenge. Ultrasonic calls are used to detect objects in the path by means of echolocation. They are given when exploring the tunnel system and are probably not used in locating foods like bats do.



Distribution of the Short-tailed Shrew

Distribution and Abundance

This shrew occurs throughout Missouri. Its North American distribution is shown on the accompanying map.

Shrew populations fluctuate violently from year to year. In peak years, there may be 25 or more Short-tailed Shrews per acre, making them the most abundant mammal in a region. More generally, however, there are fewer than 1 to 4 shrews per acre.

Habitat and Home

Short-tailed Shrews live in dark, damp, or wet localities in flooded areas or fields covered with heavy, weedy growth. They occur less often in grassy cover. Shrews run about the surface of the ground or tunnel in mossy banks, under old logs, and in the leafy cover and humus of the forest floor. Runways and tunnels of other mammals, especially those abandoned by moles or mice, are commonly used, but on occasion a shrew digs its own system of tunnels. Such tunnels are from 1 to 2 1/2 inches in diameter and are located just below the surface of the ground or deeper, sometimes as much as 22 inches underground.

Surface tunnels are constructed by merely raising and separating the leaf mold or sod from the soil beneath. Deeper tunnels are dug mainly with the front feet, although sometimes the snout and head are used

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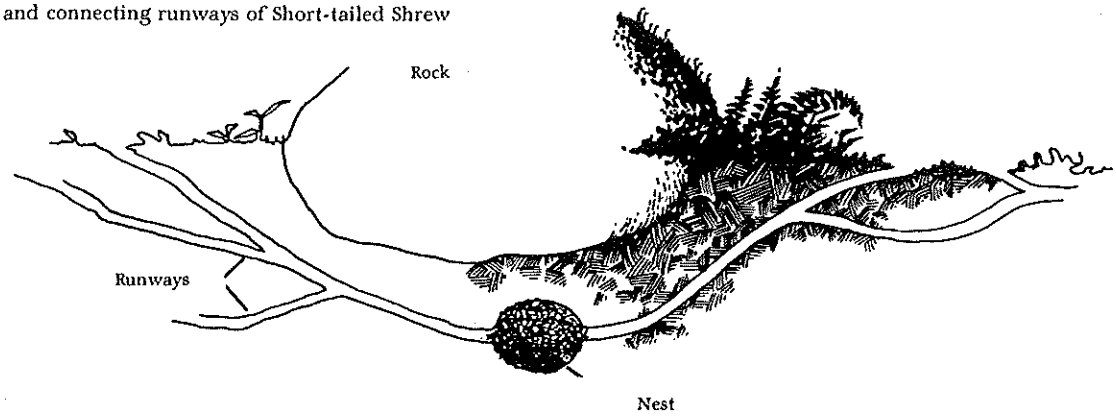
Nest and connecting runways of Short-tailed Shrew



as supplementary aids. In loose soil a shrew burrows at the rate of a foot a minute, but beneath the mossy carpet in the forest one may burrow about a yard in 15 seconds. When there is snow, they may travel on the surface of the ground but just beneath the snow.

Somewhere in the tunnel system, usually under a rock, fallen log, or stump, or as much as 12 inches underground, the female builds a bulky globe-shaped nest for her young. The nest is made of partly shredded leaves and dried grass and has from 1 to 3 openings. It is from 6 to 8 inches in diameter with an inner cavity about 2½ inches across. Similar nests, used for resting or winter homes, are also constructed in the tunnel system.

Habits

Shrews are extremely active, very nervous, and highly belligerent. They spend little time resting and, although they tend to be out mostly at night, search for food at any hour of the day or night all through the year. Their almost constant activity requires a tremendous amount of food and oxygen: this is reflected in a high basal metabolism which is 130 compared to 78 for man, a high rate of respiration that is 140–164 times a minute while at rest compared to 15 for man, and a rapid heartbeat of 160 times a minute.

Individual Short-tailed Shrews exhibit various behavioral patterns and threats, often accompanied by calls that initiate a response in another shrew. Such behavior allows this species to compete socially with reduced physical contact or fighting.

If properly cared for in captivity, Short-tailed Shrews get along well together. However, males quarrel more than females, and adults more than young. Sometimes, in confinement, one individual may kill and devour the other.

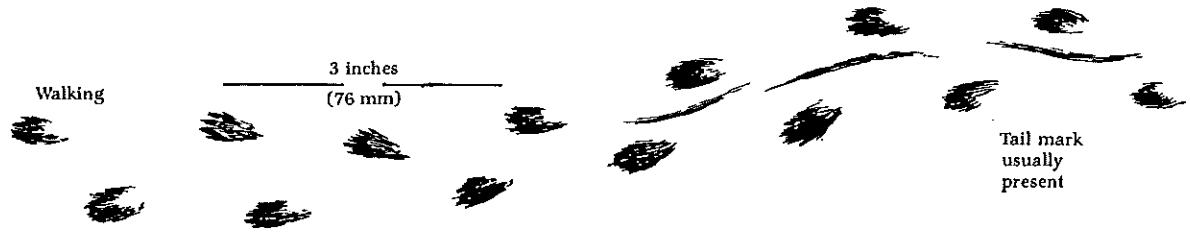
Although shrews do not defend any territory, they tend to stay in the same locality from month to month. An individual's home range is usually between ½ and 1 acre in extent with the largest, known occupied area being 4½ acres.

Shrews normally walk on the entire soles of their feet. They frequently run but rarely jump. They are able to make their way over fallen trees or to ascend inclined ones but are not good climbers. They can swim if necessary.

These mammals are very fastidious about their appearance and spend considerable time washing their fur with the tongue or combing it with their feet or toes. Droppings are deposited in unused portions of the tunnels.

Foods

Nearly the entire diet of Short-tailed Shrews consists of animal foods. Insects of all kinds predominate in the fare, but earthworms, snails, slugs, centipedes, millipedes, spiders, salamanders, snakes, birds, mice, and



Short-tailed Shrew tracks in thin snow

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Short-tailed Shrew trail in soft snow

other shrews are also included. Roots, nuts, fruits, berries, and fungi compose the vegetable foods; these are taken more in winter than in summer.

The Short-tailed Shrew may prey on mice and Least Shrews but is probably overrated as a predator on these animals. Land snails are collected and hoarded in the tunnel system and, in order to be kept immobile, are moved to the surface of the ground as the outside air temperature falls and back into the cooler tunnel as the outside temperature rises. Mutilated insects and even dead mice may be cached, but some of the food probably spoils without being eaten. Prey is located by the shrew's keen sense of touch, which compensates for its poor eyesight, and is then paralyzed by the powerful poison in the saliva.

In captivity, Short-tailed Shrews eat from one-half to more than their own weight in food daily. However, less is probably eaten in the wild. Although they are ravenous eaters, shrews have been known to fast from 24 to 36 hours without starving to death. Captive shrews take water greedily.

Reproduction

The breeding season of Short-tailed Shrews extends from early spring until late fall with peaks of production at the beginning and end of this season and only scattered breeding in winter. Most adult females have one or two litters annually. Many and prolonged matings are required by the female before the eggs are shed from the ovaries. The gestation period of Short-tailed Shrews is 21 or 22 days. From 3 to 10 young may be born per litter but numbers between 5 and 7 are most common. Although mating is promiscuous, males and females may pair for a short time before the young are born. The female takes entire care of the family.

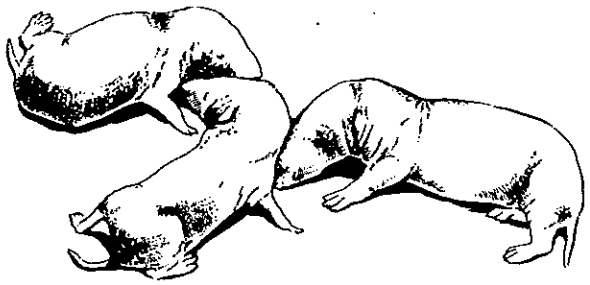
At birth the young are naked, wrinkled, and dark pink. Their eyes and ears are closed and no teeth are visible. They are only 7/8 inch long and weigh 1/100 ounce. At one week, the young shrews have doubled their birth size and short hair is evident on the body. In another week, females can be identified by the presence of teats. The young are well furred at this age, their ears are open, and they begin to crawl. About 18 days of age, the upper incisors first show through the gums and, shortly afterward, the eyes open. The young shrews are well grown at 1 month and are soon on their own. Both sexes breed the year of their birth.

Some Adverse Factors

Snakes, hawks, owls, shrikes, weasels, skunks, foxes, coyotes, bobcats, and domestic cats all kill shrews but sometimes do not eat them because of the disagreeable, musky odor.

Mites, ticks, fleas, botfly larvae, roundworms, and tapeworms are the parasites known to occur on or in Short-tailed Shrews. A nest beetle, similar to the kind found on moles, has been reported from Short-tailed Shrews. This beetle feeds on the eggs and young of mites and other small animals in the nest.

Mortality is greatest the first two months after birth. Shrews have an unusually high mortality rate from accidents and may even die of shock. Many individuals are scarred as a result of fighting with other shrews or their prey.



Short-tailed Shrews nine days old

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Importance

These numerous and widespread tiny mammals are extremely important and contribute in many ways to their environment. Through their predatory nature they help control insects and rodents; through their tunneling beneath the litter and debris of the forest floor, they aerate and mix the soil; and through their own bodies they furnish food for other animals and add to the organic content of the soil. These tiny animals help make the outdoors an interesting place.

Management or Control

No management or control measures are necessary.

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See also discussion of this species in general references, page 20.

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Least Shrew (*Cryptotis parva*)

Name

The first part of the scientific name, *Cryptotis*, is from two Greek words and means "hidden ear" (*kryptos*, "hidden," and *ous*, "ear"). It refers to the extremely large ear opening that is well concealed in the fur. The second part, *parva*, is the Latin word for "small" and emphasizes the tiny size of this animal. "Least" also refers to the size, while "shrew" is from the Anglo-Saxon name *screawa*.

Description (Plate 6)

The Least Shrew is one of the smallest mammals in Missouri, measuring only about 3 inches in total length. It has a long, pointed snout that extends considerably beyond the mouth and contains the nostrils at the end; tiny, black eyes; large ear openings hidden in the velvet-like fur; a distinct neck; moderately slender body; small front and hind limbs with 5 claw-bearing toes each; and an extremely short tail. The fur is soft and short and is not differentiated into underfur and overhair.

The Least Shrew is distinguished from Masked and Southeastern shrews by the shorter tail and from the Short-tailed Shrew by its smaller size and brown to brownish gray color on the upperparts.

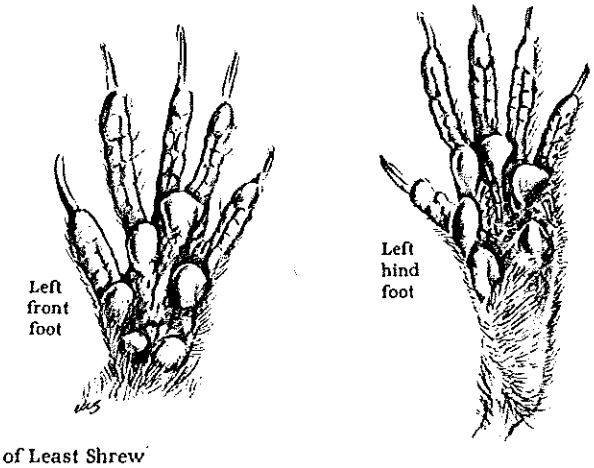
Color. The back is dark brown to brownish gray while the belly is gray. The uppersurface of the tail is colored like the back and the undersurface like the belly. There is no difference in coloration between the sexes, and all individuals are typically darker in winter than in summer. Molting occurs at any season but most commonly in late spring and late fall. Albinos are rare.

Measurements

Total length	2¾–3¼	in.	69–82	mm
Tail	½–¾	in.	12–19	mm
Hind foot	⅜–½	in.	9–12	mm
Skull length	½–⅝	in.	12–15	mm
Skull width	¼	in.	6	mm
Weight	1/10–⅕	oz.	2–5	g

Teeth and skull. The Least Shrew typically has 30 teeth, but 2 of these may not always be present. Because of little differentiation into incisors, canines, and premolars, two dental formulas are given for this species depending upon the classification of certain teeth:

$$I\ \frac{3}{1}\ C\ \frac{1}{1}\ P\ \frac{2}{1}\ M\ \frac{3}{3} \quad \text{or} \quad I\ \frac{3}{2}\ C\ \frac{1}{0}\ P\ \frac{2}{1}\ M\ \frac{3}{3} = 30$$



Feet of Least Shrew

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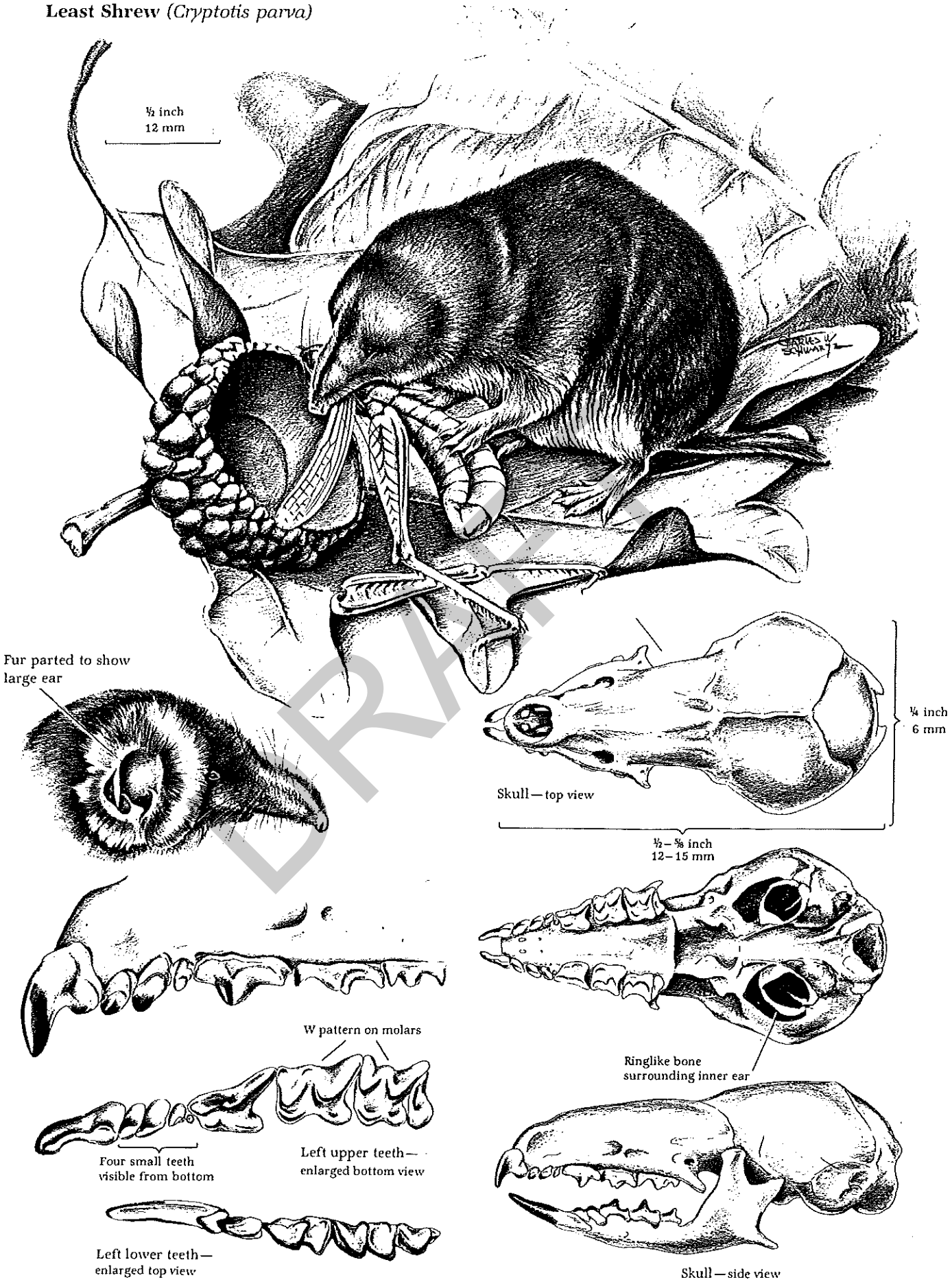
Plate 6

Least Shrew *(Cryptotis parva)*



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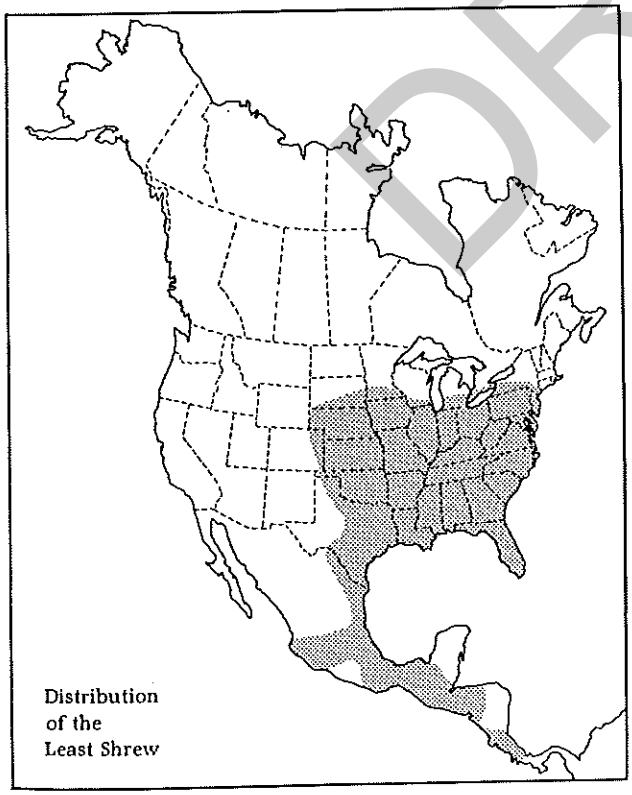
The first or central incisors on both upper and lower jaws are greatly enlarged and specialized into grasping pincers. Between these large incisors and the first, large cheek teeth on each half of the upper jaw are 4 small, single-pointed teeth (unicuspids); 3 of these are visible from the side, but the last and smallest, which may not always be present, can be seen only from below. The 2 large upper molars show a distinct *W* pattern on their surfaces. All the teeth are tipped with brown, but this color diminishes with wear and age.

The skull of the Least Shrew is distinguished from those of other shrews in Missouri by having 30 teeth and by 3 small single-pointed teeth (unicuspids) visible from the side between the first upper incisor and first upper cheek tooth. The very small fourth single-pointed tooth is visible only from the bottom view.

Sex criteria. See account of the Short-tailed Shrew for sex criteria.

Age criteria and longevity. The young are dark slaty gray. In captivity one died of "old age" at 21 months.

Glands. Wild Least Shrews have a pungent odor. This comes from the small paired glands on the flanks of both sexes. Females also have scent glands in front of the lower part of the ear. Apparently, the absence of an emission of scent, or pheromone, from these glands indicates to a male that the female is sexually receptive, while the presence of a scent means she is pregnant and not receptive.



Distribution of the Least Shrew

Voice and sounds. Least Shrews have a vocabulary of sounds that includes "clicks," "puts," "twitters," and "chirps." Clicks are the most predominant and vary from low intensity (audible to man) to high intensity (in the ultrasonic range and inaudible to man). These vary from small bursts of 5 per minute or 13 per 5 seconds to a continuous buzz in the highest frequencies. Ultrasonic calls are used during intense exploration of the tunnel system and may be interpreted as echolocation. However, the echolocation is probably not used for finding foods as bats do.

Distribution and Abundance

This species' North American distribution is shown on the accompanying map. Although these mammals are seldom seen by man, they are generally abundant.

The Least Shrew probably occurs throughout Missouri.

Habitat and Home

Open grass, brush, and dry, fallow fields are the sites preferred by Least Shrews, with marshy or timbered areas selected to a lesser extent. They dwell both on the surface of the ground and underground. These shrews use the runways and burrows of mice and the tunnels of moles or construct their own tunnels, which are about 1 inch in diameter and from 10 inches to 5 feet in length. Some Least Shrew tunnels are shallow and are made by merely separating the sod from the underlying soil; others are dug as much as 8 inches beneath the sod.

Sometimes several Least Shrews cooperate in building their tunnels. In captivity two shrews built a tunnel

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2 feet long with 4 openings, in a two-hour period. One of the shrews dug with its front feet, passed the dirt beneath the body to the hind feet, and kicked it on behind. The other pushed the dirt with its front feet and chest while using the hind feet for propulsion. Most of the dirt was packed into the sides of the tunnels and only a little was scattered near the entrances.

Somewhere in the tunnel system the female constructs a compact ball-shaped nest of dried leaves and grass for her young and lines it with similar material that is partly shredded. The nest is usually built under the shelter of a rock, log, or stump or in a shallow tunnel or deep depression. The nest is from 3 to 7 inches in diameter and has 1 or 2 openings. Larger nests may be used as a winter home for several shrews—up to 31 have been reported in one nest, probably sharing body heat. A toilet area is located at one side of the nest.

Habits

Like other shrews, Least Shrews are active at all times, resting only periodically. The breathing rate at rest is 170 times a minute. They have a very belligerent and nervous temperament but are somewhat more sociable than other shrew species. When food is scarce, however, they may fight, kill, and devour members of their own kind. They are least active during the hottest and coldest parts of the year.

These shrews frequently comb their fur with their feet or toes and cleanse it with their tongues. In captivity they use a special place in the cage for their droppings.

Captive Least Shrews do not sleep soundly but turn frequently and wake up often and stretch, yawn, and even wash themselves before going back to sleep. If several live in the same cage, they usually sleep in one pile, each one trying to burrow beneath the others and causing a constant shuffle.

Least Shrews swim well but ride high in the water.

Foods

Least Shrews eat mostly small insects and their larvae, snails, slugs, earthworms, centipedes, spiders, and the dead bodies of small animals. They take little vegetable matter.

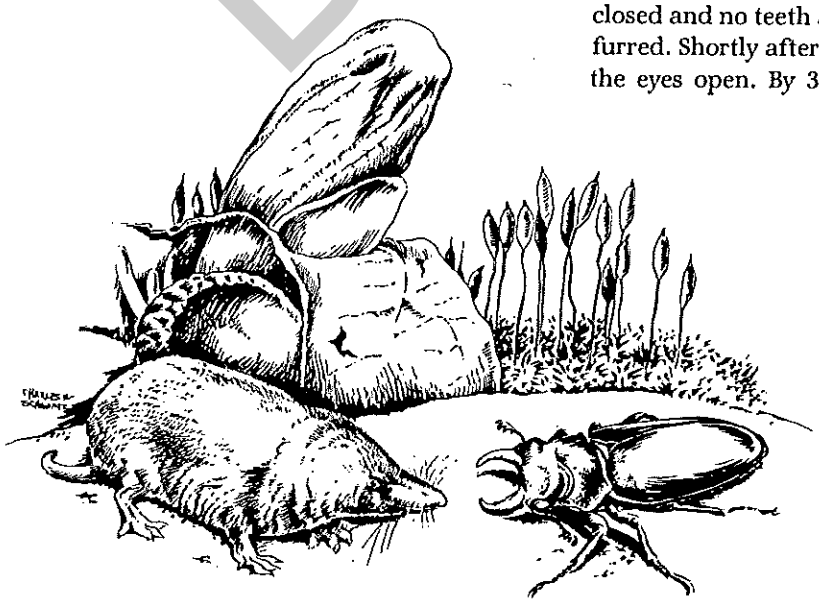
Because of very poor eyesight, these animals locate their food mostly by their well-developed sense of touch. When catching prey, Least Shrews have been observed to hamstring the knee tendons in the hind legs of frogs and attack the joints of the jumping legs of crickets and grasshoppers. Captive Least Shrews appeared afraid of live mice. Least Shrews do not possess poison and hence do not subdue their prey as do Short-tailed Shrews.

Least Shrews have tremendous appetites and consume from three-fourths of their weight to more than their weight in food daily. This large intake is necessary to supply the energy for their nearly continuous activity. Digestion is very rapid, and when the diet is altered, fecal pellets change in consistency within two hours. When the shrew is not hungry, extra food is cached. Water is taken frequently.

Reproduction

Reproduction occurs from February until November, and several litters are produced annually. From 3 to 7 young compose a litter, with most litters containing 4 or 5. The duration of pregnancy is between 21 and 23 days.

At birth the naked young measure 7/8 inch in total length and weigh 1/100 ounce. Their eyes and ears are closed and no teeth are visible. At 1 week they are well furred. Shortly after this the ears open, and at 2 weeks the eyes open. By 3 weeks of age, the young shrews



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weigh 1/8 ounce and their incisors are well developed. They are weaned at this time. When 1 month old, the young appear fully grown. Presumably both parents care for their offspring, as pairs of adults are found in nests with young.

Some Adverse Factors

One way the distribution and abundance of this seldom-seen creature is known is through recovery of parts of this mammal in the stomachs, droppings, or pellets of predators. Least Shrews are eaten by owls, hawks, snakes, weasels, and cats, as well as by Short-tailed Shrews where they occupy the same habitat.

Fleas and mites are the only parasites recorded for the Least Shrew, but doubtless many of the parasites found on the Short-tailed Shrew use this species as a host, too. Least Shrews suffer many accidents and sometimes die of shock.

Importance

Shrews are valuable mammals. Although they are very small creatures, they contribute greatly to the wildlife community. They dig and work the soil, feed on abundant insect life, aid in decomposition of dead animals by their feeding activities, and serve as food for predatory forms. Their presence enriches our environment.

Management or Control

No management or control measures are necessary.

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See also discussion of this species in general references, page 20.

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Moles (Family Talpidae)

The family name, Talpidae, is derived from the Latin word for "mole." Characteristically, the front feet, legs, and shoulders of moles are greatly enlarged and modified, an adaptation for digging their underground tunnel systems. The ears have no external flaps, and the openings are hidden in the fur. The eyes are extremely minute and in some forms are hidden in the fur and even concealed beneath the skin. The body is stout and cylindrical. As in the shrews, the body fur is plushlike.

Members of this family are found in North America, Europe, and Asia. The only representative in Missouri is the Eastern Mole.

Eastern Mole *(Scalopus aquaticus)*

Name

The first part of the scientific name, *Scalopus*, is from two Greek words and means "digging" and "foot" (*skalops*, "mole," derived from the word "to dig," and *pous*, "foot"). This name refers to the large front feet of the mole, which are used in digging. The last part, *aquaticus*, is the Latin word meaning "found in water"; this misleading term was given to the mole because the webbed structure of the feet suggested that they were used in an aquatic habitat.

The common name, "eastern," refers to the range of the species, and "mole" is from the Middle English *molle*, which is related to another Middle English word, *moldwarpe*, meaning "earth-thrower."

Description (Plate 7)

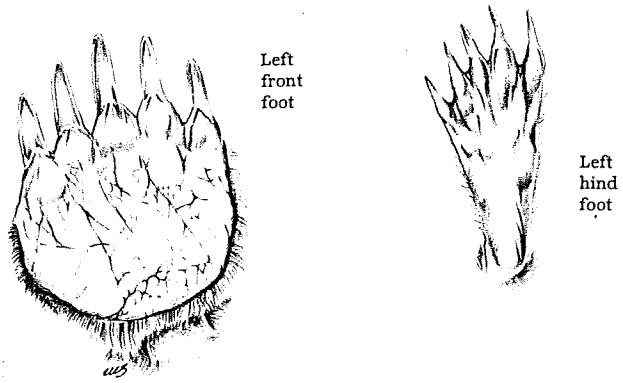
Moles live most of their lives underground and are highly specialized for their subterranean way of life. The Eastern Mole is a small, sturdy animal with a somewhat cylindrical body and an elongated head. The fleshy, movable snout, which serves as an organ of touch, projects over the mouth and contains the nostrils on the uppersurface. The tiny, degenerate eyes are concealed in the fur and covered by fused eyelids; sight is limited merely to distinguishing light from dark. Although the ear opening is small and hidden in the fur, hearing is fairly acute.

The greatly enlarged front feet, which are broader than they are long, are normally held with the soles vertical and pointing outward. They possess well-developed claws and have a specialized sesamoid bone attached to the wrist that aids in digging. The hind feet are small. Both front and hind feet have a fringe of sensory hairs that helps in the excavating operations. The short tail is thick and scantily furred; it functions as an organ of touch and guides the mole when it moves backward in the tunnel. The short, velvety fur offers little resistance to travel through tunnels.

The bones of front limb and breast are greatly enlarged, providing strong attachments for the powerful muscles used in digging. The extremely narrow hip girdle permits the mole to turn around in its tunnel; this it does by slowly performing a partial somersault or doubling back on itself.

Color. The Eastern Mole is grayish brown, being darker above and paler to browner beneath. The face, feet, and tail are whitish to pink. When viewed from different angles, the velvety fur often has a silvery sheen. The winter pelage is darker than the summer pelage. Compared to females, males tend to have a brighter orange strip on the belly caused by the secretion of skin glands in this region. Occasionally bright orange, cinnamon yellow, or white individuals occur. These variously colored moles are albinos with their white fur stained by the secretion of skin glands.

Feet of Eastern Mole



Moles molt in spring and fall. The fresh pelage comes in first on the chest and belly and then covers the underparts. On the back the new fur is first apparent near the tail and then gradually works forward. Usually there is a sharp line demarking old and new fur. There is no distinct underfur, all hairs being the same length. When viewed microscopically, the hairs can be seen to possess whiplike tips similar to the hairs of shrews but unlike those of any other mammal.

Measurements

Total length	5½–8	in.	139–203	mm
Tail	¾–1½	in.	19–38	mm
Hind foot	⅝–1	in.	15–25	mm
Skull length	1⅛–1½	in.	28–38	mm
Skull width	¾	in.	19	mm
Weight	1–5	oz.	28–141	g

Males are usually larger than females.

Teeth and skull. The dental formula of the Eastern Mole is:

$$I\ \frac{3}{2}\ C\ \frac{1}{0}\ P\ \frac{3}{3}\ M\ \frac{3}{3} = 36$$

However, the teeth of moles show little specilization into incisors, canines, premolars, and molars. The first upper incisors are large and curved downward and backward, but the second and third incisors are very small.

The Eastern Mole's skull is distinguished from the skulls of other adult Missouri mammals of similar size by the following combination of charactristics: number and shape of teeth as described above; weak zygomatic arch (the bony arch at the side of the eye socket); inflated bone surrounding the inner ear; and hard palate extending beyond the molars.

Sex criteria. The sexes are determined externally by the number of openings in the groin region. In females there are three openings. The forward one is the urinary opening, which is in the small urinary papilla, or projection; the middle one is the vagina; and the opening nearest the tail is the anus. In males only two openings occur, the combined urinary-reproductive opening in the penis, and the anus. The testes are located within the body cavity of males and never descend into a scrotum. There are six teats on the belly, of which one pair is in the chest region, one pair in the groin region, and one pair between the others.

Age criteria and longevity. Young moles are distinguished from adults by their grayer coloration. With age, the skull becomes flattened.

Moles probably have a relatively long life span, as inferred by the low reproductive rate and paucity of predators.

Glands. A rank, musky odor is given off by a scent gland on the belly. This scent is left on the floor of the tunnel as the mole passes by and may serve as a means

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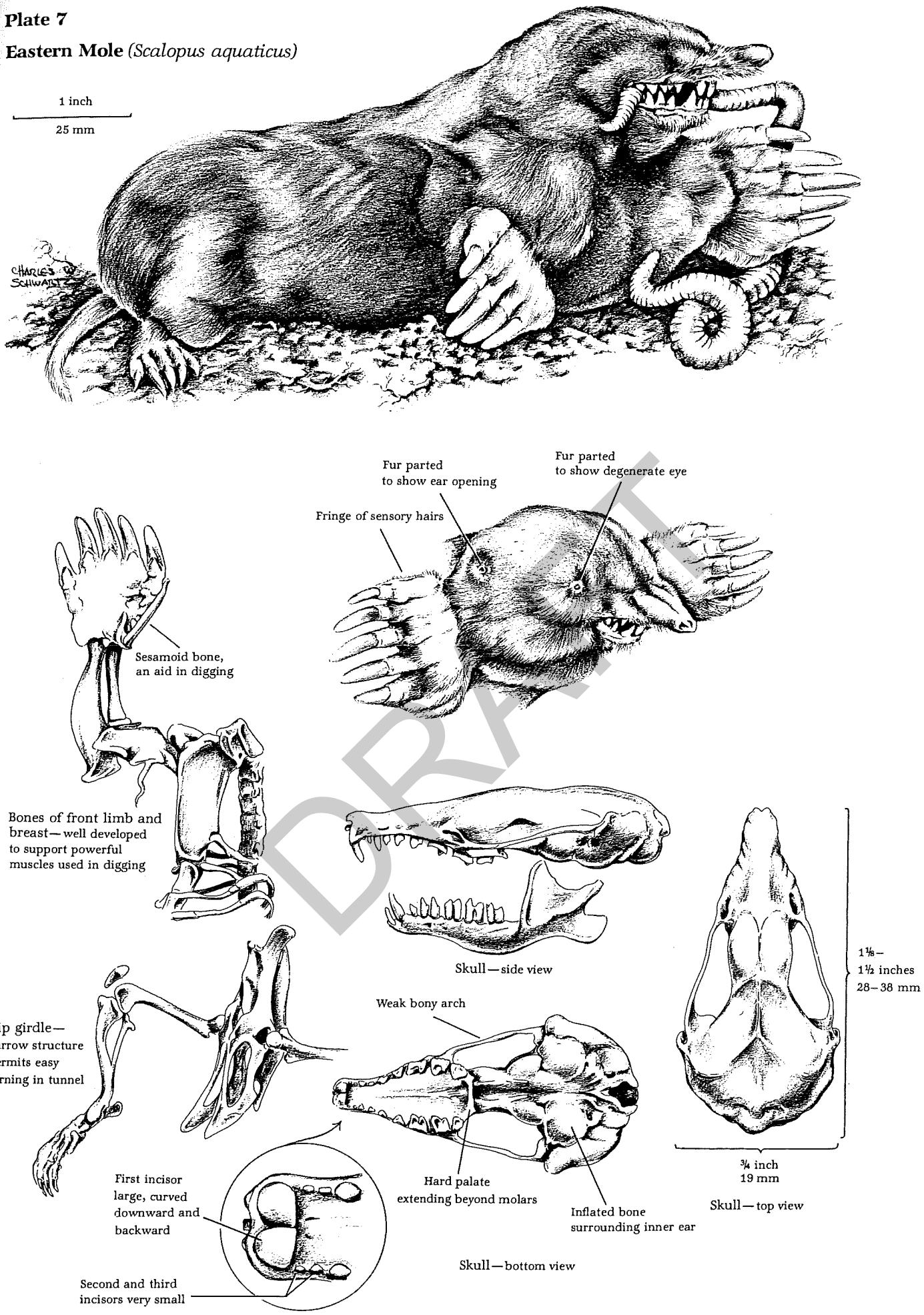
Plate 7

Eastern Mole *(Scalopus aquaticus)*



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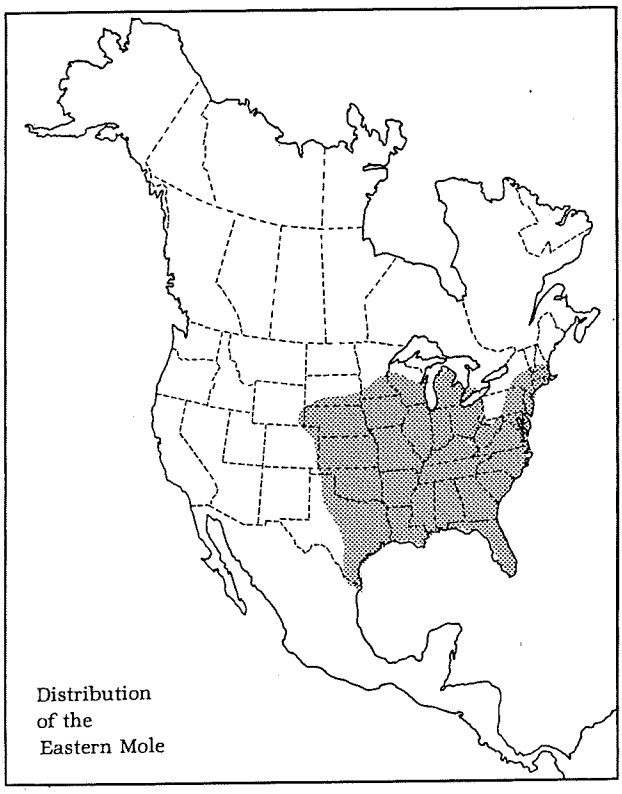

Distribution of the Eastern Mole

of communication between the sexes during the breeding season. It also renders the mole undesirable to many predators. Other large scent glands occur near the anus.

Voice and sounds. The various sounds made by moles are high-pitched squeals; harsh, gutteral squeaks; short, snorting sounds; and grating of the teeth.

Distribution and Abundance

The Eastern Mole is found throughout Missouri. A high population is 2 to 3 moles per acre.

The North American distribution of this species is shown on the accompanying map. The mole is well distributed and abundant in this range.

Habitat and Home

Moles live underground in meadows, pastures, lawns, open woodlands, gardens, and stream banks where the soil is loose, contains humus, and is well drained but moist. In such soil conditions, digging is easy and foods the mole seeks usually abound. Soils too loose in structure to support tunnels are avoided unless they have abundant food.

Moles construct a series of tunnels in the ground. Temporary ones, barely under the surface but at a uniform depth, are made by raising the sod or ground cover with the front feet. Many of these are built after rains when the mole is in search of new sources of food and are seldom reused. These are the tunnels most commonly observed by humans. Permanent tunnels occur from 10 to 18 or more inches beneath the ground surface, which in winter is below the frost line. All are just large enough in diameter to allow the mole easy passage. The tunnels of males are slightly larger than those of females because of their respective sizes.

A large chamber in the deeper runways is used as a retreat during drought, heat, or cold and as a nest for the young. This chamber, between 5 and 8 inches in diameter, is customarily located under a stump, stone wall, bush, or other feature affording some permanent protection. There are usually several openings into this home, including one from below. When this chamber is used as a nest, a lining may be added of roots, grass, or leaves.

Habits

The digging of surface tunnels normally proceeds at the rate of about 1 foot per minute, while deeper excavation goes on at approximately 12 to 15 feet per hour, including stops for rest and food. When working near the surface, a mole's movements may be detected by the upheaval of soil as it progresses with its excavations. During winter when the ground is frozen, moles dig less than at other seasons. However, they may still be active underground in their deeper tunnels.

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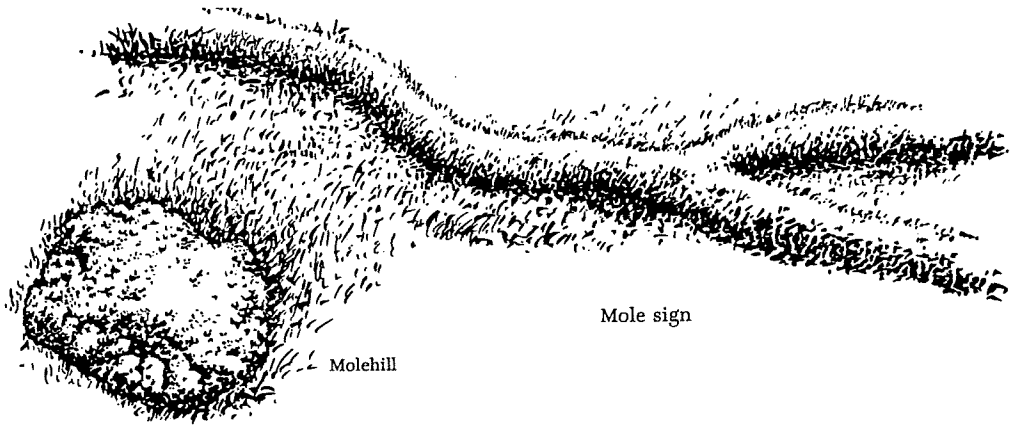


Sign in thin snow of mole tunnel underground

In digging, the mole braces itself against the sides of the tunnel with the hind feet and loosens the soil with the front feet. The dirt is passed under the body to the hind feet, which kick it further behind. When a pile has accumulated, the mole turns around and pushes the dirt ahead by using one of its front feet. The dirt is either packed into an unused tunnel or pushed out onto the surface of the ground through a vertical tunnel. At the sites of such excavations, piles of earth or "molehills" are built up. The tunnel is usually plugged from below, and no surface opening is present. However, openings sometimes occur that the mole uses for a rare visit to the surface. Molehills are not made as often by the Eastern Mole as by other kinds of moles. Molehills are occasionally confused with mounds made by pocket gophers, but the latter have soil piled fanwise around a plugged opening.

Each mole has its own system of tunnels and lives a solitary life. Other moles may be tolerated where the tunnels are next to one another or during the breeding season when some traveling probably occurs in search of mates. Often mice, shrews, and other animals use the tunnels more than do moles. There is a special place in the tunnel system for sanitation.

The home range varies from 1/2 to 4 1/2 acres. Because males have a larger home range than females, a trap set in a runway has a better chance of catching a male than a female.

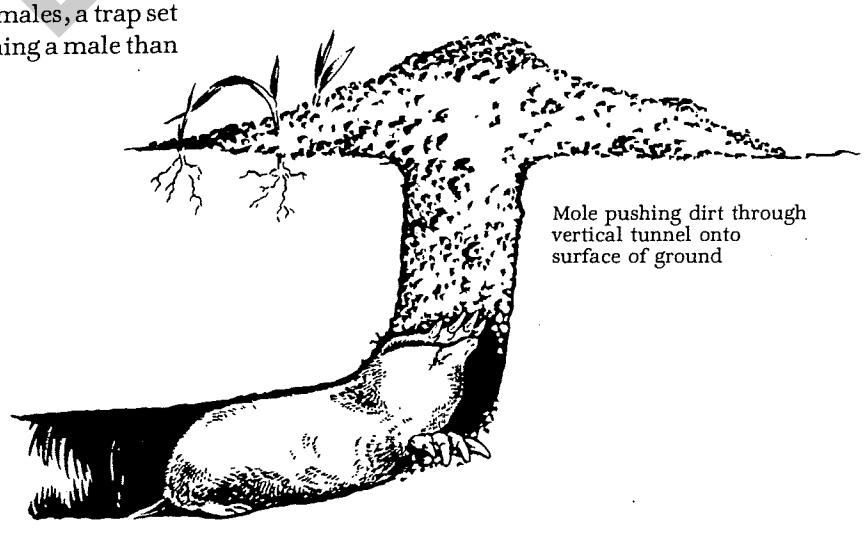
Moles are active during all hours of the day and night. They are most active on damp and cloudy days during spring and fall. By the use of telemetry, it has been determined that they rest about 3 hours, then are active about 5 hours. The resting period is spent in the nest during cold weather but in no specific location in the tunnel during warm weather.

Moles breathe very rapidly, respiring between 40 and 42 times a minute while at rest. This high metabolism seems to be associated with their general nervous disposition and high comsumption of food. Their sleep is very profound. During sleep the head is curled under the body and the front feet are directed backward.

The Eastern Mole can swim but apparently does so only in an emergency.

Foods

Animal foods constitute about 85 percent of the diet while plant matter, such as the seeds of oats, wheat, corn, and grass, sprouting corn, and rarely Irish potatoes, forms the remaining amount. Earthworms and white grubs are favorite items. Other insect larvae, adult insects, millipedes, centipedes, spiders, sowbugs,



Mole pushing dirt through vertical tunnel onto surface of ground

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snails, and slugs are taken in considerable quantities. Occasionally a meadow vole or garter snake may be added to the fare.

The mole kills its prey by crushing the animal against the side of the tunnel with its strong front feet. Sometimes the mole piles dirt on the victim and bites off its head when it attempts to escape. If the prey offers no resistance, however, the mole may start to devour the animal without killing it.

Moles are insatiable eaters and in captivity consume, on the average, the equivalent of one-half of their body weight daily. Captive moles take water readily.

Reproduction

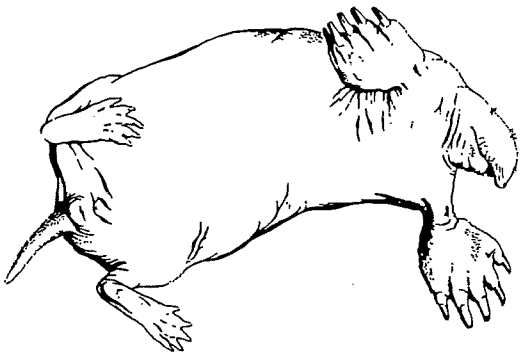
Mating occurs in the spring and, after a pregnancy of 4 to 6 weeks, the single annual litter is born in March, April, or the first week of May. The most usual number of young is 4, but from 2 to 5 may compose a litter. The female takes full care of her offspring.

The young are blind and naked at birth. Growth is rapid, and when 10 days old the young moles have a fine covering of gray, velvetlike fur that remains for several weeks. At 1 month of age, they are able to care for themselves and probably leave the nest about this time. They breed first in the spring following their birth.

Some Adverse Factors

Few animals prey upon moles because of their subterranean habits and musky odor. Snakes may overpower some moles in their tunnels, and shrews using mole runways may take some young in the nest. Hawks, owls, skunks, coyotes, foxes, and men are additional predators.

Mites, lice, fleas, and roundworms parasitize Eastern Moles. A mammal-nest beetle has been found on moles. This kind of beetle is known to live in the nests of rodents, insectivores, and bumblebees, where it probably feeds on eggs and young of mites.



Mole few days old

Importance

Moles are extremely valuable, as they till and form soil, dig tunnels into the soil permitting air and moisture to penetrate to deeper soil layers, and feed upon destructive insects such as cutworms and Japanese beetle larvae. Their unusual way of life is also interesting. Formerly the pelts of some species of moles were used commercially, but they are not in demand today. The Eastern Mole is small, and its pelt does not take dye well, so it has never been important as a commercial furbearing animal.

When moles interfere with man's interests by raising disfiguring ridges on his lawns, golf courses, and cemeteries, by inadvertently damaging the roots of garden plants in their quest for insects and earthworms in the newly plowed ground, or by taking sprouting corn, their activities are considered undesirable.

Management or Control

Because of their subterranean habits, moles are rather difficult to control. When a mole is working near the surface of the ground, it is sometimes possible to dig up the animal with a spade. Traps set in permanent runways, or in temporary ones that are being used, are the best means of controlling these mammals, especially in early spring or in fall after the first rains. Another somewhat effective method is to place peanuts coated with thallium sulphate in the runways; this poison is highly toxic to moles and other animals, including man. Users of any poison should acquaint themselves with the properties of the poison and employ it wisely. They should also consult both civil laws and the state wildlife code regarding the use of poison.

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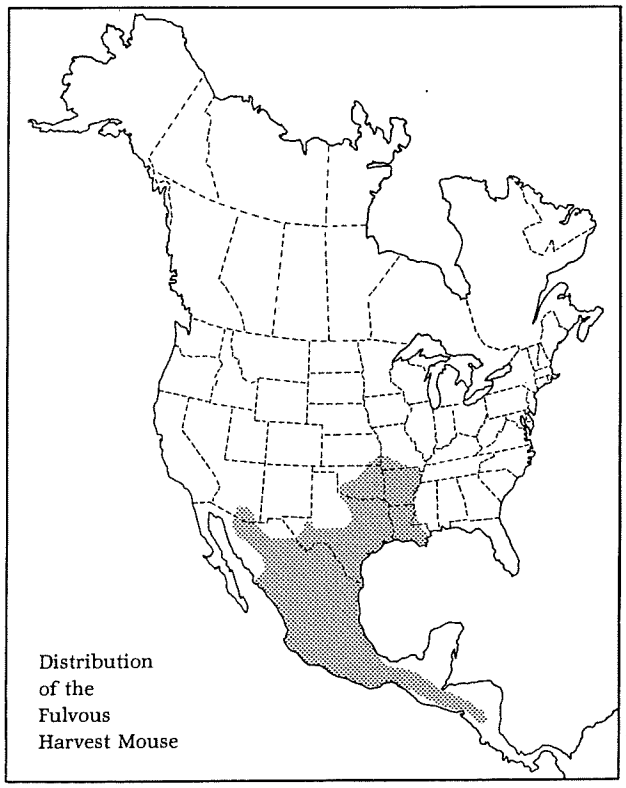

Distribution of the Fulvous Harvest Mouse

Habitat, Home, and Life History

The Fulvous Harvest Mouse is very similar to the Western Harvest Mouse in its way of life.

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Peromyscus Mice

There are four species of closely related mice that are similar in appearance and habits. These are the Deer Mouse, *Peromyscus maniculatus*, White-footed Mouse, *Peromyscus leucopus*, Cotton Mouse, *Peromyscus gossypinus*, and Texas Mouse, *Peromyscus attwateri*. For accurate identification of the individual species, specimens should be examined by a person familiar with these species.

Deer Mouse *(Peromyscus maniculatus)*

Name

The first part of the scientific name, *Peromyscus*, is from two Greek words and means "pouched little mouse" (*pera*, "pouch," and *myskos*, "little mouse"). This refers to the internal cheek pouches of this small rodent. The last part, *maniculatus*, is the Latin word for "small handed" and indicates the size of the front feet. The common name, "deer," refers to the similarity of color with the White-tailed Deer (generally brownish back and sides with whitish underparts), and "mouse" describes the typical mouse shape.

Because this species has such a wide distribution and variation in the structure, behavior, and habitat preferences of its members, the following discussion emphasizes the subspecies, *Peromyscus maniculatus bairdii*, that occurs in Missouri.

Description (Plate 37)

The Deer Mouse is a small rodent with large, protruding, black eyes; large, scantily furred ears; long, coarse whiskers; and a moderately to well furred tail from one-third to less than one-half of the animal's total length with a slight tuft at the tip. There are 4 clawed toes and an inconspicuous nailed thumb on each front foot, and 5 clawed toes on each hind foot. The soles of the hind feet are thinly furred from the heel to the 6 pads, or tubercles. Small internal cheek pouches are present. The body fur is long and soft.

The species of *Peromyscus* in Missouri are difficult to distinguish but are best told by differences in their size and characters of the tail. (See accounts of the other *Peromyscus.)*

Missouri mice, other than members of the genus *Peromyscus*, that are similar in size and general appearance to the Deer Mouse are the Golden Mouse, the harvest mice, the House Mouse, the Meadow Jumping Mouse, and the Plains Pocket Mouse. The adult Deer Mouse is readily told from the Golden Mouse by the color and by the smaller median pad on the outside of the sole on the hind foot; from the harvest mice by the absence of grooved upper incisors; from the adult House Mouse by the sharp contrast in color between back and belly and between upper and lower surfaces of the well to moderately furred tail, and by the large protruding eyes; from the Meadow Jumping Mouse by the absence of grooved upper incisors, the shorter tail, and the smaller hind feet; and from the Plains Pocket Mouse by the absence of external, fur-lined cheek pouches, smaller ears, differences in coloration, and the absence of grooved upper incisors.

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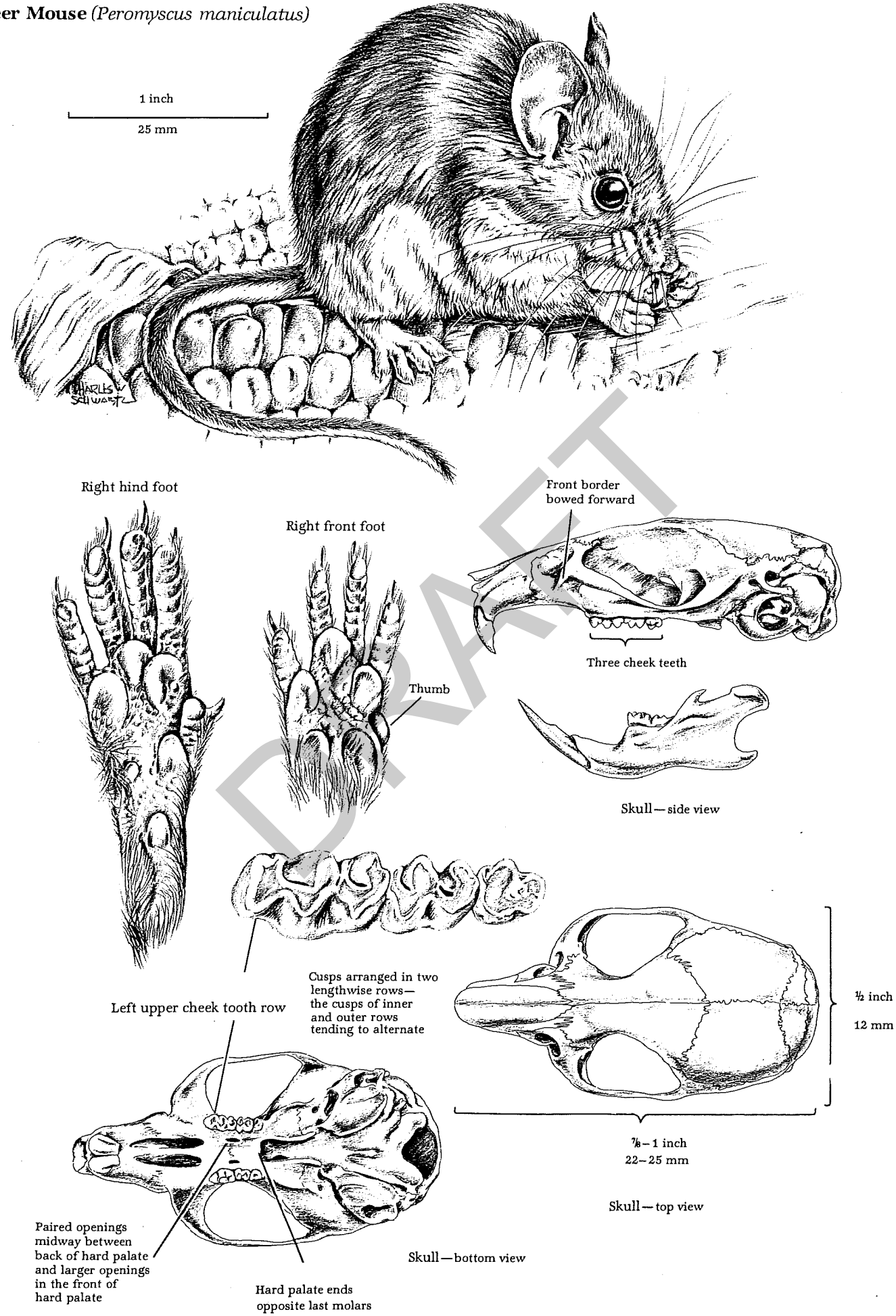
Plate 37

Deer Mouse *(Peromyscus maniculatus)*



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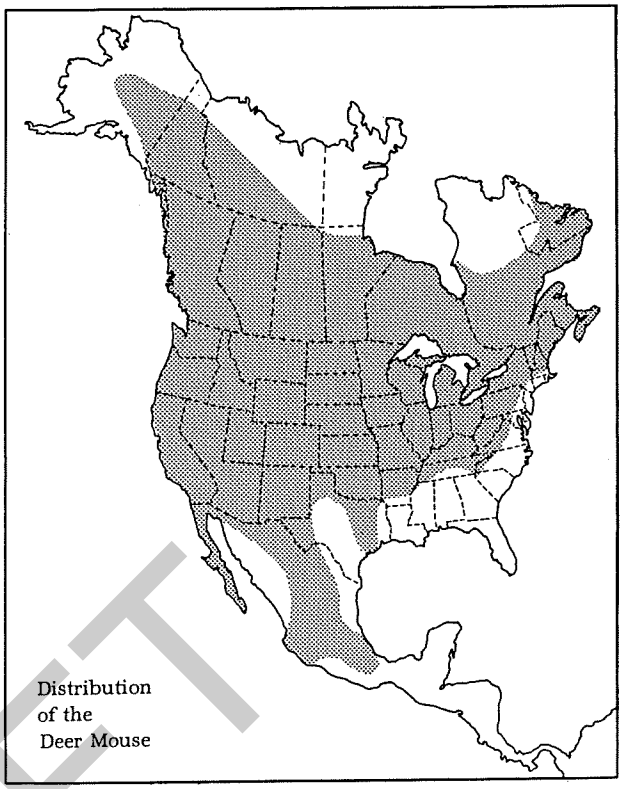
Color. There is considerable color variation in individual Deer Mice, but in general the back and sides of the adults vary from grayish to reddish brown with or without a darker area in the middle of the back. This color is sharply marked off from the lower face and underparts, which are white or sometimes grayish. The base of the hairs on both back and belly is dark gray. The feet are white. The tail is dark like the back above and sharply contrasted to light like the belly below. The ears are dark brown for approximately the outer half with a very slight grayish to whitish margin but are whitish to pinkish for the inner half. The sexes are colored alike. Adults molt in late summer or early fall. Only a few hairs are replaced at a time, beginning at the head and working toward the tail.

Measurements

Total length	4 3/8–8 in.	111–203 mm
Commonly	6 or less in.	152 or less mm
Tail	1 5/8–3 7/8 in.	41–98 mm
Commonly	2 1/2 or less in.	63 or less mm
Hind foot	5/8–1 in.	15–25 mm
Commonly	13/16 or less in.	20 or less mm
Ear	1/2–7/8 in.	12–22 mm
Commonly	5/8 or less in.	15 or less mm
Skull length	7/8–1 in.	22–25 mm
Skull width	1/2 in.	12 mm
Weight	1/3–1 oz.	9–28 g



Distribution of the Deer Mouse

Teeth and skull. The dental formula of the Deer Mouse is:

$$I\ \frac{1}{1}\ C\ \frac{0}{0}\ P\ \frac{0}{0}\ M\ \frac{3}{3} = 16$$

The grinding surfaces of the upper cheek teeth consist of small, rounded cusps capped with enamel. The cusps are arranged in 2 lengthwise rows, those of the inner and outer rows tending to alternate.

The skull of the Deer Mouse is so similar to those of the other *Peromyscus* mice in Missouri that it is difficult to distinguish between them. However, the Deer Mouse skull can be told from that of the White-footed Mouse because the Deer Mouse tends to have a narrower rostrum and to have the anterior palatine foramina (large paired openings in the front of the hard palate) parallel, while the White-footed Mouse has a broader rostrum and the anterior palatine foramina bowed out at the middle.

Among Missouri mice with 16 teeth, other than *Peromyscus*, the skull of the Deer Mouse could be confused only with that of the Golden Mouse or the Marsh Rice Rat. From the Golden Mouse, the Deer Mouse can be distinguished in two ways: in the Deer Mouse the posterior palatine foramina (tiny paired openings in the hard palate near the second molars) are about halfway between the back of the hard palate and the anterior palatine foramina (large paired openings in the front of the hard palate), and the front border of the infraorbital plate is bowed forward. From the Marsh Rice Rat, it is distinguished by the following characteristics: size, the arrangement of cusps on the upper molar teeth, the absence of a ridge above the eye socket, the absence of paired openings in the hard palate behind the last molars, and the hard palate ending opposite the last upper molars.

Sex criteria and sex ratio. The sexes are identified as in the Eastern Wood Rat. There are three pairs of teats on the belly, one pair near the front legs and two pairs in the groin region. Males outnumber females at birth and in older populations.

Age criteria, age ratio, and longevity. The young have gray to grayish black fur that changes to a duller

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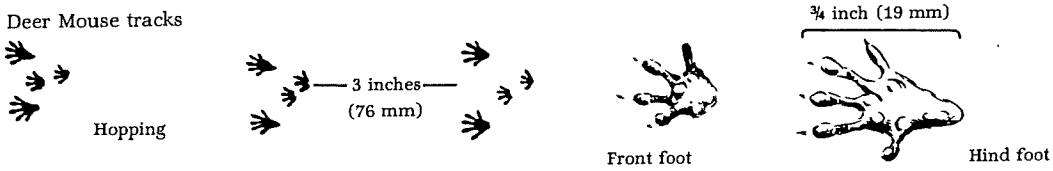
Deer Mouse tracks



and paler brown before adult coloration is acquired. Relative age is determined by the eruption and wear of the teeth. (See Sheppe, 1963.)

In Missouri, 50 percent of the population of Deer Mice in winter and spring consists of young animals, resulting from the slow maturing of young during winter; in summer only 13 percent are young because the young mature rapidly at this time of year.

In the wild, Deer Mice may live as long as 1½ or 2 years. However, fewer than ⅕ of those born usually reach sexual maturity. The record of longevity in captivity is 8 years.

Glands. Certain salivary glands (the parotid and submaxillary glands) may contain a weak poison; extracts from these glands injected into laboratory mice induced hard breathing.

Voice and sounds. Deer Mice utter high-pitched squeaks, trills, chatters, and a shrill buzz that lasts from 5 to 10 seconds and is audible for 50 feet. In addition, some sounds are ultrasonic. The young have special calls for the female but no longer give them when they are old enough to care for themselves. When disturbed or excited, Deer Mice stamp their front feet up and down very rapidly.

Distribution and Abundance

The Deer Mouse lives throughout Missouri and is one of the most abundant mammals on open lands. Highest populations in this state occur from March through June, lowest populations from July through October, and moderate ones from November through February.

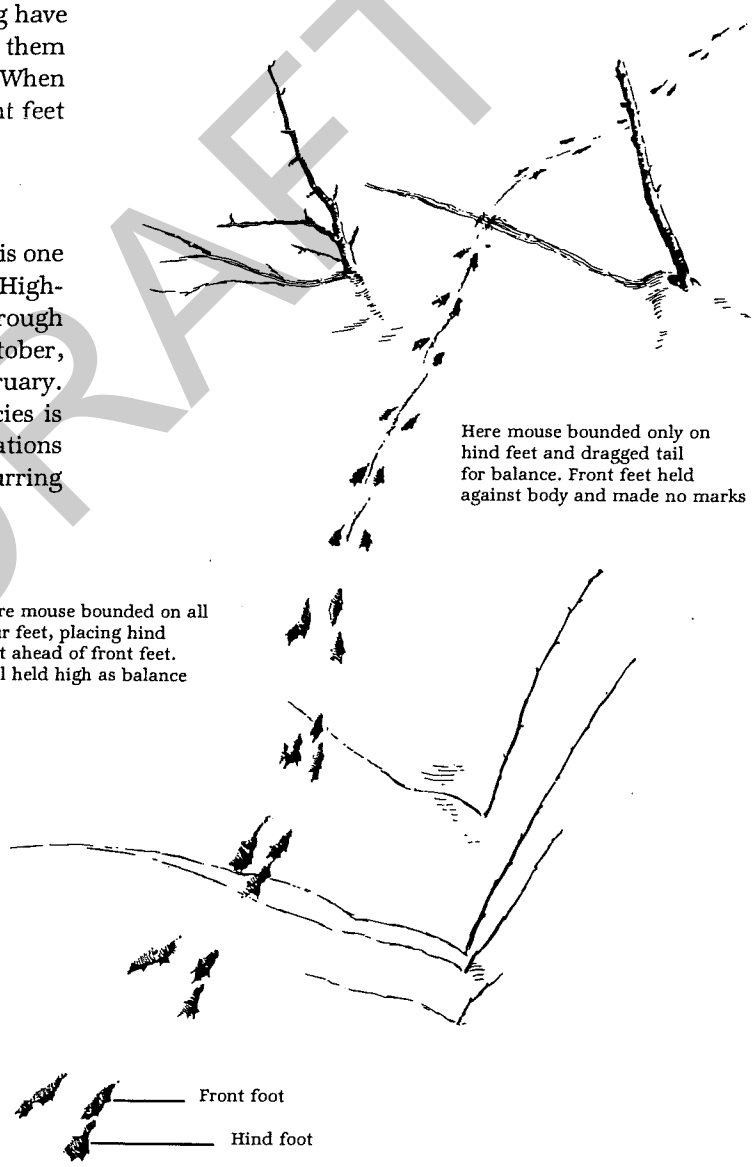
The North American distribution of this species is shown on the accompanying map. Local populations fluctuate greatly from year to year with peaks occurring about every 3 to 5 years.

Habitat and Home

In Missouri the Deer Mouse is usually found in an open habitat such as pastures, meadows, cultivated fields, and along field borders and fence rows. It may also live around and in human habitations but generally does not occur in heavy brush or wooded places. In Missouri it seldom occupies the same range as its close relative, the forest-dwelling White-footed Mouse.

The several nests are generally located underground in cavities about the roots of trees or shrubs, beneath a log or board, or in a tunnel built either by another animal, but no longer used by it, or by the mouse itself. Less often are the nests aboveground in a hollow fence post, stump, log, or an old bird's or squirrel's nest.

The Deer Mouse's nest is usually spherical with a single side entrance that the mouse closes from the inside. Leaves, stems, and roots of grasses, sedges, and



Deer Mouse tracks in snow

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other plants and shredded bark are woven into the framework; thistledown, feathers, fur, and other soft material, such as cotton or rags, are fluffed inside for the lining. When a bird's nest is used for the nest base, a dome-shaped roof is added by the mouse.

Habits

Adult Deer Mice tend to spend their entire lives in one locality. The home range is usually from 1/2 to 1 1/2 acres in extent, but in rare cases may encompass from 5 to 10 acres. In general, males range over greater areas than females, and breeding females tend to stay close to their nests. Deer Mice have a well-developed homing instinct, and some have returned to their homes when liberated up to 2 miles away. During winter, these mice live in a smaller area than during the rest of the year and may travel only about 30 feet from their nests, depending upon the food supply; in bad weather they may not leave their nests for several days at a time. The young often stay near the original nest site and rarely move farther than 550 feet before establishing their own homes.

Although Deer Mice are abroad mostly at night, they shun the hours of brightest moonlight. The customary feeding periods are during early evening and just before dawn.

During the breeding season, Deer Mice live singly or in pairs. A pregnant female may tolerate her mate in the nest with one litter of young, but she often chases both the male and young away before the birth of another litter; or she may leave the male with the weaned young in their nest and move away to establish a new home. In winter these mice often congregate in groups up to fifteen in number of mixed ages and sexes and huddle together in a common nest for warmth. This huddling helps them survive in cold climates, especially where food is scarce in winter and they cannot obtain sufficient nourishment to maintain adequate body heat. Antagonism between individuals and sexes begins with the onset of the breeding season. Certain males display a dominance over others. When White-footed Mice occur in the same areas, they show more aggression and tend to dominate Deer Mice.

These mice do not make definite runways through grass or other vegetation but often use the runways and tunnels of other kinds of mice and of shrews. They have been observed to forage for aquatic organisms in water 1/2 inch deep and to swim across 10 feet of rough water. They climb trees readily. They usually leap rather than run and have been timed to travel at the rate of 8 feet per second.

Deer Mice are very clean about their coats but insanitary about their nests. Because of their habit of

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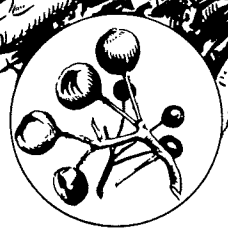
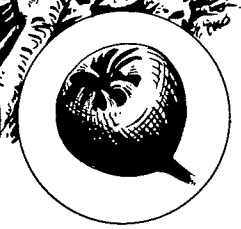
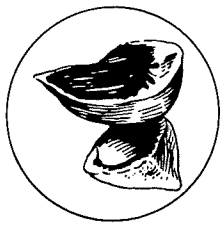
Deer Mouse at feeding platform on old bird's nest in Japanese rose fence. Empty seed hulls, rose fruit, and mouse droppings fill cup of nest and form feeding platform



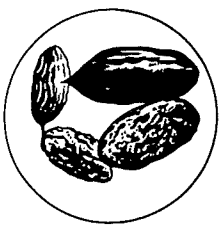
Freshly clipped rose fruit clusters sometimes present in small piles on feeding platform



Rose fruit characteristically opened at top and seeds removed



Seed hulls opened at either end or along flat side



Mouse droppings mixed with seed hulls and fruit skins

Young Deer Mice
in nest inside rotten log

leaving scraps of food in the nest and using the nest for elimination purposes, they must move from one nest to another every few weeks.

Foods

The important foods of Deer Mice are insects (beetles and larvae of butterflies and moths), nuts, wild seeds, domestic grain (corn and soybeans), fruits and fruit pits, and some leafy vegetation. Occasionally they eat fungi, snails, slugs, worms, spiders, centipedes, millipedes, eggs and young of birds, and dead mice.

In the fall, seeds and nuts are stored in holes in the ground, in old birds' nests, or in trees. Such stores may contain as much as a pint of food. Deer Mice do much of their feeding at these storehouses. The food is carried in the small cheek pouches, which together hold about a teaspoonful of seeds.

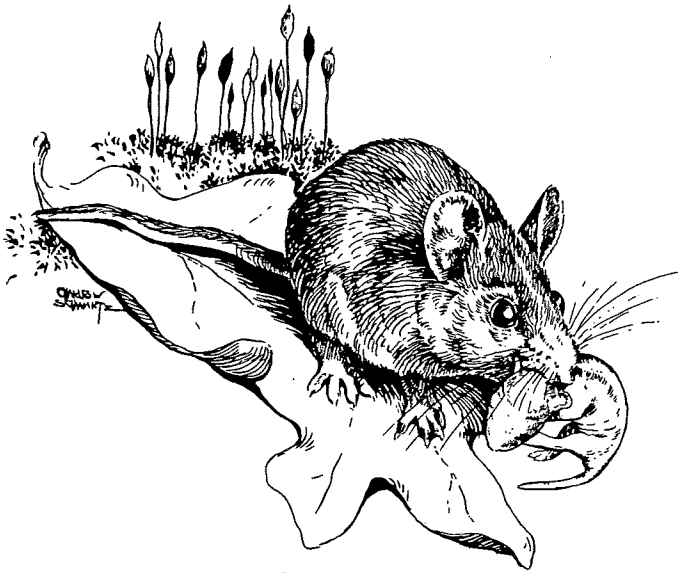
Reproduction

The principal mating periods of the Deer Mouse occur in spring and fall, but limited breeding takes place in summer and, under unusually favorable conditions of mild temperatures and abundant food, even in winter. Estrus, or heat, last for 26 hours and occurs every fifth day in unmated females. Estrus also occurs from 24 to 48 hours after birth of the young.

The gestation period is generally from 21 to 23 days but may be extended up to 37 days in nursing females. From 1 to 9 young are born per litter with 3 and 4 the most common. No female breeds continuously the year around, but a female may have 2 or more litters successively in the spring followed by a rest period in summer, and 2 or more litters again in the fall. In captivity two females bore 10 and 11 litters with totals of 45 and 42 young, respectively, during one year.

Parturition takes from 1/2 to 1 hour and usually occurs in the morning. During birth, the female may assist the young by pulling them with her teeth or front feet and by prodding them with her nose. At birth the young are wrinkled, flesh-colored, naked, and weigh about 1/15 ounce each. The eyes are closed and the ears folded. The toes and claws are formed, but the separation between the toes has not yet occurred.

The ears unfold at 3 to 4 days of age, and the incisors cut through the gums at 6 days of age. About 2 weeks



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after birth, the eyes open and the body is well furred. Weaning takes place when the young are 2 to 3 weeks old.

In brooding, the female straddles her young. When about one week old, they hold firmly to the female's nipples and are often dragged along if she moves suddenly and fails to disengage them. In transporting the young, the female rolls the young into a ball with her front feet and carries it in her mouth by the belly skin or back. She frequently permits the male to assist in caring for them.

In general, females become sexually mature between 46 to 51 days of age, while males become sexually mature when about 10 days older. The young born in early spring mature in 4½ to 9 weeks and may breed in the spring of their birth; young born late in spring or in summer breed for the first time in the fall; and young born in the fall breed for the first time in the spring following their birth.

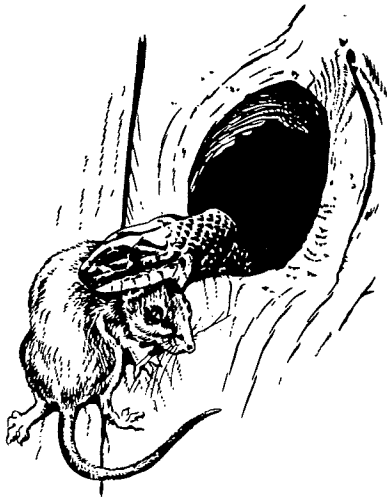
Mated mice usually stay together during the breeding season, if both survive; otherwise, new mates are acquired. If the young remain in the vicinity of the home nest, there may be considerable breeding among close relatives. In cases under laboratory conditions where two or more females have had their litters in the same nest, no antagonism was shown by the females, who nursed each other's young without apparent concern.

When Deer Mice are crossed experimentally with White-footed Mice, no offspring are produced. However, in the wild it is remotely possible that interbreeding takes place because some individuals occur that appear to be intergrades.

Some Adverse Factors

Deer Mice are preyed upon by opossums, Short-tailed Shrews, foxes, coyotes, weasels, skunks, minks, badgers, bobcats, Domestic Cats, hawks, owls, and snakes.

Parasites found on or in Deer Mice are mites, ticks, lice, fleas, botfly larvae, roundworms, and tapeworms.



The scab mite produces swollen, scabby ears and tail and causes the hair to fall out, especially on the back. This condition is most common during the summer. Botfly larvae are found most often in late summer and early fall. They are usually located in the groin region of the mouse and may contribute to mortality by making the host awkward and easier prey. Certain strains of Deer Mice reared in laboratories show an inherited tendency to develop epilepsy. Cold weather with its accompanying food shortage is a principal cause of mortality.

Importance

Deer Mice are very important as a prey species for flesh-eating animals. In fact, when they are abundant and form a ready supply of food, their predators likewise become abundant. These mice consume large quantities of weed seeds and insects. They are also important by returning their waste products to the soil as fertilizer, which, in the case of such a common animal, is considerable. Deer Mice are reared in laboratories because they make good experimental animals for research on heredity, cancer, and many other subjects.

These mice damage some crops and stores of grain and in the western states dig up seeds planted for reforestation. In the fall, they commonly enter buildings where they become a nuisance.

Management or Control

Snaptraps can effectively eradicate Deer Mice from buildings; in unoccupied dwellings, naphthalene flakes will repel them and discourage their use of mattresses for nesting material.

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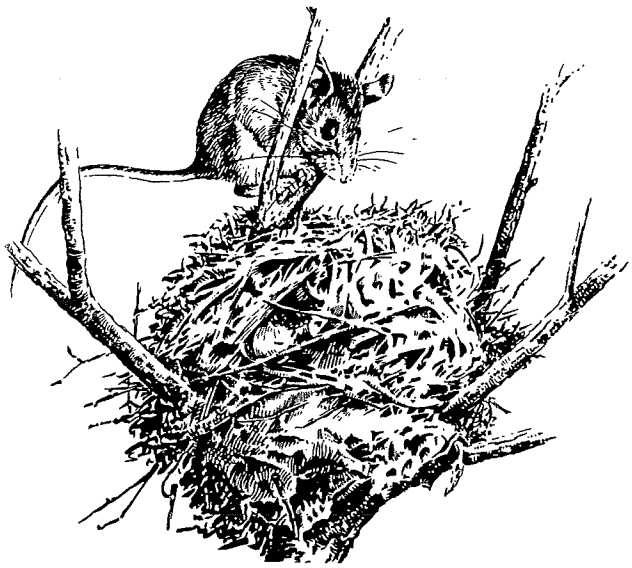


Abandoned birds' nests are frequently roofed and converted into White-footed Mouse homes

White-footed Mouse *(Peromyscus leucopus)*

Name

The first part of the scientific name, *Peromyscus*, is from two Greek words and means "pouched little mouse." The last part, *leucopus*, is from two Greek words and means "white-footed" (*leukon*, "white," and *pous*, "foot"). This and the common name describe the color of the feet.

Description (Plate 38)

The White-footed Mouse is very similar to the Deer Mouse. In general, the tail of the White-footed Mouse tends to be slightly longer (about equal to or slightly less than one-half the total length), to lack a sharp contrast in color between the upper and lower surfaces (but some tails show more contrast than others, especially in winter), and to be more sparsely furred and scaly and without a tuft of hairs at the tip. The White-footed Mouse tends to be larger than the Deer Mouse and so has a larger hind foot and ear.

Compare this description with those of other *Peromyscus* species and see the account of the Deer Mouse for distinctions between *Peromyscus* and other mice in Missouri.

Color. The color is very similar to that of the Deer Mouse.

Measurements

Total length	5½–8⅜	in.	139–212	mm
Tail	2½–4	in.	63–101	mm
Hind foot	¾–1	in.	19–25	mm
Ear	⅝–¾	in.	15–19	mm
Skull length	1–1⅛	in.	25–28	mm
Skull width	½–9/16	in.	12–14	mm
Weight	⅖–1	oz.	11–28	g

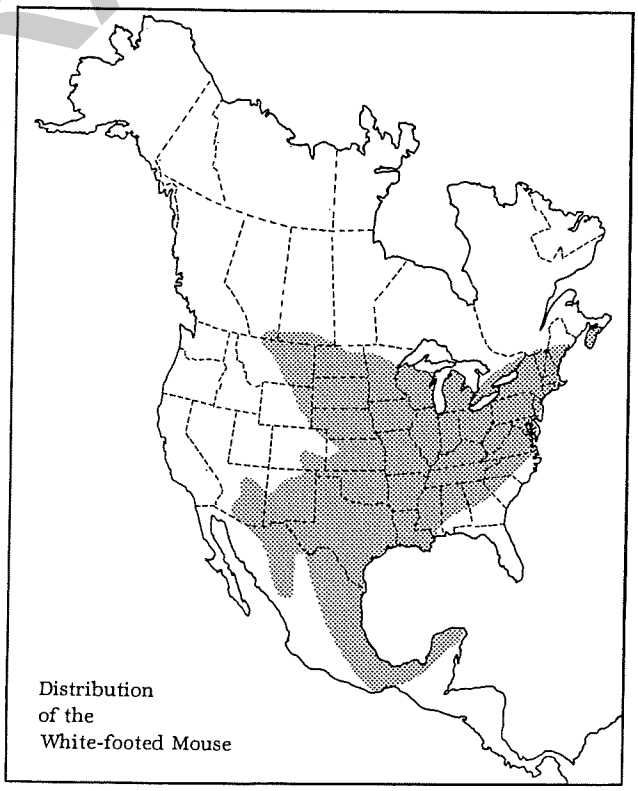
Teeth and skull. The minor differences in teeth and skull between the White-footed Mouse and the Deer Mouse are discussed with the Deer Mouse.

Distribution and Abundance

The White-footed Mouse lives throughout Missouri and is one of the most abundant mammals in wooded regions. Its North American distribution is shown on the accompanying map. Population densities usually vary from 1 to 4 White-footed Mice per acre but may reach 20.

Habitat and Home

The White-footed Mouse lives primarily in wooded areas, brushy or weedy borders, and along fence rows. It occurs only in those grassy areas or fields with harvested grain that border woods or brush. The preferred



Distribution of the White-footed Mouse

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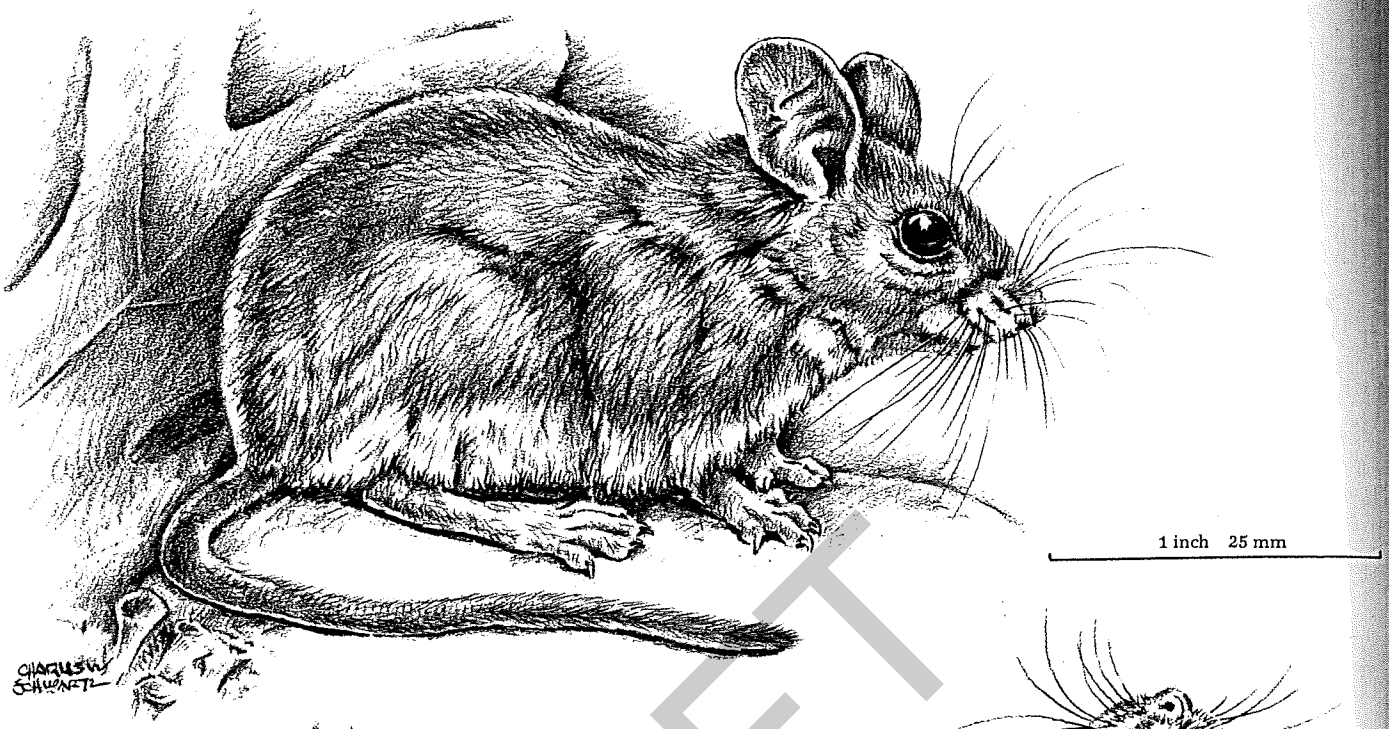
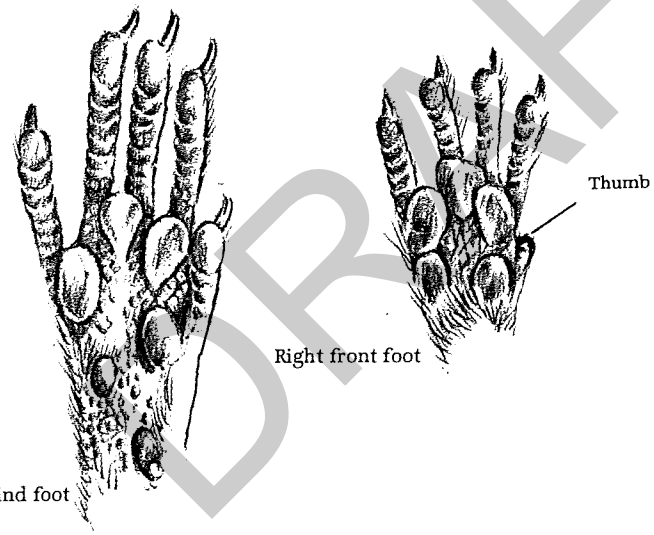
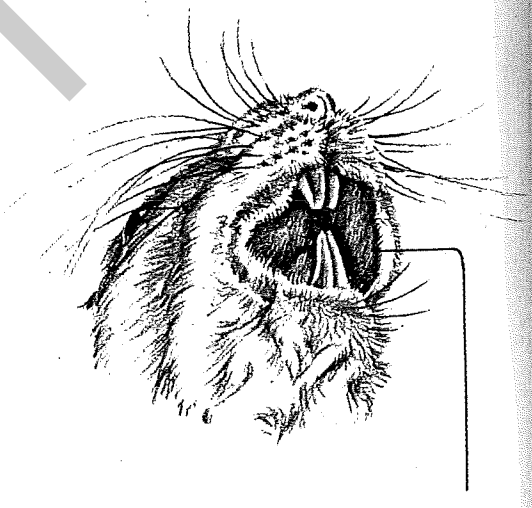
Plate 38

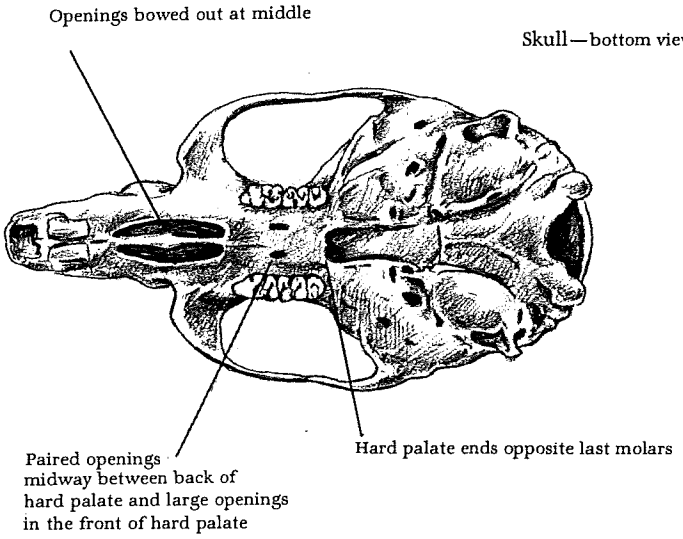
White-footed Mouse *(Peromyscus leucopus)*



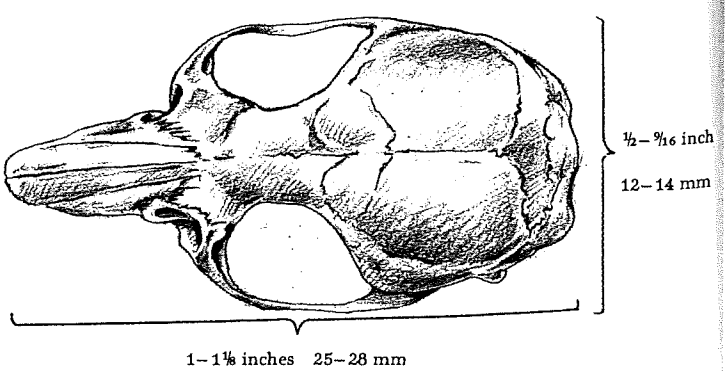




Skull—bottom view



Skull—top view



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habitat of this species rarely overlaps that of the Deer Mouse, and where ranges of these different species overlap, it is usually due to a change in the plant succession of the range.

Nests are either in tree cavities, in old birds' or old squirrels' nests, or underground beneath some protective cover such as a log or the roots of a tree.

Habits

As this species is similar in appearance to the Deer Mouse, so is it similar in life history.

Many home ranges of a White-footed Mouse tend to be circular, in other words, there is a center of activity with the mouse extending its travels in all directions from the center. Home ranges are generally small, 1/5 acre or more, but vary with the population density. They are large when the density is low and small when it is high. The different sexes tend to have home ranges that exclude the same sex but overlap the ranges of the opposite sex. Individuals are well acquainted with the landmarks in their home range and use the position of large trees, logs, and rocks to orient themselves.

There is no definite social hierarchy, which may be related to the high population turnover. In general, males are aggressive and some males tend to dominate others. Male White-footed Mice actively fight with male Deer Mice and Texas Mice where they occur in the same areas. White-footed Mouse neighbors recognize each other and are aggressive toward strangers of the same species.

White-footed Mice spend a great deal of their lives in trees. They swim well and voluntarily swim from one island to another, even when as far as 765 feet apart. Flooding of the home range has little influence on these arboreal mice. They tend to remain in trees and shrubs, then return to the ground as the water subsides.

In winter they become less aggressive and more communal. This is related to the cessation of breeding and to the need to concentrate in limited areas of good cover and available food.

Foods

The foods are generally the same as those of the Deer Mouse. The kinds and amounts of insects, seeds, fruits, and other food items reflect their availability.

Reproduction

Peaks of breeding occur in early spring and late summer, but restricted breeding may occur in mid-summer. There is some evidence that certain females breed more than others and have up to eight litters a year.

Some Adverse Factors; Importance; and Management or Control

These are similar to those discussed for the Deer Mouse.

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Their homes are often used by other animals. Their flesh serves as food for many carnivorous animals. Their droppings are good fertilizer, providing nutrients for plant growth. Animals like deer and other vegetarians benefit by this natural fertilization and are attracted to the rich plant growth in the vicinity of wood rat nests.

Management or Control

On the rare occasions when wood rats enter closed cabins and become a nuisance, large snap traps are an effective means of control.

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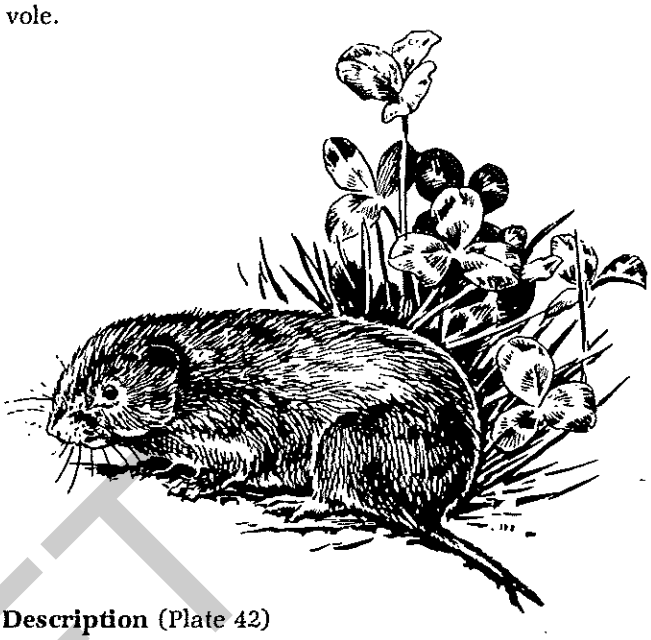
Meadow Vole *(Microtus pennsylvanicus)*

Name

The first part of the scientific name, *Microtus*, is from two Greek words and means "small ear" (*mikros*, "small," and *ous*, "ear"). This refers to the nearly concealed ears. The last part, *pennsylvanicus*, is a Latinized word referring to the locality where this animal was first collected. The common name, "meadow," describes the general habitat used by this mouse or vole.



Description (Plate 42)

The Meadow Vole is very similar in appearance to the Prairie Vole. Externally, it is distinguished by a longer tail (tail two or slightly more times the length of the hind foot), 6 plantar tubercles on the hind foot (rarely 5), body fur finer and with less white, and belly fur usually grayish. For differences in dentition, see *Teeth and Skull* below.

There are two other similar-appearing voles in Missouri, the Woodland Vole and the Southern Bog Lemming. Both of these have a shorter tail (tail nearly same length as hind foot), and the Southern Bog Lemming has grooved incisors.

Color. The Meadow Vole is uniformly dark brown above with slightly paler sides and silver-tipped hairs on the belly. The feet and tail are darker above and paler below. In winter, the color is slightly darker with more gray. Young voles are much darker then adults and have black feet and tail.

Measurements

Total length	$4\frac{3}{4}-7\frac{3}{8}$	in.	120–187	mm
Tail	$1\frac{1}{2}-2\frac{1}{2}$	in.	38–63	mm
Hind foot	$\frac{3}{4}-1$	in.	19–25	mm
Ear	$\frac{7}{16}-\frac{5}{8}$	in.	11–15	mm
Skull length	$1-1\frac{1}{8}$	in.	25–28	mm
Skull width	$\frac{1}{2}-\frac{5}{8}$	in.	12–15	mm
Weight	$\frac{3}{4}-2\frac{1}{4}$	oz.	21–63	g

Teeth and skull. The dental formula of the Meadow Vole is:

$$\begin{array}{ccccccccc} I & \underline{1} & C & \underline{0} & P & \underline{0} & M & \underline{3} & = 16 \\ & 1 & & 0 & & 0 & & 3 & \end{array}$$

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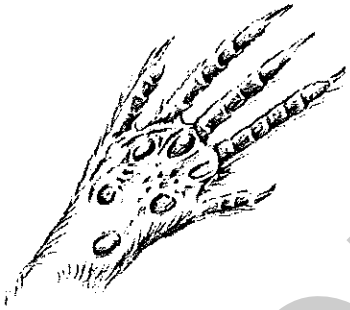
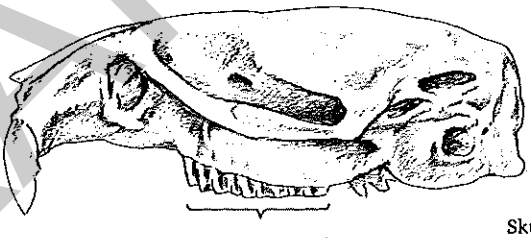
Plate 42

Meadow Vole *(Microtus pennsylvanicus)*

1 inch 25 mm

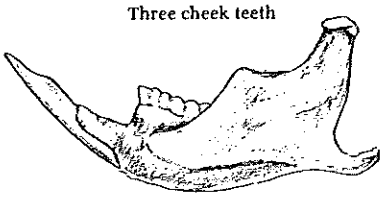


Right hind foot—showing six pads on sole



Three cheek teeth

Skull—side view

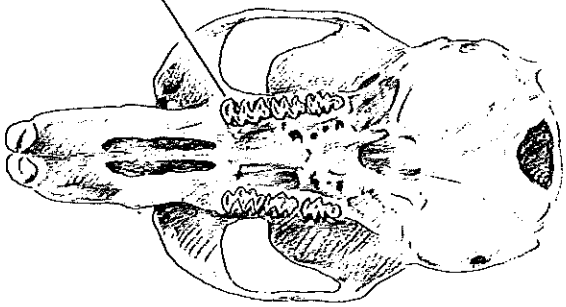


Dentine

Sharp-angled enamel fold

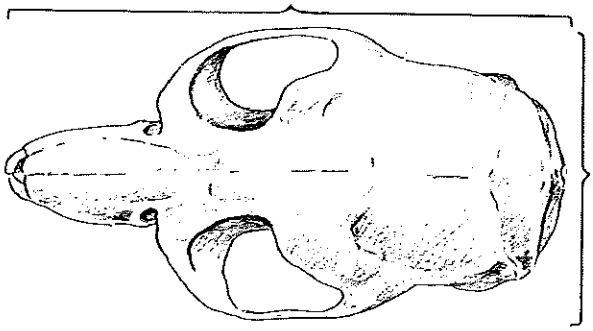
Second upper molar has five islands of dentine surrounded by enamel

Left upper cheek tooth row



Skull—bottom view

1–1⅛ inches 25–28 mm



½–⅝ inch

12–15 mm

Skull—top view

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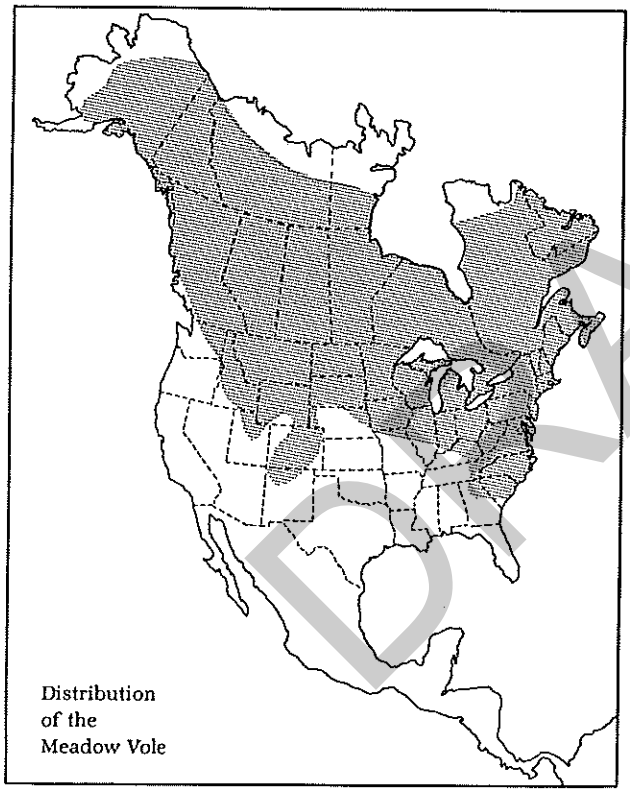
The grinding surfaces of the upper cheek teeth possess a pattern of sharp-angled enamel folds surrounding dentine. The second upper molar has 5 islands of dentine (rarely is the loop toward the rear missing). There is no lengthwise groove on the outer edge of the upper incisors.

These dental characters distinguish the skull of the Meadow Vole from the closely related Prairie Vole, the Woodland Vole, and the Southern Bog Lemming.

Sex criteria. There are four or five pairs of teats in females, two of which are in the chest region.

Distribution and Abundance

The Meadow Vole has a wide range over the northern half of North America. This is shown on the accompanying map.



Distribution of the Meadow Vole

Since the first printing of this book, specimens have been taken commonly in three counties of northern Missouri (Worth, Harrison, and Nodaway). It is not known if this species has always occurred here or has recently extended its range southward from Iowa. But because no specimens were taken here formerly and the area was trapped extensively, a recent expansion of range is considered the most likely explanation for its presence. Also, it is customary for this species to constantly invade new patches of habitat when they become available and where individuals, socially released from family ties, breed. It is probable that this vole occurs throughout much of northern Missouri in the low damp areas of stream valleys and floodplains.

The Meadow Vole is more cyclic than the Prairie Vole. There are peak populations about every four years, although these fluctuations may be local.

Habitat and Home

Meadow Voles can be found in moist, low areas where there is a heavy growth of grasses, or in drier grasslands near streams, lakes, or swamps. They construct runways in the rank cover and sometimes beneath the ground where they build a nest of dry grasses and sedges for a resting site or for their young.

The Meadow Vole has extended its range continentwide into otherwise highly cultivated areas by traveling and occupying continuous strips of dense grass along roadsides, drainage ditches, and railroads. In general, this vole occupies less dense cover than the Prairie Vole and is better adapted to moister situations than the Prairie Vole.

Life History

The life history of the Meadow Vole is similar to that of the Prairie Vole.

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See also discussion of this species in general references, page 20.

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Prairie Vole *(Microtus ochrogaster)*

Name

The first part of the scientific name, *Microtus*, is from two Greek words and means "small ear" (*mikros*, "small," and *ous*, "ear"). This name is somewhat misleading because the ear is of medium size, although it is nearly hidden in the body fur. The last part, *ochrogaster*, is from two Greek words and means "yellow belly" (*ochro*, "yellow," and *gaster*, "belly"). It describes the yellowish tinge on the belly. The common name is self-explanatory.

Sometimes authorities refer to this species as *Pedomys ochrogaster*.

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Plate 43

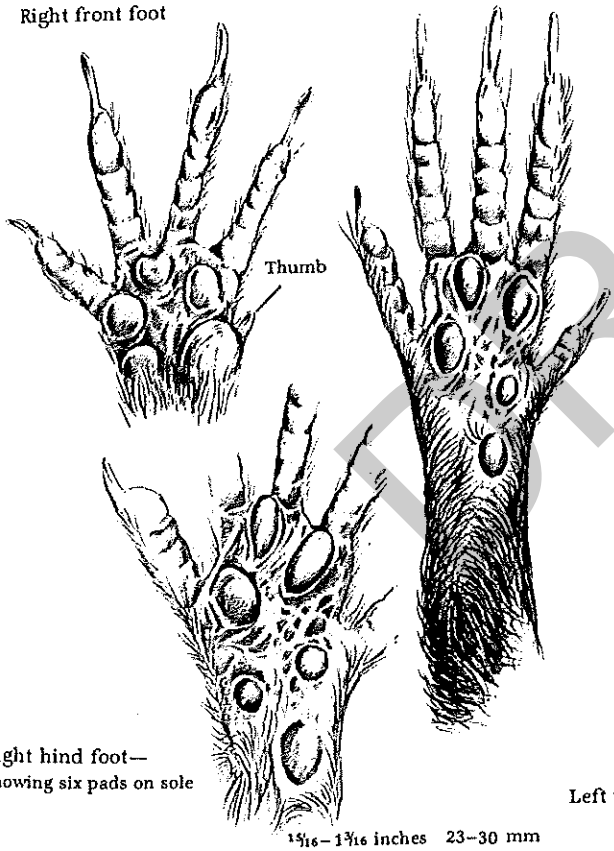
Prairie Vole *(Microtus ochrogaster)*

1 inch

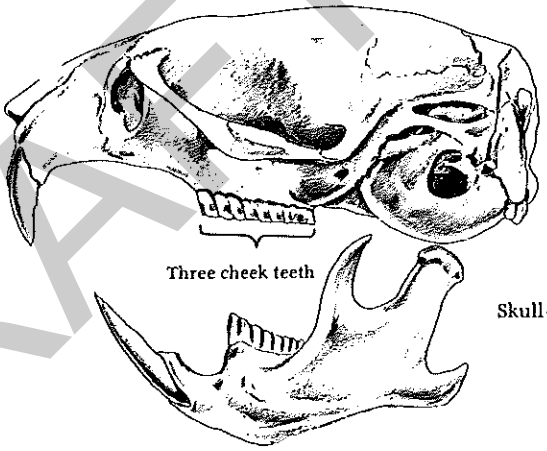
25 mm

Right hind foot—
showing five pads on sole

Right front foot

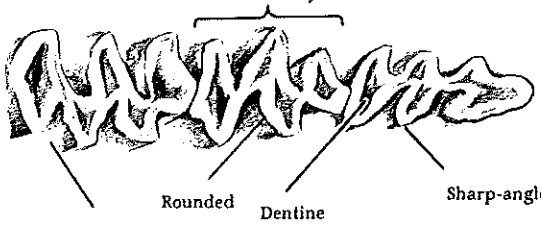


Right hind foot—
showing six pads on sole



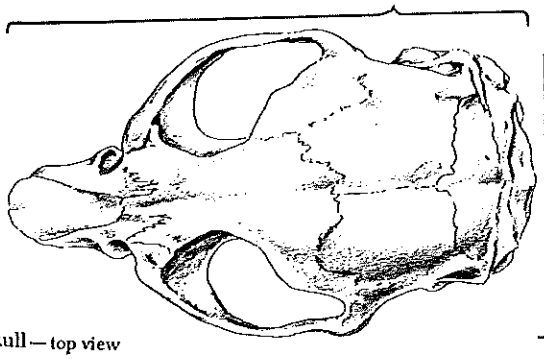
Skull—side view

Second upper molar has four islands of
dentine surrounded by enamel



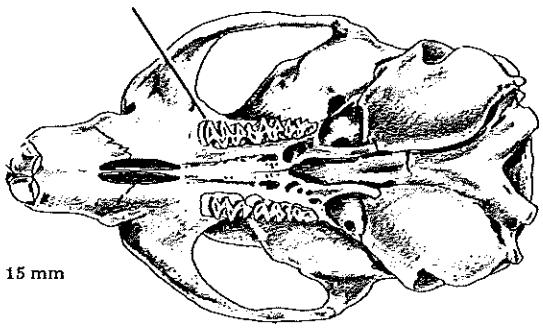
Left upper cheek tooth row

$^{15}/_{16}$–$1^{3}/_{16}$ inches 23–30 mm



⅝ inch 15 mm

Skull—top view



Skull—bottom view

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Description (Plate 43)

The Prairie Vole is a small, stocky rodent with a large head, short legs, and a short tail. The tail is moderately furred and is slightly less than twice the length of the hind foot. The black eyes are small and beady, and the well-furred ears project only slightly beyond the body fur. The lips close tightly behind the upper incisors keeping dirt out of the mouth cavity when the vole digs underground. The front foot has 4 clawed toes and a small thumb bearing a pointed nail. The hind foot has 5 clawed toes and usually 5 but sometimes 6 pads, or plantar tubercles, on the sole, which is furred from the heel to the pads. The long, loose body fur is glossy, coarse, and grizzled.

The Prairie Vole is distinguished from the closely related Meadow Vole by a shorter tail (tail slightly less than twice the length of the hind foot), 5 plantar tubercles on the hind foot, body fur coarser with more white, and belly fur usually yellowish or rusty. For differences in dentition, see *Teeth and Skull* below.

There are two other similar-appearing voles in Missouri, the Woodland Vole and the Southern Bog Lemming. Both of these have a shorter tail (tail nearly same length as hind foot), and the Southern Bog Lemming has grooved incisors.

Color. The upperparts of the Prairie Vole are grayish to blackish brown, mixed with whitish, yellowish, or rusty, imparting a grizzled appearance; the color at the base of the hairs is dark gray. The sides are paler and the belly is tan or grayish, often washed with whitish, yellow, or a rusty color especially around the base of the tail; the belly hairs are dark at the base. The feet are grayish tan. The tail is dark above and light below. Adults are rarely black, salmon colored, or albino. The sexes are colored alike. Molting occurs at any time of the year and requires three weeks for completion. In adults it begins in the chest region and works toward the head and tail.

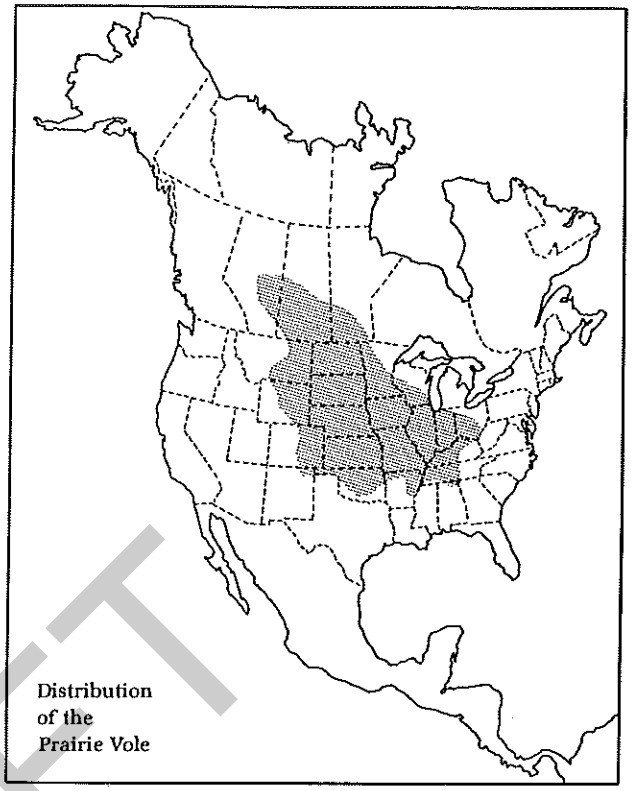
Measurements

Total length	$4\frac{5}{8}-7$	in.	117–177	mm
Tail	$\frac{7}{8}-1\frac{7}{8}$	in.	22–47	mm
Hind foot	$\frac{9}{16}-\frac{7}{8}$	in.	14–22	mm
Ear	$\frac{7}{16}-\frac{5}{8}$	in.	11–15	mm
Skull length	$\frac{15}{16}-1\frac{3}{16}$	in.	23–30	mm
Skull width	$\frac{5}{8}$	in.	15	mm
Weight	$\frac{3}{4}-2$	oz.	21–56	g

Teeth and skull. The dental formula of the Prairie Vole is:

$$\begin{array}{ccccccccc} I & \underline{1} & C & \underline{0} & P & \underline{0} & M & \underline{3} & = 16 \\ & 1 & & 0 & & 0 & & 3 & \end{array}$$

The grinding surfaces of the upper cheek teeth possess a pattern of sharp-angled enamel folds surrounding dentine. The second upper molar has 4 islands of dentine surrounded by enamel; the front border of the second island on the tongue side is rounded on most specimens. There is no lengthwise groove on the outer edge of the upper incisors.


Distribution of the Prairie Vole

These dental characteristics distinguish the skull of the Praire Vole from the closely related Meadow Vole, the Woodland Vole, and the Southern Bog Lemming.

Sex criteria. The sexes are distinguished as in the Eastern Wood Rat. There are three pairs of teats in females, two pairs in the groin region and one pair in the chest region.

Age criteria, age ratio, and longevity. The young, until 8 or 9 weeks of age, are a dull gray to black with black feet and tails. They generally weigh less than $\frac{3}{4}$ ounce. Age can be determined, in general, by an examination of the skulls. Paired ridges on the upper surface between and behind the eye sockets are farther apart in younger animals but become closer with age.

The best means for age determination in voles is by the eye-lens weight (Thomas and Bellis, 1980).

Young voles are more abundant than adults only when the population is increasing. In the wild, the life span is very short. Only a small proportion of the population exceeds 60 days of age. Few individuals become 16 months old. The heavy loss is largely the result of predation by other animals. In captivity, some Prairie Voles lived for totals of 27 and 35 months.

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Glands. Scent glands occur in the groin region of both sexes.

Voice and sounds. Squeaks, squeals, growls, and chattering of the teeth are typical sounds made by Prairie Voles. In addition, they stamp their front and hind feet.

Distribution and Abundance

The Prairie Vole lives throughout Missouri. Its North American distribution is shown on the accompanying map.

Voles show periods of abundance about every four years. An increasing population is accompanied by a longer breeding season, an increase in the number of young per litter, and proportionately more young per adult. Population decreases, which often occur suddenly in local areas of dense population, are caused by various factors such as drought in summer, severe winter weather, heavy parasitism and predation, epidemic diseases, changes in land use, or strife between individuals, which is intensified in concentrations.

Some indication of the size of the population can be ascertained from the number of runways in an area, since the number of runways is directly related to the population density. In years of low population, there may be only 15 to 40 Prairie Voles per acre, but in times of abundance there may be from 60 to 429 per acre.

Habitat and Home

Prairie Voles live in upland herbaceous fields, grasslands, thickets, fallow fields, under shocks of corn and small grain, along fence rows, and in fields of alfalfa, bluegrass, clover, or lespedeza. They do not habitually dwell in timbered areas.

Nests are made in various locations—aboveground in clumps of vegetation or under debris, at the end of short tunnels off the main runway, or belowground from 2 to 18 inches deep. The nests are usually ellipsoid and are from 7 to 8 inches long, 4 to 6 inches wide, and about 4 inches high. They are woven of coarse grass and have a lining of finer grass or other soft material. There are 1 or 2 entrances to the nest.

Habits

Voles build a system of well-defined runways both on top of the ground and under the ground. Holes about 2 inches in diameter lead from the surface runways to the series of underground tunnels where the vole spends much of its life. Dirt removed from the underground tunnels is piled near the hole or is carried and scattered along the runway forming a pavement.

The surface runways are from 1 to 2 inches in diameter and are made by clipping the grass or other vegetation very close to the ground. The floor of the runways consists either of bare ground or a thin layer of trampled grass, stems, and an occasional leaf. The sides and roof are of living vegetation. These trails lead to feeding grounds where the vole may leave the trail and feed in the near vicinity.

Many voles live in the same general area, and the runway system belongs to the entire community. Yet individual voles tend to use specific portions and each

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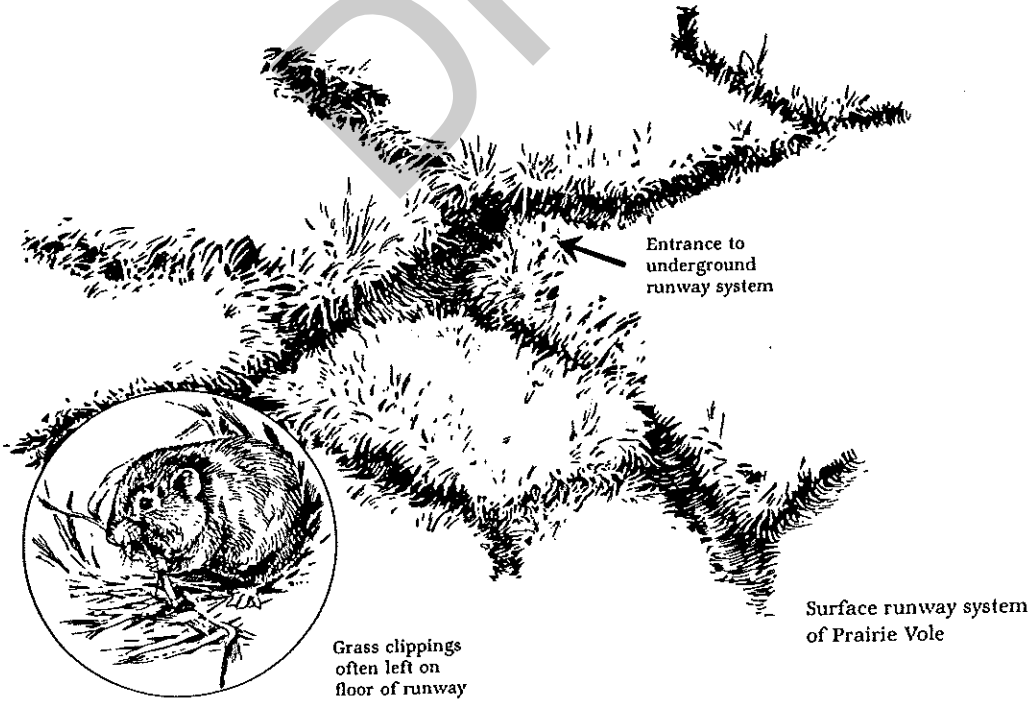


has one or more nests of its own. However, sometimes they may share nests and stores of food. Other kinds of mice, shrews, and moles travel through the runway system on occasion. There is some evidence that Southern Bog Lemmings may dominate voles when the ranges of these species overlap. However, when an area occupied by voles is taken over by lemmings, this probably is the result of successional changes in the vegetation that favor the lemmings rather than the result of physical conflict between the species.

In general, voles are sociable, but males show more aggression toward each other than toward females. On occasion, both sexes are strong fighters. Meadow Voles tend to be more aggressive toward their own kind than Prairie Voles are.

Voles have a restricted home range and usually live within 1/15 acre. However, males wander farther than females and may cover 1/2 acre or more. Individuals liberated up to 200 yards from their home have returned, but those taken farther failed to do so.

These voles are active at any time of the day or night but are most active in midday. They seemingly live on a four-hour schedule. This includes eating for the first part of the four-hour period and sleeping for the last part.

Voles are neat and clean and keep their fur carefully groomed. Their small brownish droppings are deposited along the runways and in blind alleys off the main runways.

Prairie Voles swim voluntarily and have been observed to swim distances up to 90 feet. When swimming on the surface of the water, most of the back is exposed. When swimming underwater, air bubbles become trapped in the fur and help keep the body from becoming wet.

Foods

The main foods of voles are the tender stems, leaves, roots, tubers, flowers, seeds, and fruits of grasses, sedges, and many other succulent plants. At times insects, snails, crayfish, and other mice are eaten and, when food is scarce, even the inner bark of trees, shrubs, and vines are consumed.

Food is stored in underground chambers near the nest and often aboveground in hollow stumps and similar localities. A cache may contain as much as two gallons of tubers, roots, and small bulbs. A vole is a large eater, consuming its own weight in green food every 24 hours. In addition, it often cuts and wastes more growing vegetation than it eats. In feeding on tall plants, the vole clips the stem close to the ground. Piles of cut stems are left along the runways.

Water is probably not required in the wild because of the succulent diet, but captive animals fed on grain take large amounts of water, which they lap with their tongues.

Voles, like rabbits and some other rodent species, ingest their own soft droppings when they rest following a period of feeding.

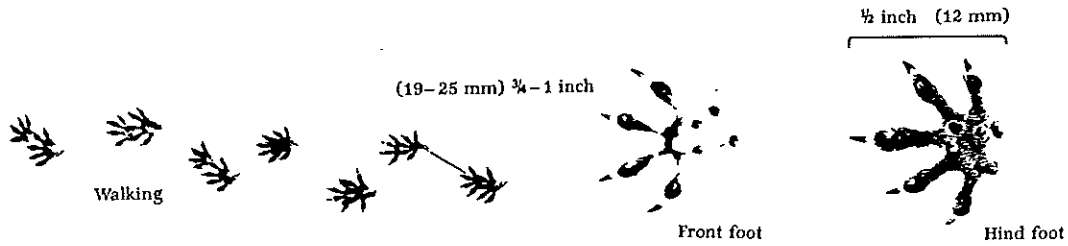
Reproduction

The breeding season may encompass the entire year, but peaks occur in spring and fall. The gestation period is 21 days, and a female can be both pregnant and nursing.

The Prairie Vole is one of the most prolific mammals known. Many litters are produced annually, the number being influenced by the food supply, temperature, amount of cover, including snow cover, abundance of mates, and other factors. Each litter contains from 1 to 7 young, but 3, 4, and 5 are the most common numbers. There are fewer young per litter at the beginning and end of the breeding season than at other times. Older and larger females average more young per litter than other females. In the closely related Meadow Vole a captive female produced 17 litters in one year, and one of her daughters produced 13 families totaling 78 offspring before she was one year old.

A litter of 6 young was born over a period of 50 minutes, each delivery taking from 4 to 5 seconds. The female aided the young by pulling with her teeth and, following the births, ate the placentas.

At birth the young are pink and hairless, and weigh about 1/10 ounce. The eyes are closed and the ears folded against the head. When nursing, the young cling tightly to the female's teats and are often carried in this way if she moves suddenly. The female protects her young and is very concerned about their welfare. If she needs to move them, she carries each one separately with her mouth.



Prairie Vole tracks

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About the fifth day after birth, the backs of the young are covered with velvety fur; in another day or two the incisors come through the gums. The eyes open and ears unfold at about 8 days of age. Weaning occurs when the young are from 2 to 3 weeks old.

At 8 or 9 weeks of age, the young are about 2 inches long and weigh between 1 and 1½ ounces. They soon begin to molt their juvenile fur and gradually acquire adult coloration. They reach adult size in 3 months.

Voles may breed at a very early age. Males are capable of breeding when 5 weeks old, but females can do so as early as 25 days of age, bearing young when they are only 45 days old. This difference in the time of reaching sexual maturity reduces the chances of mating by litter mates.

Some Adverse Factors

Because the Prairie Vole is an important food of many animals, the list of predators is long. It includes opossums, shrews, raccoons, badgers, minks, Striped Skunks, spotted skunks, dogs, Domestic Cats, bobcats, Gray Foxes, Red Foxes, coyotes, weasels, snakes, hawks, owls, crows, and even bullfrogs and snapping turtles.

These voles are parasitized by mites, ticks, lice, fleas, botfly larvae, other parasitic flies, flukes, and tapeworms. Some of these parasites carry organisms that cause several diseases in man. These are sylvatic plague, spread by fleas; tularemia, spread by insects, especially the deerfly; Rocky Mountain spotted fever, spread by ticks; and rat-bite fever, spread by the bite of an infected mouse. In addition, Prairie Voles are the hosts for many parasites, such as ticks, whose later developmental stages parasitize many larger mammals, including game, furbearing, and domestic mammals, and even man.

Occasionally voles become accidentally trapped in a tangle of weeds or briers where they may break a leg or die of starvation.

Importance

When voles are abundant, they often do considerable damage through their feeding habits. They nibble on sprouting corn, eat the corn germ in winter shocks, damage vegetables in gardens, and consume large quantities of vegetation such as alfalfa. A theoretical population of 100 voles per acre in one year will eat 300 pounds of alfalfa hay per acre, or 96 tons per section (640 acres), and waste at least twice this amount. Under conditions of prolonged snow they often girdle the base of young fruit trees or nursery stock.

Because of this damage, the contribution of voles to their environment is usually overlooked. They continually work the soil through their tunneling and other life activities, adding and mixing their stores of food and

waste products with the soil. Their clipping of stems and leaves stimulates new tender growth of plants, and their working of the soil also favors better plant growth. They convert the nutrients from the vegetation they eat into their own flesh and pass these nutrients on to the many other animals that feed on their bodies. Because they are so common and form a ready food supply for so many predators, they actually reduce predation on other and more desirable animals, like game species. Also, when they are abundant, they feed large numbers of predators and thus influence population trends in many animals, such as foxes.

Management or Control

Where it is desirable to keep voles away from cultivated fields or orchards, the elimination or close cutting of the ground cover around the crop will prove a partial barrier to their travel. Predators, especially hawks and owls, should be encouraged. Cinders or hardware cloth can be placed around individual trees to keep voles from girdling them. In small gardens, snaptraps or poisoned bait will help eliminate these rodents. Poison, however, should be used very cautiously because of the danger to other animal life that may be destroyed directly as a result of eating the poison or indirectly by eating poisoned animals. Poison should be used only after consulting the civil laws and the state wildlife code.

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Woodland Vole *(Microtus pinetorum)*

Name

The first part of the scientific name, *Microtus*, is from two Greek words and means "small ear" (*mikros*, "small," and *ous*, "ear"). This refers to the nearly concealed ears. The last part, *pinetorum*, is of Latin origin and means "belonging to the pines" (*pinetum*, "a pine woods," and *-orium*, "belonging to a place of"). This name refers to the Georgia pine forests where this species was first collected.

Other names used by different authorities are *Pitymys pinetorum* and *Pitymys nemoralis*.

Description (Plate 44)

The Woodland Vole is a small, thickset rodent with a large head, short legs, and a short tail that is about the same length as the hind foot. The eyes are small, and the ears are nearly concealed in the fur. The lips close tightly behind the upper incisor teeth, helping to keep dirt out of the mouth cavity when the vole digs underground. There are 4 toes and a small thumb on each front foot and 5 toes on each hind foot. The soles of the hind feet are furred from the heel to the 5 pads, or tubercles. The short, loose body fur is thick, soft, and glossy.

The Woodland Vole is distinguished from Prairie and Meadow voles by the shorter tail (tail nearly same length as hind foot) and from the Southern Bog Lemming by the absence of grooved incisors.

Color. The Woodland Vole is predominately reddish brown above, but the fur, when parted, shows an undercolor of dark gray. The sides are paler than the back, and the belly is grayish washed with buff; the hairs of the belly are dark at the base. The feet are grayish tan; the tail is dark above and only slightly lighter below. Occasional individuals are buffy or have white spots. The sexes are colored alike. Adults molt in early spring, throughout the summer, and again in late fall and early winter.

Measurements

Total length	$3\frac{1}{4}-5\frac{3}{4}$	in.	82–146	mm
Tail	$\frac{5}{8}-1$	in.	15–25	mm
Hind foot	$\frac{5}{8}-\frac{3}{4}$	in.	15–19	mm
Ear	$\frac{5}{16}-\frac{7}{16}$	in.	7–11	mm
Skull length	1	in.	25	mm
Skull width	$\frac{5}{8}$	in.	15	mm
Weight	$\frac{3}{4}-2$	oz.	21–56	g

Teeth and skull. The dental formula of the Woodland Vole is:

$$\begin{array}{cccccccc} I & \underline{1} & C & \underline{0} & P & \underline{0} & M & \underline{3} \\ & 1 & & 0 & & 0 & & 3 \end{array} = 16$$

The grinding surfaces of the upper cheek teeth possess a pattern of sharp-angled enamel folds surrounding dentine. The second upper molar has 4 islands of dentine surrounded by enamel; the front border of the second island on the tongue side is squared on most specimens. There is no lengthwise groove on the outer edge of the upper incisors.

These dental characteristics distinguish the skull of the Woodland Vole from the closely related Meadow Vole, the Prairie Vole, and the Southern Bog Lemming.

Sex criteria. The sexes are identified as in the Eastern Wood Rat. There are two pairs of teats in the groin region of females.

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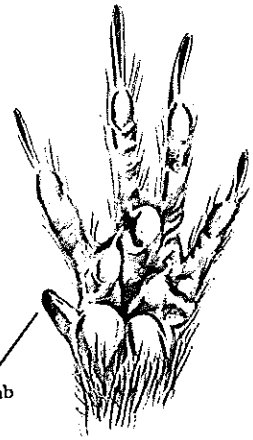
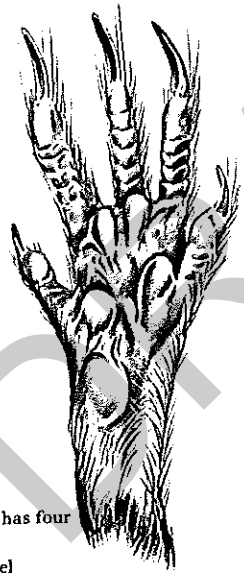
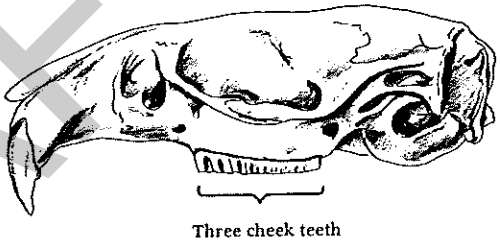
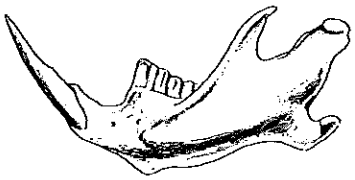
Plate 44

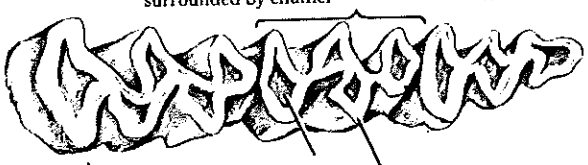
Woodland Vole *(Microtus pinetorum)*

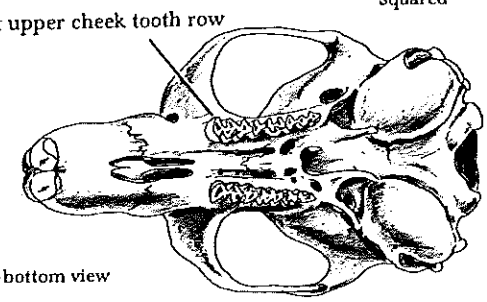


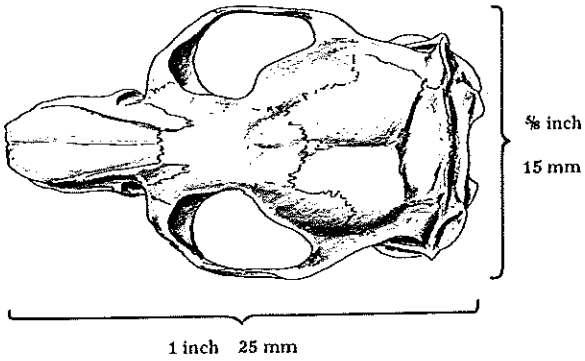












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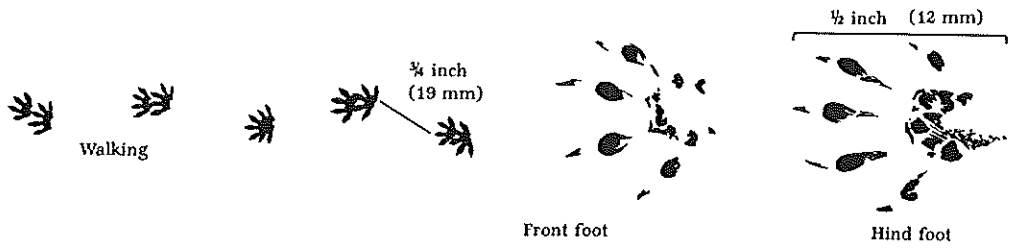
Woodland Vole tracks



Age criteria and longevity. Compared to adults, the young have darker, fuzzier, and more lead-colored fur. Age is also told by the smaller size. Woodland Voles seldom reach 10 to 12 months of age.

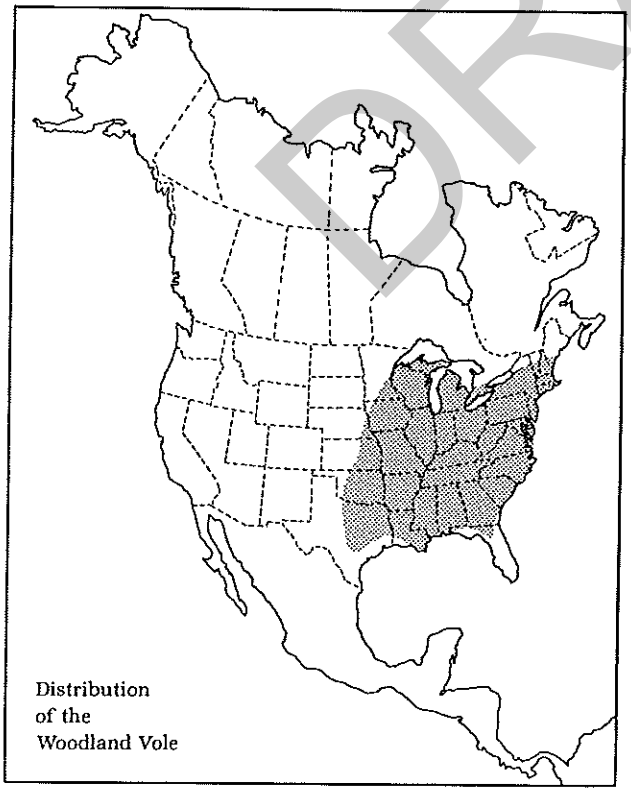
Glands. There are paired glands on the hips and in the groin region.

Voice and sounds. The Wooldand Vole gives low, birdlike chirps when alarmed or harassed. When fighting, it emits harsh chirrs.

Distribution and Abundance

The Woodland Vole occurs throughout Missouri. It is generally rare but is most common in the Ozark Highland.

The North American distribution is shown on the accompanying map. The population shows periods of abundance every three to four years. Usually between 80 and 90 voles per acre is a high density, but in one locality during a population "high," densities reached 300 Woodland Voles per acre.



Distribution of the Woodland Vole

Habitat and Home

The Woodland Vole lives underground in oak-hickory forests and sometimes in mixed hardwood and pine forests where there is a heavy layer of dead leaves or a dense mat of grass and other cover. It also lives in fields adjacent to timber, and in orchards, gardens, and scrub areas, providing they possess considerable ground litter. Loose, moist soils are preferred because they are easy to dig.

The spherical nest is built beneath a log, just below the surface litter, or several inches underground. It consists of shredded dead grass, leaves, and rootlets with a lining of short, fine pieces of grass. There may be 3 to 4 entrances.

Habits

The Woodland Vole makes tunnels from 1 to 2 inches in diameter. These are just under the carpet of leaves and grass and from 4 to 12 or more inches deep. Numerous holes open at intervals from these tunnels and lead to surface feeding grounds. Piles of dirt, excavated from the tunnel system, may occur near these openings. Woodland Voles seldom travel far on top of the ground; they feed mostly in the tunnels.

In digging, the Woodland Vole uses its teeth, head, and front feet to loosen the soil and the hind feet to push the dirt behind. When a pile of dirt has accumulated, the vole pushes it out of the tunnel with its head. Although voles dig their own tunnels, they may use nearby tunnels of moles, shrews, and other kinds of mice.

Woodland Voles have a normal home range of about ¼ to ⅓ acre. They may remain in this area for their entire lives or gradually shift their home, occupying new areas. Voles released up to 150 feet from the point of capture have returned to the home area.

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These voles tend to live in colonies. They are not very aggressive but are more so than the Prairie Vole. There is a report of one nest containing 3 litters, presumably belonging to 2 or 3 females.

Woodland Voles do not hibernate and are slightly more active during the night than during the day. They are poor jumpers and climbers but are capable of swimming. They normally walk slowly but have been timed to run at a rate 3.8 miles per hour for 25 feet.

Foods

Because of its subterranean habits, much of this vole's food comes from below the surface of the ground. Succulent roots and tubers of many kinds of plants, sprouts, the tender bark of tree roots, stems, leaves, seeds, nuts, berries, apples, and an occasional insect or body of a dead Woodland Vole compose the diet. A fallen apple is consumed from the bottom by digging up underneath it.

Some food is stored in underground chambers that may contain as much as a gallon of tubers. When filled, the chamber entrance is closed with dirt. In captivity, Woodland Voles drink large amounts of water.

Reproduction

The breeding season encompasses most of the year, beginning in January and ending in November. The peak occurs in March and April. Several litters of 2 to 4 young, with extremes of 1 and 8, are born annually. The gestation period is about 21–24 days. From 1 to 6 litters may be born a year. The annual production is generally lower than that of Meadow and Prairie voles.

At birth the young are blind, naked, weigh about $^1/_{10}$ ounce, and are between $1^1/_4$ and $1^3/_8$ inches long. They hang onto the female's teats very tenaciously and are often dragged about when she moves suddenly. At 5 to 6 days of age, fur appears and the incisors cut through the gums. The ears unfold on the eighth day, and the eyes open between the ninth and twelfth days. The young leave the nest for short periods by 2 weeks of age and are weaned between 16 and 21 days of age. The young develop adult fur and coloration when between 7 and 10 weeks old; they are ready to breed at 2 months.

Some Adverse Factors

Owls, hawks, snakes, opossums, coyotes, foxes, Domestic Cats, raccoons, and minks are known predators on Woodland Voles, although shrews, weasels, and other carnivorous mammals may also prey on this species. Predation, however, is probably light because of the Woodland Vole's subterranean existence. The reproductive rate also indicates that mortality is low in contrast to the Prairie Vole, which is more prolific and more heavily preyed upon.

The external parasites found on Woodland Voles are mites, ticks, lice, and fleas; the recorded internal parasites are tapeworm larvae, eggs of roundworms, and adult spiny-headed worms. A fatal skin disease frequently occurs in high populations.

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Importance

Where Woodland Voles are abundant, they may damage orchard trees. They do this by their underground tunneling, severing of smaller roots, and girdling of larger roots. These voles may also reduce yields of truck crops by digging along rows of potatoes and other root vegetables. Rabbits are often blamed for the work of Woodland Voles.

The tunneling by this species contributes to aeration of the soil and helps prevent the rapid runoff of rain. Other interrelations of voles in general with their environment are given under the Prairie Vole.

Management or Control

Because of their underground habits, the control of Woodland Voles is often difficult. Where they are destructive in small areas, traps or poisoned bait placed in the tunnel system are effective methods of control. In orchards, clean cultivation eliminates the habitat of the Woodland Vole.

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Muskrat *(Ondatra zibethicus)*

Name

The first part of the scientific name, *Ondatra*, is the Iroquois Indian name for this animal. The last part, *zibethicus*, is the New Latin word for "musky-odored" and refers to this rodent's characteristic odor. Part of the common name, "musk," also refers to this scent while the rest, "rat," comes from the Anglo-Saxon *raet*.

Description (Plate 45)

The muskrat is a medium-sized rodent with a broad head, stocky body, short legs, and a vertically flattened, sparsely haired, and scaly tail that is slightly shorter than the combined length of head and body. The eyes are small, and the ears barely project beyond the fur. The lips close behind the incisor teeth permitting the muskrat to gnaw underwater. The small front feet have 4 clawed toes and a nailed thumb; the large hind feet have 5 clawed toes that are webbed at their bases. There is a fringe of stiff hairs on the edge of the web, the sides of the toes, and edge of the foot. Five tubercles are present on the soles of the hind feet. The pelage consists of a dense coat of waterproof underfur and long glossy overhairs.

Color. The back of the adult muskrat is dark blackish brown, while the sides are lighter brown with a reddish, or sometimes yellowish, tinge. The underparts are still lighter, shading to white on the throat, and have a silvery cast caused by the underfur showing through the overhairs. There is a small blackish spot on the chin and blackish fur at the wrists and ankles. The feet are dark brown and the tail is blackish brown. Some individuals are almost entirely black, tan, or white. The sexes are colored alike.

Adult muskrats molt continuously, but young ones molt twice during their first year. In fall, the fur of adults is more prime, or best for wearing quality and appearance, than the fur of young animals, but the fur of both ages is most prime in late winter or early spring.

Measurements

Total length	16–25¼	in.	406–641	mm
Tail	7–11½	in.	177–292	mm
Hind foot	2½–3⅝	in.	63–92	mm
Ear	¾–1	in.	19–25	mm
Skull length	2⅜–2¾	in.	60–69	mm
Skull width	1½–1¾	in.	38–44	mm
Weight	1½–4	lb.	680–1,814	g

Males are heavier than females.

Teeth and skull. The dental formula of the muskrat is:

$$\begin{array}{cccccccc} I & \underline{1} & C & \underline{0} & P & \underline{0} & M & \underline{3} \\ & 1 & & 0 & & 0 & & 3 \end{array} = 16$$

The grinding surfaces of the cheek teeth have a characteristic pattern of sharp-angled enamel folds surrounding dentine. There are 4 or more islands of dentine in each tooth.

Among Missouri rodents with 16 teeth, the skull of the muskrat could be confused only with skulls of the Norway Rat, the Black Rat, or the wood rat on the basis of size. One way to distinguish these skulls is by the pattern on the grinding surfaces of the upper cheek

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APPENDIX G

QAPP for Natural Resource Damages

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QUALITY ASSURANCE PROJECT PLAN FOR NATURAL RESOURCES DAMAGES

REVISION 3
APRIL 13, 2011

Prepared by the
Missouri Department of Natural Resources
Division of Environmental Quality
Hazardous Waste Program
Superfund Section

Department of Natural Resources
P.O. Box 176
Jefferson City, Missouri 65102-0176

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A. PROJECT MANAGEMENT

A1. Signature Page

PROGRAM APPROVALS

HWP NRD Project Officer _____ Signature _____ Date 5/4/11

HWP QA Project Officer _____ Signature _____ Date 5-4-2011

HWP Director _____ Signature _____ Date 5/4/2011

ESP Director _____ Signature _____ Date 5/12/11

DEQ Quality Assurance Manager _____ Signature _____ Date May 24, 2011

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A2. Distribution List

Missouri Department of Natural Resources (MDNR)

Keith Bertels - Quality Assurance (QA) Manager, Division of Environmental Quality (DEQ)

Hazardous Waste Program (HWP)
David Lamb - Director
Dennis Stinson – Chief, Superfund Section
Frances Klahr - Natural Resources Damages Coordinator, Superfund Section
Julieann Warren – Chief, Site Assessment Unit

Environmental Services Program (ESP)
Alan Reinkemeyer – Director
Chris Boldt – Chief, Chemical Analysis Section (CAS)
Eric Sappington – Chief, ESP Field Services Unit
Karla Wiseman - Quality Assurance Project Plan (QAPP) Coordinator

A3. Project/Task Organization

The following list identifies key individuals and organizations participating in the project, and discusses their specific roles and responsibilities as they pertain to this QAPP.

Project Management Staff for NRD, Superfund Section, HWP, DEQ, MDNR

Role: NRD Coordinator, HWP, DEQ, MDNR

Responsibilities: Overall management of Natural Resource Damage Assessment (NRDA) projects. Coordinate all site-specific activities related to conducting the NRDA including correspondence, communication and scheduling. Conduct sample collection by appropriate methods to provide data of sufficient quality. Prepare NRD assessment reports ensuring that site-specific activities conducted pursuant to this QAPP meet project Data Quality Objectives (DQOs).

Julieann Warren, Chief, SAU, HWP, DEQ, MDNR

Role: QA Project Officer

Responsibilities: As QA Project Officer reviews the NRD QAPP and subsequent revisions, and ensures that the most current revision is available to all staff. Ensures that hardcopy and electronic versions of the QAPP are maintained and available to all Unit staff. Assists in the development of project DQOs and project sampling plans. Review data collected and resolve QA issues that arise. Evaluate analytical data to ensure that

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DQO are met. Utilize the data collected to complete NRDA and/or to evaluate remediation objectives. Review and approve all project and Quality Assurance/Quality Control (QA/QC) data. Ensures that all QA requirements of the QAPP are met. Coordinate overall project activities.

Roles: HWP QA Coordinator

Responsibilities: As HWP QA Coordinator, serves as the program's point of contact on all QA issues. Coordinates all QA activities for the program. Provides QA/QC information and reviews all HWP QA/QC activities. Informs QA Manager of all program QA needs, problems, and status. Assists in the completion of the QA status reports to the EPA. As Supervisor of SAU, reviews the data and validates that the project DQOs are met. Assists as appropriate in the performance auditing of all activities performed by the HWP and contractual staff.

Environmental Specialist Staff of Field Services Unit, ESP, DEQ, MDNR

Role: Field Staff, FSU, ESP, DEQ

Responsibilities: Prepare and implement site specific sampling plans to collect environmental samples according to ESP Standard Operating Procedures (SOPs) at NRD sites. Conduct sample collection by appropriate methods to provide data of sufficient quality. Prepare and implement health and safety plans for investigations conducted by the Department at NRD sites. Prepare formal reports of sampling investigations for SAU staff to evaluate and include in NRDA reports.

Eric Sappington, Chief, Field Services Unit, ESP, DEQ, MDNR

Role: Chief, FSU, ESP, DEQ

Responsibilities: Supervises field staff conducting investigations and assists in scheduling their activities. Assures staff is qualified and trained to perform the work, familiar with the required SOPs, including those related to QA/QC, and have the equipment necessary to perform the work. Reviews reports of investigation for completeness, clarity, and accuracy.

Chris Boldt, Chief, CAS, ESP, DEQ, MDNR

Role: Supervisor, CAS, ESP

Responsibilities: Ensures that appropriate analytical methods, CAS SOPs, QC procedures, documentation, and training are implemented and routinely followed by all supervisory and technical staff of the CAS. Utilizes data, review checklists, and QC

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charts for both precision and accuracy data in the data quality review process. Conducts reviews of data files following review and approval by staff at the unit chief level.

Alan Reinkemeyer, Director, ESP, DEQ, MDNR

Role: Director, ESP

Responsibilities: Ensures overall validation and final approval of data generated by the ESP. Assists as appropriate in the performance auditing of all activities performed by ESP personnel.

Keith Bertels, Quality Assurance Manager, DEQ, MDNR

Role: DEQ QA Manager

Responsibilities: Monitors the overall QA operations for the division. Develops and maintains the Quality Management Plan (QMP). Reviews and approves all QAPPs for the division. Prepares QA status reports for the EPA.

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A4. Project Background/Definition

A4.1 Project Background

The Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), as amended, and the Oil Pollution Act (OPA) provide for the restoration of natural resources lost or injured by hazardous substance releases (§107(f)) and discharges of oil (§1006) and require the designation of certain Federal and State officials to act on behalf of the public as Trustees for natural resources. Natural resources include land, fish, biota, water, groundwater, drinking water supplies, and other resources belonging to, managed by, held in trust by, appertaining to, or otherwise controlled by the United States, any State or local government, or Indian Tribe.

The designated federal Trustees include the Secretaries of the Departments of the Interior, Commerce, Defense, Energy and Agriculture. State natural resource Trustees are designated by the Governor of each state, and for Native American Tribes, the Tribal chairman or a person designated by Tribal officials act as the Trustee. The Director of the Department of Natural Resources is the designated Trustee for the State of Missouri. Trustees generally have overlapping management interests in the same resources or "co-extensive authority". The statutory prohibition against double recovery requires Trustees to coordinate their natural resource damage (NRD) actions as co-trustees.

Trustees conduct NRDAs. The purpose of conducting a NRDA is to identify and document the extent of injuries to natural resources; quantify the injuries and all associated losses to the public; and select restoration projects. Section 301(c) of CERCLA requires the promulgation of

regulations for the assessment process. The President, pursuant to Executive Order 12580 (January 23, 1987), vested the rulemaking authority with the Department of the Interior (DOI).

The DOI regulations (43 CRF 11) provide a framework and standards for the NRDA process in coastal and marine environments (Type A) and other environments (Type B). The Type A process involves the use of a computer model to assess damages, in a standard and simplified manner, that result from chemical or oil discharges into coastal and marine environments. Type B is used in situations that require an individual approach. Both types follow four sequential phases.

Pursuant to section 1006(e)(1) of OPA, the National Oceanic and Atmospheric Administration (NOAA), the agency designated by the Department of Commerce (DOC) to act as Trustee for the natural resources administered by DOC, was charged with developing NRDA regulations (Type A) resulting from a discharge of oil (except for any part of oil defined as a "hazardous substance" by CERCLA). The regulations provide a framework for conducting NRDAs that achieve restoration and include four distinct phases: prespill planning; preassessment, restoration planning and restoration implementation.

Under CERCLA, preassessment screens (Phase I) are conducted using readily available information to determine if certain criteria have been met to warrant further action. The assessment plan (Phase II) defines the procedures used to confirm the exposure of trust resources and identifies how the potential injuries will be evaluated. During assessment implementation (Phase III) necessary data is gathered to quantify the injuries and determine damages. During post-assessment (Phase IV) the Trustees prepare a report of assessment outlining the results of the implementation phase. A reasonable number of restoration alternatives, including natural recovery, are usually proposed. A preferred alternative is selected based on several factors. All proposed alternatives are subject to public review and comment prior to implementation of any project.

The goal of the NRD is restoration and Trustees who comply with the regulations have the advantage of creating a rebuttable presumption in litigation. This means the potentially responsible parties (PRPs) have the burden of proof. Trustees do not have to follow the established regulations, but can perform the assessment in cooperation with the PRPs to promote integration of NRD into the response and site cleanup process as well as advancing the speed of the restoration. Additionally, coordination among the Trustees and response agencies ensures integration of NRD into the remedial process, promoting timely and efficient cleanup and expedited restoration by reducing injury, investigative costs, and thus, overall NRD liability.

A4.2 Project Definition

This QAPP covers the field and analytical activities related to CERCLA and OPA NRDAs. In the absence of cooperative agreements with PRPs and/or federal or tribal Trustees, total funding will be provided from the Natural Resource Protection Fund (NRPF). If co-trustees are involved, only the state Trustee's portion will be paid from the NRPF. Once damages have been awarded

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or settlement reached, the Trustees establish an account for the recovered damages that are to be used to restore, replace, rehabilitate or acquire the equivalent of the injured resources. Pursuant to the *Memorandum of Understanding Between the Missouri Department of Natural Resources and the United States Department of the Interior*, any funds recovered as a result of joint assessment and restoration activities will be deposited into the DOI Natural Resource Damage Assessment and Restoration Fund unless all Trustees agree otherwise.

A NRD checklist may be utilized by the NRD Coordinator or HWP personnel, at the request of the NRD Coordinator, during a site reconnaissance visit to collect cursory NRDA information for referral purposes to other Trustees (i.e., federal and/or tribal). No sampling will be conducted at this stage until the cursory information is evaluated and a need identified.

The PRPs will be invited to participate in a cooperative assessment (CA) process whereby both the PRPs and Trustees will jointly develop the sampling plan. Each sampling plan will include a site description and history, sampling strategies, sample collection order and quantity, sample containers and preservation requirements, chain-of-custody, analyses requested, data quality objectives developed using the 7-step systematic planning process, investigation-derived waste, site safety and reporting requirements. When PRPs are conducting the sampling for NRDAs, split sampling will be required for verification purposes. ESP may collect split samples as necessary.

If the PRPs do not participate in the CA process, the Trustees may conduct a formal NRDA in accordance with the DOI regulations (43 CFR 11). During the pre-assessment screen phase, where appropriate, existing sampling data will be used for injury determination and pathway exposure purposes. Additional sampling could be required to fill data gaps. Data needs will vary based on what stage (e.g., pre-remedial or remedial action) the site is in when the NRDA is initiated. ESP assistance may be required at this phase. When the NRD Coordinator requests assistance from ESP at the pre-assessment phase, a formal sampling plan may or may not be required. If a formal sampling plan is not required ESP personnel will collect samples according to MDNR's SOPs for sampling (Appendix 5) and will prepare an abbreviated trip report.

If the pre-assessment screen meets the five established criteria and there is a determination that an assessment should be conducted, the NRDA will move to the second phase – assessment planning. ESP will prepare sampling plans for the assessments that will focus on developing data to support a NRD claim and could potentially be used to support other actions within the Hazardous Waste program. Prior to preparing sampling plans, ESP personnel, in conjunction with HWP personnel, may conduct a site reconnaissance to select sampling points, or a map indicating potential sampling locations will be prepared by HWP and transmitted to ESP. ESP will develop the sampling plan based upon the information from the site reconnaissance or map prepared. The sampling plan will include a site description and history, sampling strategies, sample collection order and quantity, sample containers and preservation requirements, chain-of-custody, analyses requested data quality objectives developed in using the 7-step systematic planning process, investigation-derived waste, site safety and reporting requirements.

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All NRD field activities and public contacts will be coordinated through the NRD Coordinator. Draft sampling plans will be sent to the NRD Coordinator and HWP QAC for approval on sampling points and parameters. Sampling plan approval will be documented on the signature page, which will include the signature of the ESP personnel who prepared the report and approval signature of the NRD Coordinator and HWP QAC. Since the NRD Coordinator and HWP QAC are independent of those responsible for generating the data (i.e., ESP), approval of the sampling plan in terms of QA requirements is sufficient to ensure that data is of known and usable quality.

Changes in site conditions between the time of the site reconnaissance and the on-site sampling visit may alter sampling points and parameters in the field. Such deviations or changes to the sampling plan while in the field will be made and approved by the NRD Coordinator or HWP QAC who originally approved the sampling plan. The deviations or changes will be documented in the final Sampling Report prepared by ESP and submitted to the NRD Coordinator. Safety considerations will be made prior to arriving on-site for sampling and more in-depth site investigation. Sampling Plans will include a Site Health and Safety Plan. Sampling will generally be limited to sediment, surface water, groundwater, containerized wastes, air, surface and subsurface soil, and background media, but could include plant and/or animal tissue and whole or fillet fish sampling. When sufficient information is known about a site, specific monitoring points and parameters will be recommended. The sampling plan will estimate the number of samples to be collected, but existing site conditions and the visual appearance at the time of sampling will determine the actual number of samples collected. On-site screening chemical analyses may be conducted by ESP when a variety of unknown materials or media are present on-site, or when field screening analyses could result in significant economies in laboratory analytical work.

It is estimated that ESP personnel will be requested to conduct sampling on one site for natural resources damages purposes. The ESP field personnel will be responsible, in conjunction with HWP personnel as appropriate, for field activities involving the collection of samples, including decontamination procedures and disposal of investigation derived wastes.

A5. Project/Task Description

A5.1 Task Description

The tasks included in projects addressed by this QAPP can be grouped into the following general categories: site reconnaissance, sampling plan preparation, sample collection, sample analysis, data verification and validation, documentation and reporting, and auditing. The various tasks in these categories are briefly described below.

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A5.1.1 Site Reconnaissance

At the beginning of each project, ESP and HWP personnel may jointly conduct a site reconnaissance for the purposes of selecting sampling points. In the absence of a site reconnaissance, HWP personnel will prepare a map indicating potential sampling locations and provide to ESP.

A5.1.2 Sample Collection

Projects conducted under this QAPP will generally require the collection of site samples from environmental media. The media to be sampled will vary based on site-specific conditions, and may include, sediment, surface water, groundwater, containerized wastes, background media, air, surface and subsurface soil, plant and/or animal tissue, or whole or fillet fish samples. Other media may require sampling on a site-specific basis.

Based on available site information, the NRD Coordinator or HWP staff will prepare a sampling request memo to ESP. The memo will provide general site background information, describe the number, type, and location of samples to be collected, along with analytical parameters requested for each sample. The NRD Coordinator or HWP staff will use the DQO process described in Section A6 of the QAPP to develop the sampling request memo. Based on the sampling request memo, ESP and the NRD Coordinator or HWP staff will prepare and implement a sampling plan. Sample collection is typically conducted by ESP personnel, with on-site oversight by HWP personnel. However, for some projects with limited sampling needs, HWP personnel will assist with sampling or conduct sampling independently

A5.1.3 Sample Analysis

Samples collected for projects under this QAPP will be submitted to the ESP CAS for laboratory analysis. The CAS will conduct sample analysis using standard EPA testing methods, and provide analytical results to the NRD Coordinator. The analytical parameters requested will vary by project. Further information about sample analysis is provided in Section B.

On-site field screening analyses may be conducted by the ESP or HWP personnel when a variety of unknown materials or media are present on-site, or when field screening analyses could result in significant economies in laboratory analytical work.

A5.1.4 Data Verification and Validation

In general, data verification and validation are performed by the staff and supervisors of ESP FSU and CAS. Further data validation is conducted by the HWP QA Project Officer during review of the reports generated by ESP, and review of the final project report. Data verification and validation methods are as described in ESP FSU and CAS SOPs. Data quality assessment is conducted by the HWP QA Project Officer/HWP QA Coordinator. Details on validation, verification, and data quality assessment process are provided in Section D.

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A5.1.5 Documentation and Reporting

Documentation and reporting tasks are completed at various steps along each project's duration. Notes from the site reconnaissance and sampling events are recorded in a field notebook, and formalized in a site reconnaissance memo (prepared by ESP) and a sampling report (prepared by ESP or HWP personnel). A sampling plan and health and safety plan are prepared by ESP working together with the NRD Coordinator and approved by the HWP QA Project Officer/HWP QA Coordinator. Following sample analysis, CAS provides analytical data reporting sheets to the NRD Coordinator containing sample results. The sample collection event is summarized in a sampling report prepared by ESP. Summary reports are prepared by ESP following audits of both laboratory and field sampling performance. Further information on documenting and reporting is provided in each of the following main sections of the QAPP.

A5.1.6 Auditing

Periodic auditing is done both of laboratory performance and field activities.

A5.2 Special Equipment and Services

Some of the projects initiated under this QAPP will require the use of special equipment and/or services. Where used, this equipment and services will be fully described in the project sampling plan. A brief description of this equipment and services along with information on how they will be implemented is provided below.

A5.2.1 Excavation and Well Installation

The NRD Coordinator and/or HWP personnel will identify the need to perform limited excavation at sites to obtain samples of buried material or to document other subsurface conditions. The NRD Coordinator or HWP personnel will also identify the need for installation of any permanent or temporary monitoring wells. The ESP will manage the procurement, selection, and oversight of contractual services for excavation or installation work using procedures acceptable for expenditure of federal funds. The ESP will involve the NRD Coordinator and/or HWP personnel in concurrence of scopes of work, Requests for Proposals (RFP), and other procurement documents and will involve the NRD Coordinator and/or HWP personnel in contractor selection.

A5.2.2 Global Positioning System

The NRD Coordinator and/or HWP personnel will either request the ESP to collect Global Positioning System (GPS) readings for all sites or collect such data themselves. The decision on which staff will collect GPS data will be made on a site-specific basis, and will be specified in

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the sampling plan. The readings should include one locational point for the general site position and a reading for each sample collection point. All GPS points should be collected in accordance with Department data collection policy using a Trimble GPS, and the data post-processed. The GPS readings will be used to create Geographic Information System (GIS) site maps using ARCGIS®.

A5.2.3 X-Ray Fluorescence Detector (XRF)

For some projects, the NRD Coordinator and/or HWP personnel will identify the need to conduct screening of site samples for specific metals using the HWP's XRF analyzers. The NRD Coordinator and/or HWP personnel may conduct XRF screening independently or they may request that ESP screen soil samples from a site using one of the three following methods: in-situ screening, screening samples collected and homogenized in plastic bags, or screening fully prepared samples (dried, ground and sieved). Analysis of soil and sediment samples with the XRF will be conducted in accordance with the manufacturer's user's guide and applicable EPA SW-846 methods

A5.3 Project Scheduling

This QAPP covers the field and analytical activities related to CERCLA NRD assessments. These ongoing assessments will be funded through the Natural Resource Protection Fund. As these assessments are ongoing, the QAPP is designed to continue in effect indefinitely. The NRD Coordinator and QA Project Officer will review the QAPP at least once a year, and will provide any significant changes in the content of the QAPP. This annual QAPP review will be completed no later than August 15th of each year.

A description of the types of services anticipated to be requested from the ESP FSU, along with the estimated volume of these services, is provided in a workplan prepared between the HWP and ESP annually. A list of the estimated number and type of laboratory analyses anticipated to be requested from the ESP CAS are provided in a workplan between the HWP and ESP prepared annually.

A6. Systematic Planning Process (Data Quality Objectives)

DQOs are qualitative and quantitative statements derived from the Systematic Planning and DQO processes, developed by EPA and further described in *Guidance for the Data Quality Objectives Process* (U.S. EPA, 2006), *Data Quality Objectives Process for Hazardous Waste Investigations* (U.S. EPA, 2006a), and Guidance on Systematic Planning Using the Data Quality Objectives Process (U.S. EPA, 2006b). The DQO process is the Systematic Planning Process used to develop this QAPP. The DQO process is an iterative, strategic planning approach designed to ensure that the type, quality, and quantity of environmental data used in decision making are appropriate for the intended application. The following section describes general DQOs applicable to all projects conducted under this QAPP. Since this QAPP is generic, in that it does not pertain to a specific project, DQOs cannot be fully developed here. Instead, the

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general steps will be described, and any portions of the DQOs that apply to all projects will be provided. The specific DQOs for each project will be developed and documented in the sampling plan prepared by the NRD Coordinator together with ESP.

A6.1 Problem Statement

A6.1.1 Background Information

Historical and background information relevant to the general process of NRD assessments is presented in section A3. When available, a summary of background information, specific to each site assessed under this QAPP, will be provided by the NRD Coordinator to ESP at the beginning of each project requiring ESP field and analytical services.

A6.1.2 Conceptual Site Model

A conceptual site model (CSM) will be prepared for each project and documented in the sampling plan. The CSM will be described in the narrative of the sampling plan, and a graphic diagram will be included as a figure in the plan. An example CSM diagram is included as Appendix 1.

A6.1.3 Available Resources and Constraints

Workplans will be negotiated annually between HWP and ESP identifying the amount of field and analytical services HWP will be requesting from ESP each State Fiscal Year for conducting site assessment activities.

A6.2 Decision Statements

The primary overall goal of NRD assessments is to identify and document the extent of injuries to natural resources; quantify the injuries and all associated losses to the public; and select restoration projects.

A6.3 Inputs into the Decision

The types of information inputs required to resolve the decision statements presented in Section A6.2 are listed below. The information is gathered from numerous sources including the site reconnaissance, interviews of site owners, operators, employees, and/or others related to the site, analytical data generated by MDNR's ESP or other laboratory, published reference books and resources, MDNR databases, U.S. Fish and Wildlife and other co-Trustees, internet resources, and evaluations of site conditions by MDNR geologists.

- Historical site data including: property use, surrounding land use, site operations, ownership history, regulatory history
- Previously collected environmental sampling data
- Site reconnaissance observations
- Waste sources and ecological receptors

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- Census Data
- Meteorological and Climatic Data
- Geologic data provided by the DGLS geologists
- Groundwater resource and usage data
- Surface water resource and usage
- Sensitive Environments or Species data
- Physical, chemical and toxicological data on hazardous substances of concern
- Analytical results from waste and environmental media
- Background concentrations (measured or published) of hazardous substances of concern

Waste source and affected media sampling data will be compared to, but not limited to; established ecological benchmarks or criteria outlined in the DOI NRDA regulations.

A6.4 Study Boundaries

43 CFR Part 11.61 (Injury Determination Phase) establishes non-mandatory procedures for determining whether an injury to natural resources has occurred and if the injury resulted from the discharge of oil or release of a hazardous substance based upon the exposure pathway and the nature of the injury. The injury determination phase consists of general information; injury definition; pathway determination; and testing and sampling methods.

A6.5 Decision Rules

The primary goal of NRD assessments conducted under this QAPP is to identify and document the extent of injuries to natural resources; quantify the injuries and all associated losses; and select restoration projects. Separate decision rules for each NRD assessment will be based on site-specific project goals, and documented in the sampling plan.

A6.7 Design Optimization

For each NRD assessment, the NRD Coordinator, in consultation with the QA Project Officer and ESP FSU sampling staff, will review the DQO output from Sections A6.1 through A6.6 together with existing environmental data for the site, and develop a sample collection design based on this review. The sample collection design will specify the type, location, timing, number of analyses per sample, and, if different than specified in Section B, the sample size, field sampling or analytical methods, and QC samples. Rationale for the location of samples and types of analyses will be thoroughly developed and supported. This information will all be documented in the sampling plan prepared by ESP and approved by the NRD Coordinator and/or HWP personnel.

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A7 Special Training Requirements/Certification

In accordance with 40 CFR Part 311, which references 29 CFR 1910.120, all staff are required to successfully complete a 40-hour Hazardous Waste Operations and Emergency Response (HAZWOPER) site safety course, with 8-hour annual refreshers and medical monitoring prior to conducting any field work on a site where hazardous substances are present or suspected.

A8 Documentation and Records

Documentation procedures are outlined in the following MDNR SOPs: ESP-CAS-2020 "Data Review, reduction and Transfer to LIMS", ESP-CAS-2090 "Quality Control Procedures and Quality Control Charts" for the CAS and MDNR-ESP-004 "Field Documentation" for the ESP FSU.

The reports and documents generated throughout NRD assessments are listed below. An example of each type of report and document is included in Appendix 2: Example Sampling Plan and Report Outlines.

- Site Sampling Plan
  This plan is generated by the NRD Coordinator together with ESP FSU, reviewed by the HWP QA Project Officer and signed by the NRD Coordinator before sampling occurs. The Site Sampling Plan includes a Site Health and Safety Plan as an appendix.

- Results of Sample Analyses Report
  The laboratory will report sample results on the Results of Sample Analysis sheets. The laboratory result sheets will be generated by the ESP CAS and sent to the NRD Coordinator within 30 days of receipt of the samples. The sheets will include the information detailed in Table 2 on the following page.

- Site Sampling Report
  This report will be generated by the ESP FSU for all NRD assessment sampling events thatrequire a sampling plan. The Site Sampling report will be submitted to the NRD Coordinator as soon as possible after all analytical data has been reported.

The ESP will provide a minimum of Level II QC data reporting for each NRD assessment under this QAPP. This level of data quality is generally accepted by the USEPA as qualitative, quantitative and legally defensible. The minimum Level II QA/QC data to be included in each laboratory analysis report is defined below.

1. Sample Data. See Table 2 below.
2. Results of blanks (i.e., trip, equipment, and lab blanks).
3. Results of field duplicates identified as such.
4. Results of laboratory control data for replicates and spikes. Calculated as Percent Relative

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5. Standard Deviations (%RSD) or replicates and Percent Recovery (%R) of spikes, and the control limits values utilized for each parameter/matrix.
6. Results of field spikes, if any, identified as such.

The above list, which applies to both inorganic and organic analysis, will ensure that the Project Managers are apprised of the quality level of the analytical data through each laboratory report.

Table 2. Data To Be Included in Laboratory Results of Sample Analysis Sheets

Report Sheet Element	Comment
Site name	From the COC form "Site/Study Name:" field
County	From the COC form "County" field
Program Contact	
LDPR and Job Code	
Sample number	From the COC form "Sample Number" field
Sample description	From the COC form "Description (permit/station number, sample type, etc.):" field.
Date and time of sample collection	From the COC form "sample Collected" field
Sample collector & affiliation	From the COC form "Collector's Name" and "Affiliation" fields
Date of Analysis	
Report Date	
Analytical method used	
QC Batch ID	
Sample Matrix	From the COC form "Matrix" field
Practical Quantitation Limit (PQL)	Included in the "Result" column when qualifier "ND" is used.
Units (e.g. ug/l, mg/kg, etc.)	
Qualifier	From the list in Appendix 8.

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A Laboratory Information Management System (LIMS) at the ESP maintains all information and data on all environmental samples received. The system is utilized to log in samples collected, record results of analyses, and generate sample analyses and management reports. The LIMS is backed up daily through the off-site DNR tape backup system. System maintenance is performed weekly. This includes checking for operating system errors, LIMS system errors, and database integrity.

All original copies of site-specific reports and documents will be stored in site specific files in

the HWP Records Center. All non-site specific quality management reports and documents relating to this QAPP will be stored in the QA/QC Superfund in the HWP Records Center. The QA Manager for the department identifies all QA and QC documents listed in the DEQ Agency Records Disposition Schedule. The NRD Coordinator and ESP will follow the current Agency Records Disposition Schedule approved by the Secretary of State's Office for all QA and QC documents and records of environmental data.

B. MEASUREMENTS/DATA ACQUISITION

B1 Sampling Process Design

Sampling conducted during NRD assessments will be designed to meet the general DQOs developed for the specific project type as discussed in Section A6. Site-specific DQOs will be developed for individual projects, and these will be specified in the sampling plan prepared by the NRD Coordinator and ESP FSU. Depending on the scope of the NRD assessment, the results may be used to determine the appropriate next course of action (e.g. further assessment if conducted during the pre-assessment screen, no action, etc.). .

Based on available site information, and/or data gathered during the site reconnaissance, the NRD Coordinator together with ESP FSU will prepare a sampling plan. The sampling plan will provide general site background information, document the data quality objectives using the DQO process, describe the number, type, and location of samples to be collected, along with analytical parameters requested for each sample. The plan will be reviewed by the HWP QA Officer prior to submission to ESP. Because the QA Project Officer is independent of those responsible for generating the data, (i.e. ESP), their review of the sampling plan in terms of QA requirements is sufficient to ensure the project sampling design is adequate to meet the DQOs and will be of usable quality. The NRD Coordinator will also notify the CAS by e-mail in advance of sampling to indicate the anticipated number and type of samples to be collected, the date(s) of sampling, and the analyses required.

An example sampling plan outline is provided as Appendix 4. Sampling plan approval will be documented on the signature page, which will include the signature of the ESP personnel who prepared the report and the approval signature of the NRD Coordinator. Sample collection is generally conducted by ESP personnel, with on-site oversight by the NRD Coordinator and/or HWP personnel. However, for some projects with limited sampling needs, the HWP personnel may conduct the sampling.

The sampling plan will provide a best estimate of the number and types of samples to be collected, but site conditions at the time of sampling will determine the actual number and type of samples collected. Decisions on deviations from the sampling plan in terms of sampling points and parameters will be made and approved by the QA Project Officer or NRD Coordinator who approved the sampling plan. The deviations or changes will be documented in a field notebook, and in the final sampling report prepared by the ESP and submitted to the NRD Coordinator.

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Background sample(s) will be collected for each type of environmental media sampled (e.g. soil, sediment, groundwater, surface water, air) for each project in accordance with the guidance provided in MDNR-ESP-210 "Quality Assurance/Quality Control for Environmental Data Collection".

B2 Sampling Methods and Procedures

The field investigations and sample collection activities for all projects will adhere to the methods described in established department SOPs. A list of Department SOPs is provided in Appendix5. Additional sample volume will be collected from one background sampling location of each matrix sampled for each project. The additional volume will provide enough sample for the laboratory to conduct matrix spike and matrix spike duplicate analyses on the background sample. Collection of twice the optimum volume specified in Appendix 3 will provide sufficient sample volume.

The NRD Coordinator and/or HWP personnel, in consultation with the ESP sampling staff will be responsible for corrective action regarding any failures in sampling encountered in the field. Unanticipated needs to deviate significantly from these sampling methods and procedures in the field will be approved by the NRD Coordinator in consultation with ESP sampling staff.

B3 Sample Handling and Custody Requirements

Chain-of-custody and field documentation of samples collected for this project will be in accordance with MDNR-ESP-002 "Field Sheet and Chain-of-Custody Record" and MDNR-ESP-004.

B4 Analytical Method Requirements

The specific analytical methods required for a specific project will be included in the sampling plan. All analyses will be conducted in accordance with applicable ESP CAS SOPs.

The HWP may occasionally request that ESP conduct a tentatively identified compound (TIC) search on a sample or group of samples. ESP will provide a list of compounds tentatively identified together with an estimated concentration for each compound. Estimated concentrations will be calculated using a relative response factor (RRF) of 1.0 unless data is available to indicate that a more specific RRF is warranted.

Any analytical work not performed by the ESP will be conducted at a laboratory under contract with the ESP. The contract will specify that EPA SW-846 methods or other methods as specified will be utilized and that the QC procedures specified in these methods will be followed. The contract will require that all QC documentation be provided with each analytical deliverable package. The ESP will be responsible for ensuring all analytical data provided under contract for the project meets the contract requirements and the requirements of this QAPP.

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B4.1 List of Target Analytes

The specific target analytes required will vary on a project-specific basis, and will be specified in the sampling plan and in the chain of custody submitted to the CAS with the samples. Some analyses are requested by referencing commonly grouped analytes such as Volatile Organic Compounds (VOCs), Semi-Volatile Organic Compounds (SVOCs), pesticides and herbicides, and RCRA Metals. The specific analytes to be included in these groups when requested are listed in the tables of Appendix 4. For some projects analytes, other than those listed in Appendix 4 will be required. The NRD Coordinator and/or HWP personnel will consult with the CAS on special analytical needs for these projects well in advance of sampling.

B4.2 Sensitivity Requirements

Method Detection Limits (MDLs) for each analytical parameter will be established by the CAS as specified in 40 CFR 136 Appendix B and Section 5, Chapter 1, Quality Control, of SW-846. PQLs as defined in 40 CFR Part 300 Appendix A, Section 1.1 will be developed by the CAS. The CAS may use either the PQL or the MDL as reporting limits for analyses conducted under this QAPP, however the reporting limit used must be identified on the laboratory reporting form. Project-specific sensitivity requirements will be documented in the sampling plan.

Analytical results obtained for projects conducted under this QAPP will be compared to various action limits established for the project, or to screening benchmarks, the most common of which include ecological benchmarks and/or criteria established in the DOI NRD regulations (43 C.F.R. Part 11). Ideally, the laboratory reporting limits would be at or below each benchmark value in each environmental media.

It is important to note that interference caused by difficult sample matrices and highly contaminated samples may cause PQLs to be elevated above those specified in the project-specific sampling plan.

The NRD Coordinator will consult with ESP CAS well in advance of sampling regarding the appropriate analytical method, to verify that the laboratory PQL will meet the project DQOs, and to determine the appropriate course of action where applicable (e.g. the use of an alternative analytical method or subcontracting to another laboratory).

Data that do not meet the project DQOs for sensitivity will be qualified by the ESP CAS as described in the applicable verification/validation procedure (Section D), and documented in the project report.

B4.3 Laboratory Turnaround Time Requirements

All analyses will be conducted within the EPA-specified maximum sample holding time limits specified in the tables of Appendix 5. ESP will provide the analytical data report sheets to the NRD Coordinator within 30 calendar days of the delivery of samples to the ESP laboratory for

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analysis. In the event that the 30-day turn around time cannot be met, the ESP will notify the NRD Coordinator. The NRD Coordinator authorizes the ESP to contract out analysis for those samples that will not meet the 30-day turnaround time due to workload at the ESP. The NRD Coordinator may request expedited turnaround time (10 days) for laboratory analysis of samples at certain sites. If the NRD Coordinator requests expedited turnaround ESP CAS will be notified by e-mail well in advance of sampling to specify the analytes, number of samples, and date by which results are needed.

Any data obtained from analyses conducted on samples after the holding time limits specified in Appendix 3 will be qualified by the CAS as described in the applicable validation procedure (Section D) and discussed in the project report.

B5 Field and Laboratory Quality Control Elements

A number of field and laboratory QC checks will be required to ensure data meet the project DQOs. The principal quality attributes important to NRD assessments are precision, accuracy, comparability, representativeness, and completeness. Criteria for these attributes are discussed below. All QC samples, including field blanks, trip blanks, equipment rinsate blanks, replicate splits and duplicate samples will be collected in accordance with MDNR-ESP-210 "Quality Assurance/Quality Control for Environmental Data Collection."

B5.1 Precision

Precision is a measure of mutual agreement among individual measurements of the same property, under prescribed similar conditions. It is typically expressed in terms of the standard deviation among a set of data or as the relative percent difference between two measurements. For the purposes of this QAPP the components of precision have been grouped into those associated only with the laboratory analysis and those associated with the overall sampling and analysis process.

B5.1.1 Laboratory Precision

Precision of laboratory analyses is assessed by the analysis of Matrix Spike/Spike Duplicates (MS/MSD), laboratory duplicate samples, and blind performance evaluation samples. The frequency with which laboratory precision is assessed, and the performance criteria vary by analyte, analytical method, and environmental media. The criteria and methods for assessment of laboratory precision are specified in the analytical methods and are developed in accordance with MDNR-CAS-2090, MDNR-CAS-2070, and CAS SOPs for the various analyses. Data that do not meet the laboratory precision criteria 3 will be qualified by the CAS as described in the applicable validation procedure (Section D), and discussed in the project report.

B5.1.2 Overall Sampling and Analysis Precision

Precision will be measured as data variability between replicate and duplicate field samples collected at various points in the sample collection process. The scale at which the dups/reps are

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collected will determine how the estimate of precision will be interpreted. For example, the data variability between two or more separate soil samples collected very near each other and submitted for separate analysis provide a measure of short-scale heterogeneity present at the site. In other cases a soil sample may be collected and manually mixed in a pan prior to subsampling into two or more separate jars for analysis. In that case the data variability between the results provides a measure of within-sample heterogeneity and precision of the subsampling process. At the smallest scale, two or more analytical subsamples will be collected at the lab from the same sample jar. In that instance, data variability will measure micro-scale heterogeneity and precision of the lab subsampling process.

Specific QC measures to be taken for a given project will be detailed in the Sampling Plan. Overall precision of the entire sampling and analytical process will be assessed using analyses of blind field duplicate and replicate split samples. Aqueous and air precision QC samples will be collected as duplicates, while non-aqueous precision QC samples will be sampled as replicate splits. Definitions of the terms "duplicate" and "replicate split" are provided in MDNR-FSS-210. Non-aqueous samples to be analyzed for VOCs cannot be homogenized prior to collection due to the potential for loss of VOCs. Therefore, in place of replicate split samples, for projects involving the collection of non-aqueous samples for VOC analysis, duplicate non-aqueous samples will be collected. Duplicate air samples collected in accordance with EPA Method TO-15 will consist of two samples analyzed from the same Summa canister, while replicate split samples will be samples analyzed from two separate canisters collected from the same air mass.

Precision will generally be measured using the Relative Standard Deviation (RSD) when three or more measures are taken, and Relative Percent Difference (RPD) when two measures are taken.

$$RSD = 100\left[\frac{S\tan dardDeviation}{\bar{x}}\right]$$

$$RPD = 100\left[\frac{x_1 - x_2}{\bar{x}}\right]$$

The RSD/RPD criterion for aqueous samples is ≤ 30% for each contaminant measured above the laboratory reporting level. For non-aqueous VOC samples the criterion will be ≤ 50%. The criterion for air samples will be 25%. If data fall within these limits, then the overall precision of the sampling and analytical process is adequate to meet the project DQOs. Data that do not meet these precision criteria will be qualified as described in the applicable validation procedure (Section D), and discussed in the project report.

Because this QAPP is generic, covering many different NRD assessments, these precision criteria will be applied to a large number of analytes in various complex sample matrices. It is not likely that the precision limits for the overall sampling and analytical process will be met for every contaminant in every sample for every project. This is especially true for projects involving the sampling of non-aqueous matrices. When released to the environment, many contaminants distribute themselves extremely unevenly in soils; even on the small scale at which sampling occurs. This problem is further confounded by the heterogeneous nature of the dense clayey and silty clay soils found in many areas of the state. The need to collect duplicate non-

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aqueous samples for VOC analysis exacerbates the problem further still, since the primary and duplicate samples may not be homogenized prior to analysis. The variability measured at the different scales using these QC practices should be used to quantify uncertainty in estimates made of concentration. Great care will be taken when interpreting overall sampling and analysis precision data for non-aqueous duplicate and replicate split samples. The NRD Coordinator and/or HWP personnel, in consultation with appropriate ESP personnel, will evaluate all qualified data on a project-specific basis, and determine how/whether to use the data.

B5.1.3 Accuracy

The accuracy of laboratory analyses will be assessed by analysis of preparation/method blanks, laboratory control samples, surrogates, internal standards, matrix spikes, and blind performance samples. The frequency with which laboratory accuracy is assessed, and the performance criteria vary by analyte, analytical method, and environmental media. Criteria for laboratory accuracy are specified in the analytical methods and will be developed and maintained in accordance with the following CAS SOP: MDNR-CAS-2090.

Field accuracy will be assessed through the analysis of trip blanks, field blanks, and field equipment rinse blanks. For all projects involving the collection of aqueous samples, a trip blank will be included at a frequency of one per separate sampling event (mobilization) per sample cooler. If aqueous samples are collected from multiple projects during the same mobilization for the same analytical parameters, a single trip blank per cooler may be used to assess accuracy for all of the projects. A field blank may be requested by the NRD Coordinator and/or HWP personnel for some projects where the potential for contamination of samples by atmospheric pollutants is suspected. An equipment rinsate blank will be collected for projects where the sampling equipment is decontaminated in the field for reuse. The equipment rinsate blank will be collected at a frequency of one per separate sampling event (mobilization) for each different combination of sampling equipment, decontamination method, and analytical parameter.

Contaminants should not be detected above the laboratory reporting level in trip blanks, field blanks, and equipment rinse blanks. Any data that do not meet these accuracy criteria will be qualified as described in the applicable validation procedure (Section D). The NRD Coordinator and/or HWP personnel in consultation with appropriate ESP personnel will evaluate all qualified data on a project-specific basis, and determine how/whether to use the data.

B5.1.4 Data Comparability

Comparability is an expression of the confidence with which one data set can be compared to another. The objective of comparability for this QAPP is to ensure that sampling data developed during the project investigation may be readily compared to each other and to the appropriate screening benchmarks. All data will be reported as ^{o} Celsius (flash point) pH units, µg/l or mg/l for water, liquids or Toxicity Characteristic Leaching Procedure (TCLP), µg/kg or mg/kg for soil, sediment or other solids, and µg/m^3 for air. Comparability is further addressed by using appropriate field and laboratory methods that are consistent with current standards of practice as approved by EPA.

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B5.1.5 Data Representativeness

Data representativeness addresses the degree to which measurements are made and physical samples are collected in a manner that the resulting data appropriately reflect the environment or condition being studied or measured.

Representativeness is ensured for projects under this QAPP in several specific ways that are further discussed in other sections of this QAPP:

- Use of correct sampling procedures and equipment (Section B2)
- Adherence to QA and QC requirements for ensuring sample integrity (Section B5)
- Collection of an adequate amount of sampled material (Section B2 and Appendix 3)
- Selection and implementation of appropriate analytical measurement method, including sample preparation (Section B4 and Appendices 3 and 4).

B5.1.6 Data Completeness

Completeness is expressed as a percentage of the amount of valid data obtained compared to the amount that was planned. One hundred percent of data completeness is desired for the collection of field samples for all project investigations. If less than 100 percent is received, the QA Project Officer will decide if the valid data obtained from a measurement system compared to the amount that was expected to be obtained under normal conditions is sufficient to meet the project DQOs. If not, additional sampling will be required.

B6 Instrument/Equipment Maintenance and Calibration Requirements

Field analytical instruments used during this project will be maintained and calibrated according to instructions provided by the instrument manufacturer, and applicable field analytical methods. All major laboratory instruments used for quantitative sample analysis in the CAS are covered by service/maintenance contracts with the instruments' vendors. In addition to the detailed maintenance procedures performed as part of these contracts, the analytical staff at the laboratory performs the routine daily maintenance and calibration procedures which are necessary to ensure that the analytical data produced is of definable quality and meets the DQOs of the projects. Maintenance and calibration procedures are conducted in accordance with manufacture's instrument manuals, MDNR-CAS-2040, and other CAS SOPs for specific instruments/analyses. A full list of applicable CAS SOPs is included as Appendix 5.

B7 Inspection/Acceptance Requirements for Supplies and Consumables

These requirements are specified in MDNR-CAS-2140 "Supplies Procurement, Inspection and Acceptance."

B8 Non-direct Measurements

Several types of data and information will be obtained from non-measurement sources for use in projects conducted under this QAPP. The primary types of non-measurement data are listed in

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Section A6.3. These data will be used with the directly measured data collected during each project to evaluate potential uncontrolled hazardous substance sites as described in Section A3. Non-direct measurement data must meet the documentation and referencing provisions of the EPA Guidance Document, *Regional Quality Control Guidance for NPL Candidate Sites*, (U.S. EPA, 1991b).

B9 Data Management

Data management will be in accordance with the following SOPs: MDNR-CAS-2000, MDNR-CAS-2020, MDNR-CAS-2090, and MDNR-CAS-2130.

Documentation will be in accordance with MDNR-ESP-004, and will include the sampling reports, copy of the chain-of-custody, and field QA controls with the analytical results. Data reduction will occur in accordance with MDNR analytical SOPs for each parameter. If difficulties are encountered during sample collection or sample analyses, a brief description of the problem will be provided in the sampling report prepared by ESP. The laboratory qualifiers listed in Appendix 6 will be used where applicable on the results of analysis report sheets provided by the CAS. Data reporting will be in accordance with MDNR-CAS-2020.

Adequate precautions will be taken during the reduction, manipulation, and storage of data in order to prevent the introduction of errors or the loss or misinterpretation of data. The LIMS maintains all information and data on all environmental samples received. The system is utilized to log in samples collected, record results of analyses, and generate sample analyses and management reports. The LIMS is backed up daily through the off-site DNR tape backup system. System maintenance is performed weekly. This includes checking for operating system errors, LIMS system errors, and database integrity.

The current Agency Records Disposition Schedule approved by the Secretary of State's Office for all QA and QC documents and records of environmental data will be followed.

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C. ASSESSMENT/OVERSIGHT

C1 Assessment and Response Action

This section describes the internal and external checks necessary to ensure that all elements of the QAPP are correctly implemented as prescribed, that the quality of the data generated by implementation of the QAPP is adequate, and that any necessary corrective actions are implemented in a timely manner.

C1.1 Laboratory Performance Assessment

EPA, Region VII conducts periodic Laboratory On-Site Evaluations to assess the laboratory procedures in order to maintain certification under the requirements of the Safe Drinking Water Act and for other state operated, federally-funded programs.

C1.2 Field Performance Assessment

The auditor in charge of ESP field QA will conduct audits of field activities according to MDNR-ESP-211 "Quality Assurance Field Auditing Procedures." The process of choosing when field audits are conducted is not based on a particular project or site-sampling event, but rather is based on assuring that each ESP staff member involved in sample collection is audited at least once every two years. The time of year, and thus the particular site-sampling event field personnel are working on, is randomly chosen.

For this project, the ESP field QA auditor is authorized to issue a stop work order upon finding a significant condition that would adversely affect the quality and usability of the data. The ESP field QA auditor will have the responsibility for initiating and implementing response actions associated with findings identified during the field audit. The procedures require that the field personnel properly address any response actions needed.

C1.3 Overall Project Performance Assessment

Overall performance auditing of projects conducted under this QAPP will be undertaken annually by the EPA Site Assessment Manager. These audits will evaluate the effectiveness of the projects in attaining the stated DQOs, documentation practices, and the overall quality of project reports.

EPA Region VII conducts periodic evaluations of the state's environmental programs. These evaluations normally include some type of review of the department's quality management system, and may include examination of DEQ QAPPs.

C1.4 Data Validation

All field and laboratory data will be subject to validation by review for accuracy, precision, completeness, representativeness and comparability. The acceptance criteria for measurement data are discussed in Section B5. Data validation procedures are presented in Section D2.

C2 Reports to Management

Field performance assessment audits will be documented by the ESP field QA auditor in a written report that shall be kept on file at the ESP. Copies of the written report shall be provided to the subject of the audit, his/her supervisor, and the DEQ QA Manager upon request.

Results from the laboratory's semi-annual participation in the round robin audit studies, and from EPA Region VII's periodic On-Site Laboratory Evaluations, will be kept on file at ESP. Copies of these results will be provided to the HWP QA Coordinator.

Findings from the EPA Site Assessment Manager annual overall project evaluation are documented in a letter to the HWP QA coordinator, who facilitates the implementations of any

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recommendations and/or corrective actions needed.

Comments and recommendations from the EPA Region VII periodic evaluations of state environmental programs are provided to the DEQ QA manager and used by DEQ management and staff to take any corrective actions which may be needed.

D. DATA VALIDATION AND USABILITY

D1 Data Verification, Validation, and Data Quality Assessment

This section describes the process for documenting the degree to which the collected data meet the project objectives, individually and collectively, and to estimate the effect of any deviations on the ability to use the data for addressing the decision rules described in Section A6.5.

D1.1 Sampling Design

The HWP QA Project Officer will verify that the sampling design in the sampling plan prepared by the NRD Coordinator and ESP FSU is adequate to meet the project DQOs. During preparation of the sampling report, ESP FSS personnel will verify that the actual number, type, location, and requested lab analyses collected conforms to that specified in the sampling plan. Any deviations noted during sampling design verification will be documented by the ESP FSU personnel in the sampling report.

D1.2 Sample Collection and Handling Procedures

The ESP FSU personnel responsible for the project and the ESP Director will provide verification and validation that the field portions of all sample collection and handling procedures used conform with those specified in Sections B2, B3, and Appendix 5 of this QAPP. The CAS supervisor will provide verification and validation that the laboratory portions of all sample handling procedures used, conform to those specified in Section B3 and Appendix 5 of this QAPP. The data will be further validated by the NRD Coordinator and/or HWP personnel during review of the sampling report, and by the QA Project Officer during review of the project report.

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D1.3 Analytical Procedures

The CAS supervisor will provide verification and validation of each sample to ensure that the procedures used to generate the data were implemented as specified in Section B4 of the QAPP. Any deviations will be documented in the sampling report. The data will be further validated by the NRD Coordinator and/or HWP personnel during review of the sampling report, and by the QA Project Officer during review of the project report.

D1.4 Quality Control

The ESP FSU personnel responsible for the project will provide verification and validation that the data generated conform to the field QC elements in Section B5 of this QAPP. The CAS supervisor will provide verification and validation that the data generated conform to the laboratory QC elements of Section B5. Any QC deviations noted during verification and validation will be documented in the sampling report. The QC data will be further validated by the NRD Coordinator and/or HWP personnel during review of the sampling report, and by the QA Project Officer during review of the project report.

D1.5 Calibration

The CAS supervisor will provide verification and validation that the data generated conform with the instrument/equipment maintenance and calibration requirements in Section B6 of this QAPP. Any deviations noted during verification and validation will be documented in the sampling report.

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D2 Validation and Verification Methods

Data validation methods are described in the analytical CAS SOPs for specific analyses and in MDNR-CAS-2020, MDNR-CAS-2070, MDNR-CAS-2090, MDNR-CAS-2130, MDNR-ESP-002, MDNR-ESP-003, MDNR-ESP-004, MDNR-ESP-018, MDNR-ESP-210, and MDNR-ESP-211.

Results of data verification and validation performed by ESP will be documented in the sampling report provided to the NRD Coordinator for each project. Validation activities conducted by the QA Project Officer will be documented in the project report.

D3 Reconciliation with User Requirements (Data Quality Assessment)

Results of each project will be reconciled with data user requirements using the Data Quality Assessment (DQA) process described in *Guidance for Data Quality Assessment*, EPA QA/G-9, July 2000. The DQA guidance was developed primarily for projects whose DQOs are amenable to evaluation by statistical analyses. The limited number of samples collected for most NRD assessment projects conducted under this QAPP are not readily evaluated by statistical analyses.

At the completion of the project, the NRD Coordinator, together with the QA Project Officer will review the sampling design, and data collection and analysis documentation to evaluate their consistency with the project DQOs specified in the QAPP and sampling plan. If it is determined that the DQOs are not met, the NRD Coordinator, together with the QA Project Officer/HWP QA Coordinator, will identify the appropriate corrective measures necessary, and ensure they are implemented. These measures will most commonly include laboratory re-analysis, re-sampling, and/or the collection of additional samples.

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E. REFERENCES

Missouri Department of Natural Resources, 1999, Locational Data Method Accuracy Description. Air and Land Protection Division. Version 6.1.1.

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U.S. EPA, 1992a, Guidance for Performing Site Inspections under CERCLA – Interim Final. Office of Emergency and Remedial Response, Washington, DC. EPA/540/R-92/021, Publication PB92-963375.

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U.S. EPA, 1999a, Improving Site Assessment: Combining PA/SI Assessments. Office of Solid Waste and Emergency Response, Washington, DC. EPA/540/F-98/038, OSWER 9375.2-10FS.

U.S. EPA, 1999b, Improving Site Assessment: Pre-CERCLIS Screening Assessments. Office of Solid Waste and Emergency Response, Washington, DC. EPA/540/F-98/039, OSWER 9375.2-11FS.

U.S. EPA, August 2000, Guidance for Choosing a Sampling Design for Environmental Data Collection, Use in the Development of a Quality Assurance Project Plan – Peer Review Draft, EPA QA/G-5S. Office of Environmental Information, Washington, DC.

U.S. EPA, 2000b, Guidance for Data Quality Assessment, Practical Methods for Data Analysis, EPA QA/G-9. Office of Environmental Information, Washington, DC. EPA/600/R-96/084.

U.S. EPA, 2006, Data Quality Objectives Process for Hazardous Waste Site Investigations, EPA QA/G-4HW, Office of Environmental Information, Washington, DC. EPA/600/R-00/007.

U.S. EPA, 2006a, Guidance for the Data Quality Objectives Process, EPA QA/G-4. Office of Environmental Information, Washington, DC. EPA/600/R-96/055.

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U.S. EPA, 2006b, Guidance on Systematic Planning Using the Data Quality Objectives Process, EPA QA/G-4, February 2006.

U.S. EPA, June 2001, Guidance on Environmental Data Verification and Data Validation – Peer Review Draft, EPA QA/G-8. Office of Environmental Information, Washington, DC.

U.S. EPA, September 2001, Guidance on Data Quality Indicators – Peer Review Draft, EPA QA/G-5I. Office of Environmental Information, Washington, DC.

U.S. EPA, 2001, EPA Requirements for Quality Assurance Project Plans, EPA/QA/R-5. Office of Environmental Information, Washington, DC. EPA/240/B-01/003.

U.S. EPA, July 2002, Guidance for Quality Assurance Project Plans - Peer Review Draft, EPA/QA/G-5. Office of Environmental Information, Washington DC.

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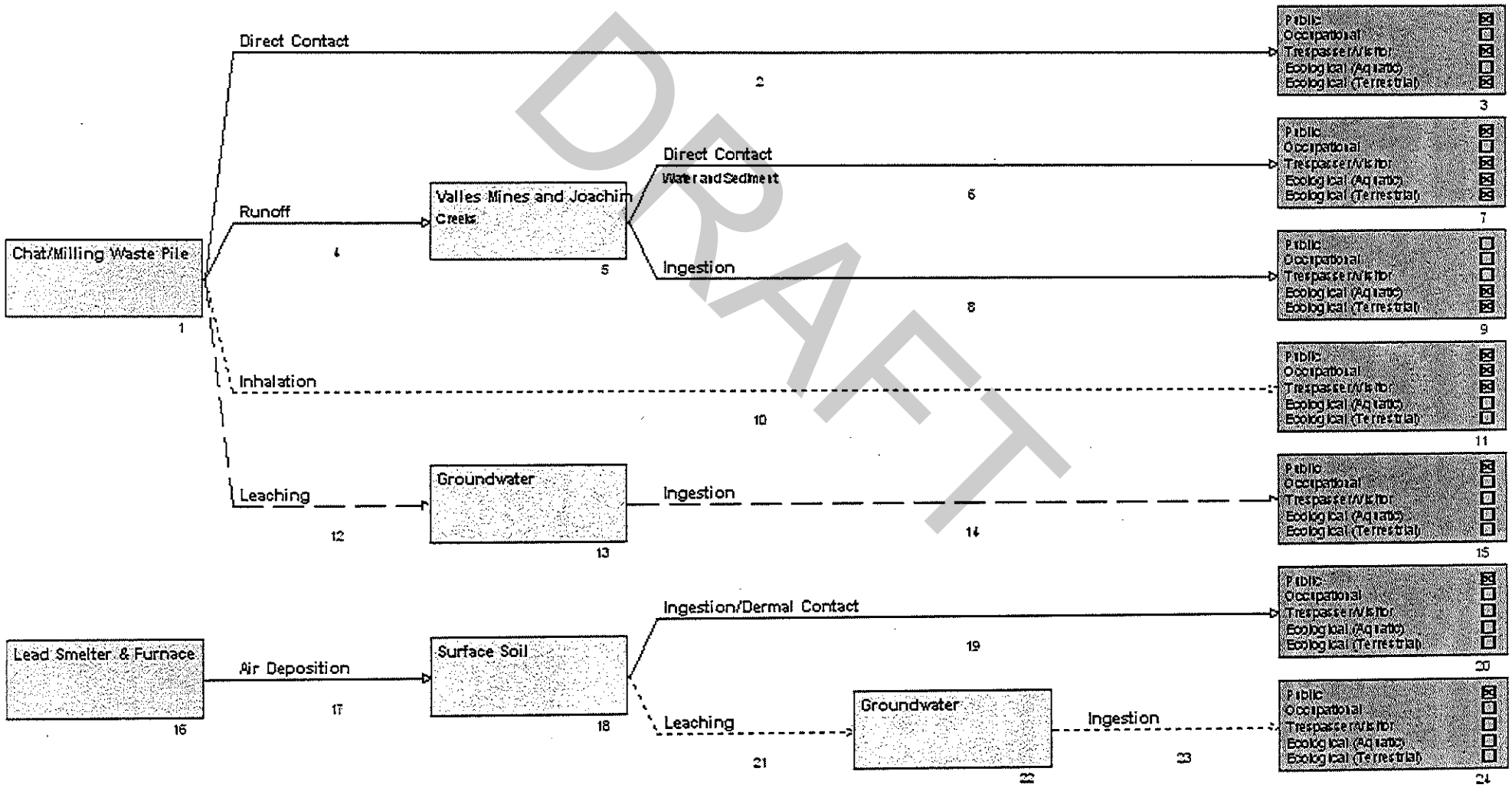
APPENDIX 1: EXAMPLE CONCEPTUAL SITE MODEL (CSM) DIAGRAM



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APPENDIX 2: EXAMPLE SAMPLING PLAN AND REPORT OUTLINES

OUTLINE FOR SAMPLING PLAN

1.0 INTRODUCTION

2.0 SITE INFORMATION

2.1 LOCATION
2.2 DESCRIPTION
2.3 HISTORY/CONTAMINANTS OF CONCERN

3.0 SITE RECONNAISSANCE

4.0 DATA QUALITY OBJECTIVES

4.1 PROBLEM STATEMENT
4.1.1 Background Information
4.1.2 Conceptual Site Model
4.1.3 Available Resources and Constraints
4.2 DECISION STATEMENTS
4.3 INPUTS INTO THE DECISIONS
4.4 STUDY BOUNDARIES
4.5 DECISION RULES
4.6 LIMITS ON DECISION ERROR
4.7 SAMPLING DESIGN

5.0 FIELD ACTIVITIES

4.1 SAMPLING METHODS
4.1.1 Soil sampling
4.1.1.1 Surface soil sampling
4.1.1.2 Depth-discrete soil sampling
4.1.2 Water sampling
4.1.2.1 Surface water sampling
4.1.2.2 Groundwater sampling
4.1.3 Sediment sampling
4.1.4 Air sampling
4.1.5 Fish tissue sampling
4.1.6 Monitoring well installation
4.2 SAMPLING ORDER
4.3 SAMPLE QUANTITY
4.4 ANALYSES AND SENSITIVITY REQUESTED
4.5 SAMPLE CONTAINER AND PRESERVATION REQUIREMENTS
4.6 CHAIN-OF-CUSTODY

6.0 QUALITY CONTROL

5.1 FIELD METHODS
5.2 FIELD DECONTAMINATION
5.3 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) SAMPLES
5.3.1 Trip blank
5.3.2 Duplicate (co-located) samples
5.3.3 Replicate (split) samples
5.3.4 Equipment Rinsate blank samples
5.3.5 Field blank samples

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7.0 INVESTIGATION DERIVED WASTES (IDW) PLAN

8.0 SITE SAFETY

9.0 REPORTING

APPENDICES

APPENDIX A - Conceptual Site Model Diagram
APPENDIX B - Site Map
APPENDIX C - Site Health & Safety Plan

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APPENDIX 2: EXAMPLE SAMPLING PLAN AND REPORT OUTLINES

OUTLINE FOR SITE HEALTH AND SAFETY PLAN

1.0 INTRODUCTION

2.0 KEY PERSONNEL

3.0 SITE INFORMATION

3.1 OVERALL INCIDENT/RISK/HAZARD ANALYSIS
3.2 CONTAMINANT(S) OF CONCERN
3.2.1 Pysical State and Chemcial Characteristics
3.2.2 Physical Hazards
3.3 TASK SPECIFIC RISK ANALYSIS

4.0 MEDICAL SURVEILLANCE AND PERSONNEL TRAINING REQUIREMENTS

5.0 PERSONAL PROTECTIVE EQUIPMENT

6.0 FREQUENCY AND TYPE OF AIR MONITORING/SAMPLING

7.0 SITE CONTROL MEASURES

7.1 THE "BUDDY -SYSTEM"
7.2 SAFE WORK PRACTICES
7.3 SITE COMMUNICATIONS
7.4 WORK ZONES

8.0 DECONTAMINATION PROCEDURE/SOLUTIONS

9.0 EMERGENCY INFORMATION

10.0 ADDITIONAL EMERGENCY INFORMATION/NUMBERS

11.0 SIGNATURES

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APPENDIX 2: EXAMPLE SAMPLING PLAN AND REPORT OUTLINES

OUTLINE FOR SAMPLING REPORT

1.0 INTRODUCTION

2.0 SITE INFORMATION

2.1 LOCATION
2.2 DESCRIPTION
2.3 HISTORY/CONTAMINANTS OF CONCERN

3.0 METHODS

3.1 FIELD PROCEDURES
3.1.1 Soil sampling
3.1.1.1 Surface soil sampling
3.1.1.2 Depth-discrete soil sampling
3.1.2 Water sampling
3.1.2.1 Surface water sampling
3.1.2.2 Groundwater sampling
3.1.2.2.1 Residential well sampling
3.1.2.2.1 Municipal well sampling
3.1.2.2.1 Monitoring well sampling
3.1.2.2.1 Temporary well sampling
3.1.3 Sediment sampling
3.1.4 Air sampling
3.1.5 Fish tissue sampling
3.1.6 Monitoring well installation
3.2 SAMPLING ORDER
3.3 SAMPLE QUANTITY
3.4 ANALYSES REQUESTED
3.5 CHAIN-OF-CUSTODY

4.0 DATA QUALITY

4.1 FIELD METHODS
4.2 FIELD DECONTAMINATION
4.3 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) SAMPLES
4.3.1 Trip blank
4.3.2 Duplicate (co-located) samples
4.3.3 Replicate (split) samples
4.3.4 Equipment rinsate blank samples
4.3.5 Field blank
4.4 QA/QC DATA INTERPRETATION
4.4.1 Trip blanks
4.4.2 Equipment rinsate samples
4.4.3 Background samples

5.0 INVESTIGATION DERIVED WASTES (IDW)

6.0 OBSERVATIONS

7.0 REPORTING

APPENDIXS

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APPENDIX 2: EXAMPLE SAMPLING PLAN AND REPORT OUTLINES

OUTLINE FOR SAMPLING REPORT (CONT.)

TABLE 1 - Sample Listing/Analytes
TABLE 2 - Sample Description
TABLE 3 - Geographic Coordinates of Sample Locations
APPENDIX A - Site Maps
APPENDIX B - Chain-of-Custody Copies/Analytical Results
APPENDIX C - Photographs
APPENDIX D - Copies of Field Notes

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APPENDIX 3: HOLDING TIMES, PRESERVATION, AND SAMPLE VOLUMES

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AQUEOUS MATRICES					
Parameter	**Minimum Volume (mls)**	**Optimum Volume (mls)**	**Container Type**	**Preservative**	**Holding Time**
INORGANIC NONMETALLIC CONSTITUENTS					
Cyanide (CN), Total	100	1000	P,G	Cool, NaOH to pH > 12	14 days
Cyanide (CN), Amenable to Chlorination	250	1000	P,G	Cool, NaOH to pH > 12	14 days
METALLIC CONSTITUENTS					
Total Metals (As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Pb, Ni, Ag, Zn, Al, Sb, Be, Se, Mg, Ca, Hg)	250	1000	P,G	Cool, HNO_3 to pH < 2	6 mos. 28 days for Hg
Dissolved Metals (As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Pb, Ni, Ag, Zn, Al, Sb, Be, Se, Mg, Ca, Hg)	150	1000	P,G	Filter on-site Cool HNO_3 to pH < 2	6 mos. 28 days for Hg
Dissolved Hexavalent Cr	100	500	P,G	Filter on-site; none	24 hrs.
TCLP Metals (Ag, As, Ba, Cd, Cr, Pb, Se, Hg)	750	1000	P,G	Cool	6 mos.

APPENDIX 3: HOLDING TIMES, PRESERVATION, AND SAMPLE VOLUMES

AQUEOUS MATRICES					
Parameter	Minimum Volume (mls)	Optimum Volume (mls)	Container Type	Preservative	Holding Time
ORGANIC CONSTITUENTS					
Semivolatile Organic Compounds (SVOCs)	1000	3000	G	Cool	7 days to extract
Chlorophenoxy Acid Herbicides	1000	3000	G	Cool	7 days to extract
Organochlorine Pesticides & PCBs	1000	3000	G	Cool	7 days to extract
Volatile Organic Compounds (VOCs)	40 (1 vial)	120 (3 vials)	G	Cool, HC1 to pH < 2	14 days
Total Petroleum Hydrocarbon (8015/OA2)	1000	3000	G	Cool	7 days to extract
2,3,7,8 – Tetrachloro-dibenzo-P-Dioxins (TCDD)	1000	3000	G	Cool	30 days to Extract

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APPENDIX 3: HOLDING TIMES, PRESERVATION, AND SAMPLE VOLUMES

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NON-AQUEOUS MATRICES					
Parameter	Minimum Volume (fl oz, gm, or oz)	Optimum Volume (fl oz or gm)	Container Type	Preservative	Holding Time
INORGANIC NONMETALLIC CONSTITUENTS					
Cyanide (CN), Total	8 fl.oz jar	8 fl oz jar	G	Cool	14 days
METALLIC CONSTITUENTS					
Total Metals (As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Pb, Ni, Ag, Zn, Al, Sb, Be, Se, Mg, Ca, Hg)	100gm (~3oz)	8 fl oz jar	G	Cool	6 mos. 28 days for Hg
TCLP Metals (Ag, As, Ba, Cd, Cr, Pb, Se, Hg)	8 fl oz jar	(2) 8 fl oz jar	G	Cool	6 mos. 28 days for Hg
ORGANIC CONSTITUENTS					
Semi-volatile Organic Compounds (SVOCs)	50gm (~2oz)	8 fl oz jar	G	Cool	14 days to extract
TCLP SVOCs	8 fl oz jar	(2) 8 fl oz jar*	G	Cool	14 days to extract
Chlorophenoxy Acid Herbicides	50gm (~2oz)	(2) 8 fl oz jars	G	Cool	14 days to extract
TCLP Herbicides	8 fl oz jar	(2) 8 fl oz jar*	G	Cool	14 days to extract
Organochlorine Pesticides & PCBs	100gm (~4oz)	(2) 8 fl oz jars	G	Cool	14 days to

APPENDIX 3: HOLDING TIMES, PRESERVATION, AND SAMPLE VOLUMES

NON-AQUEOUS MATRICES					
Parameter	Minimum Volume (fl oz, gm or oz)	Optimum Volume (fl oz or gm)	Container Type	Preservative	Holding Time
					extract
TCLP Pesticides	8 fl oz jar	(2) 8 fl oz jar*	G	Cool	14 days to extract
Volatile Organic Compounds (VOCs) (includes Total Petroleum Hydrocarbons 8015/OA1)	(1) 5gm Encore™ sample	(2) 5gm Encore™ samples**	E	Cool	14 days
TCLP VOCs	(1) 25gm Encore™ sample	(1) 25gm Encore™ samples**	E	Cool	14 days to extract
Total Petroleum Hydrocarbons (8015/OA2)	50gm (~2oz)	(1) 8 fl oz jar	G or E	Cool	7 days to extract
2,3,7,8 – Tetrachloro-dibenzo-P-Dioxins (TCDD)	50gm (~2oz)	8 fl oz jar	G	Cool	30 days to Extract

* If total and TCLP organics are needed, a total of (2) 8fl oz jars per analyte group will be adequate to conduct both analyses

** Optimal volume on sample(s) collected for precision QC analysis (replicates and duplicates) will be (4) 5gm Encore™ samples and (2) 25 gm Encore™ samples.

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APPENDIX 4: PARAMETER LISTS AND SAMPLE QUANTITATION LIMITS

Organochlorine Pesticides, Chlorophenoxy Acid Herbicides, and Polychlorinated Biphenyls			
Methods[1]	Parameter	Water PQL ug/L	Soil PQL ug/Kg
3510C or 3545A/8081A	Aldrin	0.25	5
3510C or 3545A /8081A	alpha-BHC	0.25	5
3510C or 3545A /8081A	beta-BHC	0.25	5
3510C or 3545A /8081A	gama-BHC (Lindane)	0.25	5
3510C or 3545A /8081A	delta-BHC	0.25	5
3510C or 3545A /8081A	Chlordane	2.5	50
3510C or 3545A /8081A	4,4'-DDE	0.25	5
3510C or 3545A /8081A	4,4'-DDD	0.25	5
3510C or 3545A /8081A	4,4'-DDT	0.25	5
3510C or 3545A /8081A	Dieldrin	0.25	5
3510C or 3545A /8081A	Endosulfan I	0.25	5
3510C or 3545A /8081A	Endosulfan II	0.25	5
3510C or 3545A /8081A	Endosulfan sulfate	0.25	5
3510C or 3545A /8081A	Endrin	0.25	5
3510C or 3545A /8081A	Endrin aldehyde	0.25	5
3510C or 3545A /8081A	Heptachlor	0.25	5
3510C or 3545A /8081A	Heptachlor Epoxide	0.25	5
3510C or 3545A /8081A	Methoxychlor	0.25	5
3510C or 3545A /8081A	Toxaphene	2.5	50
3510C or 3545A /8151A	2,4-D	1	20
3510C or 3545A /8151A	2,4,5-T	0.1	2
3510C or 3545A /8151A	2,4,5-TP (Silvex)	0.1	102
3510C or 3545A /8151A	Pentachlorophenol	0.1	NA
3510C or 3545A /8082	Polychlorinated Biphenyls (PCBs)[2]	0.5	10
1311/3510C/8081A	TCLP Pesticides[3]	1	NA
1311/3510C/8151A	TCLP Herbicides[3]	1	NA

1. Most Recent revision of *EPA SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, or Methods for the Determination of Organic Compounds in Drinking Water (EPA/600/4-88-039)*.
2. Quantitated as total PCBs.
3. PQL is for each pesticide or herbicide listed in 40 CFR Part 261 Subpart C (261.24).

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APPENDIX 4: PARAMETER LISTS AND SAMPLE QUANTITATION LIMITS

Volatile Organic Compounds (VOCs)			
Methods[1]	Parameter	Water PQL ug/L	Soil PQL ug/Kg
5030B or 5035/8260B	1,1,1,2-Tetrachloroethane	1	5
5030B or 5035/8260B	1,1,1-Trichloroethane	1	5
5030B or 5035/8260B	1,1,2,2-Tetrachloroethane	1	5
5030B or 5035/8260B	1,1,2-Trichloroethane	1	5
5030B or 5035/8260B	1,1-Dichloroethane	1	5
5030B or 5035/8260B	1,1-Dichloroethene	1	5
5030B or 5035/8260B	1,1-Dichloropropanone	2	10
5030B or 5035/8260B	1,1-Dichloropropene	1	5
5030B or 5035/8260B	1,2,3-Trichlorobenzene	5	25
5030B or 5035/8260B	1,2,3-Trichloropropane	1	5
5030B or 5035/8260B	1,2,4-Trichlorobenzene	5	25
5030B or 5035/8260B	1,2,4-Trimethylbenzene	1	5
5030B or 5035/8260B	1,2-Dibromoethane (EDB)	1	5
5030B or 5035/8260B	1,2-Dichlorobenzene	1	5
5030B or 5035/8260B	1,2-Dichloroethane	1	5
5030B or 5035/8260B	1,2-Dichloropropane	1	5
5030B or 5035/8260B	1,3,5-Trimethylbenzene	1	5
5030B or 5035/8260B	1,3-Dichlorobenzene	1	5
5030B or 5035/8260B	1,3-Dichloropropane	1	5
5030B or 5035/8260B	1,4-Dichlorobenzene	1	5
5030B or 5035/8260B	1.2-Dibromo-3-chloropropane	1	5
5030B or 5035/8260B	1-Chorobutane	1	5
5030B or 5035/8260B	2,2-Dichloropropane	1	5
5030B or 5035/8260B	2-Butanone (MEK)	5	25
5030B or 5035/8260B	2-Chlorotoluene	1	5
5030B or 5035/8260B	2-Hexanone	2	10
5030B or 5035/8260B	2-Nitropropane	1	5
5030B or 5035/8260B	4-Chlorotoluene	1	5
5030B or 5035/8260B	4-Methyl-2-pentanone(MIBK)	1	5
5030B or 5035/8260B	Acetone	20	100
5030B or 5035/8260B	Acrylonitrile	2	10
5030B or 5035/8260B	Allyl Chloride	1	5
5030B or 5035/8260B	Benzene	1	5
5030B or 5035/8260B	Bromobenzene	1	5
5030B or 5035/8260B	Bromochloromethane	1	5
5030B or 5035/8260B	Bromodichloromethane	1	5
5030B or 5035/8260B	Bromoform	1	5
5030B or 5035/8260B	Bromomethane	5	25
5030B or 5035/8260B	Carbon disulfide	1	5
5030B or 5035/8260B	Carbon Tetrachloride	1	5
5030B or 5035/8260B	Chloroacetonitrile	25	125
5030B or 5035/8260B	Chlorobenzene	1	5
5030B or 5035/8260B	Chloroethane[2]	5	25
5030B or 5035/8260B	Chloroform	1	5
5030B or 5035/8260B	Chloromethane[2]	25	125
5030B or 5035/8260B	cis-1,2-dichloroethene	1	5
5030B or 5035/8260B	cis-1,3-Dichloropropene	1	5
5030B or 5035/8260B	Dibromochloromethane	1	5
5030B or 5035/8260B	Dibromomethane	1	5
5030B or 5035/8260B	Dichlorodifluoromethane	1	5
5030B or 5035/8260B	Diethyl ether	20	100
5030B or 5035/8260B	Ethylbenzene	1	5

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APPENDIX 4: PARAMETER LISTS AND SAMPLE QUANTITATION LIMITS

Volatile Organic Compounds (VOCs)			
Methods[1]	Parameter	Water PQL ug/L	Soil PQL ug/Kg
5030B or 5035/8260B	Ethylmethacrylate	1	5
5030B or 5035/8260B	Hexachlorobutadiene	2	10
5030B or 5035/8260B	Hexachloroethane	1	5
5030B or 5035/8260B	Iodomethane	5	25
5030B or 5035/8260B	Isopropylbenzene	1	5
5030B or 5035/8260B	m,p-xylene	1	5
5030B or 5035/8260B	Methacrylonitrile	1	5
5030B or 5035/8260B	Methyl Acrylate	10	50
5030B or 5035/8260B	Methylene chloride[2]	20	100
5030B or 5035/8260B	Methylmethacrylate	1	5
5030B or 5035/8260B	Methyl-t-butyl ether	1	5
5030B or 5035/8260B	Naphthalene	5	25
5030B or 5035/8260B	n-Butylbenzene	1	5
5030B or 5035/8260B	Nitrobenzene	10	50
5030B or 5035/8260B	n-Propylbenzene	1	5
5030B or 5035/8260B	o-Xylene	1	5
5030B or 5035/8260B	Pentachloroethane	1	5
5030B or 5035/8260B	p-isopropyltoluene	1	5
5030B or 5035/8260B	Propionitrile	20	100
5030B or 5035/8260B	sec-Butylbenzene	1	5
5030B or 5035/8260B	Styrene	1	5
5030B or 5035/8260B	Tert-Butylbenzene	2	10
5030B or 5035/8260B	Tetrachloroethene	1	5
5030B or 5035/8260B	Tetrahydrofuran	5	25
5030B or 5035/8260B	Toluene	1	5
5030B or 5035/8260B	Total Xylenes	2	10
5030B or 5035/8260B	Trans-1,2-Dichloroethene	1	5
5030B or 5035/8260B	Trans-1,3-Dichloropropene	1	5
5030B or 5035/8260B	Trans-1,4-Dichloro-2-butene	1	5
5030B or 5035/8260B	Trichloroethene	1	5
5030B or 5035/8260B	Trichloroflouromethane	5	25
5030B or 5035/8260B	Vinyl Chloride	1	5
1311/5030B/8260B	TCLP VOCs[3]	40	NA

1. Most Recent revision of *EPA SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods.*
2. A lower PQL may be needed for this analyte on specific projects covered by this QAPP. Arrangements will be made with ESP CAS in advance of sampling to take special analytical precautions or to subcontract analysis.
3. PQL listed is for each volatile organic compound listed in 40 CFR Part 261 Subpart C (261.24).

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APPENDIX 4: PARAMETER LISTS AND SAMPLE QUANTIATION LIMITS

Semivolatile Organic Compounds (SVOCs)			
Methods[1]	Parameter	Water PQL ug/L	Soil PQL ug/Kg
3510C or 3545A /8270C	1,2,4-Trichlorobenzene	5	100
3510C or 3545A /8270C	1,2-Dichlorobenzene	5	100
3510C or 3545A /8270C	1,3-Dichlorobenzene	5	100
3510C or 3545A /8270C	1,4-Dichlorobenzene	5	100
3510C or 3545A /8270C	2,4,5-Trichlorophenol	10	100
3510C or 3545A /8270C	2,4,6-Trichlorophenol	510	200
3510C or 3545A /8270C	2,4-Dichlorophenol	5	100
3510C or 3545A /8270C	2,4-Dimethylphenol	5	100
3510C or 3545A /8270C	2,4-Dinitrophenol	50	1000
3510C or 3545A /8270C	2,4-Dinitrotoluene	520	400
3510C or 3545A /8270C	2,6-Dinitrotoluene	5	100
3510C or 3545A /8270C	2-Chloronaphthalene	10	200
3510C or 3545A /8270C	2-Chlorophenol	5	400
3510C or 3545A /8270C	2-Methyl-4,6-dinitrophenol	10	400
3510C or 3545A /8270C	2-Methylnaphthalene	5	100
3510C or 3545A /8270C	2-Methylphenol	5	100
3510C or 3545A /8270C	2-Nitroaniline	5	100
3510C or 3545A /8270C	2-Nitrophenol	5	100
3510C or 3545A /8270C	3,3'-Dichlorobenzidine	5	100
3510C or 3545A /8270C	3-Nitroaniline	5	100
3510C or 3545A /8270C	4-Bromophenyl phenyl ether	5	100
3510C or 3545A /8270C	4-Chloro-3-methylphenol	5	100
3510C or 3545A /8270C	4-Chloroaniline	5	400
3510C or 3545A /8270C	4-Chlorophenyl phenyl ether	10	200
3510C or 3545A /8270C	4-Methylphenol	10	200
3510C or 3545A /8270C	4-Nitroaniline	5	100
3510C or 3545A /8270C	4-Nitrophenol	50	1000
3510C or 3545A /8270C	Acenaphthene	5	100
3510C or 3545A /8270C	Acenaphthylene	5	100
3510C or 3545A /8270C	Anthracene	5	100
3510C or 3545A /8270C	Azobenzene	5	100
3510C or 3545A /8270C	Benzo(a)anthracene	5	100
3510C or 3545A /8270C	Benzo(a)pyrene	5	100
3510C or 3545A /8270C	Benzo(b)fluoranthene	5	100
3510C or 3545A /8270C	Benzo(ghi)perylene	5	100
3510C or 3545A /8270C	Benzo(k)fluoranthene	5	100
3510C or 3545A /8270C	Benzoic Acid	10	200
3510C or 3545A /8270C	bis(2-Chloroethoxy)methane	20	400
3510C or 3545A /8270C	bis(2-Chloroethyl)ether	5	100
3510C or 3545A /8270C	bis(2-chloroisopropyl)ether	5	100
3510C or 3545A /8270C	bis(2-Ethylhexyl) phthalate	10	200
3510C or 3545A /8270C	Butyl benzyl phthalate	10	200
3510C or 3545A /8270C	Chrysene	5	100
3510C or 3545A /8270C	Dibenzo(a,h)anthracene	5	100
3510C or 3545A /8270C	Dibenzofuran	5	100
3510C or 3545A /8270C	Diethyl phthalate	20	400
3510C or 3545A /8270C	Dimethyl phthalate	5	100
3510C or 3545A /8270C	Di-n-butyl phthalate	5	100
3510C or 3545A /8270C	Di-n-octyl phthalate	5	100
3510C or 3545A /8270C	Fluoranthene	5	100
3510C or 3545A /8270C	Fluorene	5	100
3510C or 3545A /8270C	Hexachlorobenzene	5	100

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ESP-CAS-2520	Total Nitrogen – Autoclave Method
ESP-CAS-2600	Analysis of Base Neutral and Acid Extractable Semi-Volatile Organics by GC/MS
ESP-CAS-2605	Petroleum Fractions –OA2
ESP-CAS-2610	Analysis of Drinking Water for Carbamate Pesticides
ESP-CAS-2620	Analysis of Drinking Water for Haloacteic Acids
ESP-CAS-2625	Analysis of PCBs on Swabs
ESP-CAS-2630	Analysis of PCBs in Water
ESP-CAS-2632	Analysis of PCBs in Soil by Heated Pressurized Soxhlet Extraction
ESP-CAS-2650	Analysis of Pesticides in Water/Wastewater
ESP-CAS-2655	Analysis of Drinking Water by Method 525.2
ESP-CAS-2657	Analysis of Drinking Water by Methods 507/508
ESP-CAS-2660	Analysis of Samples for Total Petroleum by Fingerprint Analysis
ESP-CAS-2665	Analysis of Petroleum Tank Samples by MRBCA Method
ESP-CAS-2670	Analysis of Volatile Organics by GC/MS
ESP-CAS-2680	Analysis of Volatile Organics in Drinking Water by GC/MS
ESP-CAS-2690	Analysis of Chlorinated Acid Herbicides in Drinking Water
ESP-CAS-2695	Analysis of Chlorinated Acid Herbicides in Soil/Sludge
ESP-CAS-2696	Analysis of Chlorinated Acid Herbicides in Non-Potable Water
ESP-CAS-2700	Flashpoint
ESP-CAS-2710	Oil and Grease (O&G)
ESP-CAS-2720	TCLP Extraction for Volatile Organics
ESP-CAS-2750	TO-15
ESP-CAS-2755	Analysis of Organochlorine Pesticides in Non-Drinking Water, Soil and Organic Samples
ESP-CAS-2760	Analysis of Organophosphorus Pesticides in Non-Drinking Water, Soil and Organic Samples

Hazardous Waste Program

Superfund Section, Site Assessment Unit

MDNR-SAU-100	Writing Pre-CERCLIS Site Screening Reports
MDNR-SAU-101*	Writing Site Assessment Reports
MDNR-SAU-102*	Formatting Site Assessment Reports
MDNR-SAU-103*	Creating Site Maps
MDNR-SAU-104	Creating an Analytical Data Table
MDNR-SAU-107*	Obtaining Information for Site Assessment Investigations
MDNR-SAU-200*	Completing the Desk Top Review Form
MDNR-SAU-201*	Completing the Pre-CERCLIS Site Initiation Form
MDNR-SAU-202	Completing the Pre-CERCLIS Site Screening Form

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MDNR-SAU-203*	Completing Preliminary Assessment Scoresheets
MDNR-SAU-204*	Completing Site Investigation Scoresheets
MDNR-SAU-205*	Completing the Removal Site Evaluation Form
MDNR-SAU-300*	Operation of Trimble GPS Receiver
MDNR-SAU-301*	Operation of Portable X-Ray Fluorescence (XRF) Analyzers
MDNR-SAU-302	Operating Digital Cameras
MDNR-SAU-303*	Operating 35mm Cameras
MDNR-SAU-400*	Documenting Field Notes
MDNR-SAU-401	Naming Sites
MDNR-SAU-402*	Entry of Site Data into the Site Management and Reporting System Database (SMARS)
MDNR-SAU-403*	Filing Procedures
MDNR-SAU-404*	Electronic File Management
MDNR-SAU-405*	Requesting an Missouri Department of Conservation (MDC) Ecological Review

* SOP is planned, but has not been written as of the date of this QAPP revision.

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APPENDIX 6: LABORATORY ANALYTICAL DATA QUALIFIERS

Data Qualifier	Explanation
1	Improper collection method
2	Improper preservation
3	Exceeded holding time
4	Estimated value; detected below the PQL
5	Estimated value; quality control data outside limits
6	Estimated value; analyte outside calibration range
7	Analyte present in blank at > ½ reported value
8	Sample was diluted during analysis
9	Laboratory error
10	Estimated value; matrix interference
12	Insufficient sample quantity
13	Estimated value; true result is > reported value
14	Estimated value; non-homogenous sample
15	No result; failed quality control requirements
16	Not analyzed – related analyte not detected
17	Results in dry weight
18	Sample pH is outside the acceptable range
19	Estimated Value
20	Not analyzed – Instrument failure
21	No result – spectral interference
22	pH was performed at the Laboratory
23	Contract Lab specific qualifier – see sample comments
24	No result – matrix interference
25	No Result: Excessive Chlorination
26	No Result: Excessive Dechlorination
ND	Not detected at reported value

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APPENDIX 7: ACRONYM LISTING

DEQ	Division of Environmental Quality
CAS	Chemical Analysis Section
CERCLA	Comprehensive Environmental Response Compensation & Liability Act
COC	Chain of Custody
DGLS	Division of Geology and Land Survey
DQA	Data Quality Assessment
DQO	Data Quality Objective
EPA	Environmental Protection Agency
ES	Environmental Specialist
ESP	Environmental Services Program
FSU	Field Services Unit
FTE	Full Time Employee
GC/MS	Gas Chromatrography/Mass Spectrometry
GIS	Geographic Information System
GPS	Global Positioning System
DGLS	Division of Geology and Land Survey
HAZWOPER	Hazardous Waste Operations and Emergency Response
HWP	Hazardous Waste Program
LIMS	Laboratory Information Management System"
LMAD	Locational Data Method Accuracy Description
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MDL	Method Detection Limit
MDNR	Missouri Department of Natural Resources
MIP	Membrane Interface Probe
MRBCA	Missouri Risk Based Corrective Action
MS/MSD	Matrix Spike/Matrix Spike Duplicate
NELAC	National Environmental Laboratory Accreditation Conference
NIST	National Institute of Standards and Technology
NPL	National Priorities List
NRD	Natural Resources Damages
NRDA	Natural Resources Damages Assessment
PAH	Polynuclear Aromatic Hydrocarbons
PQL	Practical Quantitation Limit
PQL	Sample Quantitation Limit
PRG	Preliminary Remediation Goals
PRP	Potentially Responsible Party
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
QMP	Quality Management Plan
RCRA	Resource Conservation Recovery Act
RFP	Request for Proposal
RPD	Relative Percent Difference
RRF	Relative Response Factor
SARA	Superfund Reauthorization Act
SCDM	Superfund Chemical Data Matrix
SOP	Standard Operating Procedure
SVOC	Semi-Volatile Organic Compound
TCLP	Toxicity Characteristic Leaching Procedure

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APPENDIX 7: ACRONYM LISTING

VOA	Volatile Organic Analysis
VOC	Volatile Organic Compound
WQS	Water Quality Standards
XRF	X-Ray Fluorescence

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APPENDIX H

ACUC Form

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Attachment 1: ACUC Long Form – New Studies & Triennial Review

Instructions: The Long Form is for new proposed studies and triennial review of on-going studies. Using Microsoft Word or similar word processing software, please complete the following form by typing your responses into the provided answer boxes. Type as much information as needed into the box, as the box will expand to accommodate your answers. This form must accompany the final project plan when sent for approval by the appropriate Research Manager and Center Director.

Study Plan Title:	Sampling and Analysis Plan for the Southeast Missouri Lead Mining District Small Mammal Study
Principal Investigator:	Eric Gramlich , Missouri Department of Natural Resources Local Contacts: W. Nelson Beyer, Barnett A. Rattner
Research Manager:	John B. French

Question:	**Answer:**
1. Will vertebrate animals be used in this project? Mark Yes or No.	__X__ Yes, continue at # 2 ____ No, proceed to Signature
2. Does the project only involve the use of free-living wild animals in their natural habitat without disturbing or handling them? Mark Yes or No.	____ Yes, proceed to Signature __X__ No, continue at # 3
3. Will live animals be used in this project? Mark Yes or No.	__X__ Yes, continue at # 3.1 ____ No, proceed to #4
3.1. Species, sex and number to be used:	Short-tailed shrew (*Blarina brevicauda*) and least shrew (*Cryptotis parva*), Deer mouse (*Peromyscus maniculatus*) and white-footed mouse (*Peromyscus leucopus*), meadow vole (*Microtus pennsylvanicus*), prairie vole (*Microtus orhrogaster*), woodland vole (*Microtus pinetorum*); adult males and females, N=120 total animals.
3.2. Method of identification (bands, transmitters, etc.):	None
3.3. Potential suppliers or source of animals:	Live-captured in Missouri
3.4. Method of trapping and bait used:	Sherman live traps (baited with rolled

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	oats and peanut butter) , pitfall traps
3.5. Will live animals be housed in Center facilities? Mark Yes or No.	____ Yes, continue at #3.5.1 __X__ No, proceed to #4
3.5.1. Expected arrival date	
3.5.2. Identify quarantine space that has been schedule with the Attending Veterinarian:	
3.5.3. Where will animals be held for project?	
3.5.4. Number per cage:	
3.5.5. Cage size:	
3.5.6. Type, source and storage of food:	
3.5.7. Frequency of waste removal:	
3.5.8. Duration of project:	2 weeks
3.5.9. Name(s) and positions of animal caretakers	
4. Will animals be caught, trapped, handled or experimentally manipulated or treated in this project? Mark Yes or No.	__X__ Yes, continue at # 4.1 ____ No, proceed to #5
4.1. What types and frequency of handling or manipulation are required in this project?	Small smalls will be trapped, transported to a necropsy facility, deeply anesthetized (Plane 3 of anesthesia), bled, and euthanized
4.2. Are substances being administered to the animals? Mark Yes or No.	__X__ Yes, continue at # 4.2.1 ____ No, proceed to #4.3
4.2.1. If yes, what materials and methods of administration will be used?	Carbon dioxide
4.3. Is surgery or other invasive procedure required? Mark Yes or No.	__X__ Yes, continue at # 4.3.1 ____ No, proceed to #4.4
4.3.1. Describe surgery or other invasive procedure:	Animal will be carefully restrained in a gloved hand, anesthetized to plane 3 using carbon dioxide delivered via a tube attached to a small funnel covering the face of the subject. The axillary plexus will be cut with a scalpel and the animal exsanguinated, and blood for hematocrit and biochemical assays will be collected in capillary tubes. The animal will then be necropsied, portions of vital organs fixed

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	in phosphate buffered formalin for histopathology, and portions of the liver and kidney collected for metals analysis.
4.3.2. Will the procedure cause more than momentary or slight pain or distress to the animal(s)? Mark Yes or No.	__X__ Yes, continue at # 4.3.3 ____ No, proceed to #4.4
4.3.3. If yes to 4.3.2, federal regulations require that you search for alternative. Provide a written narrative description (in space provided above Signature Line) of sources used to determine that less painful alternatives are not available. To fulfill this requirement, bibliographical searches may be performed though the Animal Welfare Information Center (AWIC) of the National Agriculture Library (http:www.nalusda.gov/awic/).	
4.4. In some cases, treatments or procedures have unintended or unexpected adverse effects on animals and procedures must be terminated. How will end points for procedures (e.g., death, sickness or other signs in experimental animals) be defined?	Non-target and immature small animals will be released when traps are checked. Any moribund animals will be sacrificed by cervical dislocation in the field. Only adult animals will be collected and transported or blood and tissue collection.
4.5. How will live animals be disposed of at the end of the study?	__X__ Euthanasia, continue at #4.5.1. ____ Other (explain), proceed to #6:
4.5.1. Justification for euthanasia	Chemical analyses for exposure and adverse effects of metals as part of a Natural Resource Damage Assessment for the Department of the Interior and the State of Missouri.
4.5.2. Method of euthanasia	Carbon dioxide
4.5.3. Is method considered a recommended method for the species by AVMA?	__X__ Yes, proceed to #5 ____ No, continue at #4.5.3.1
4.5.3.1.If not a recommended method by AVMA, provide justification for why recommended methods can not be used.	
5. How will dead animals or animal parts be disposed of?	Incineration
6. Describe the disposition of live animals at the end of the study. (If animals are to be released: where, when, and what permits are needed? If releases are to occur this year, show evidence that applications for	All targeted animals that are captured will be euthanized.

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permits have been made.)	

Question #4.3.3: Written narrative on alternatives (if required). Type in the box provided.
As per Act's policy on alternatives, the minimal written narrative should include: the databases searched or other sources consulted, the date of the search and the years covered by the search, and the key words and/or search strategy used by the PI when considering alternatives or descriptions of other methods and sources used to determine that no alternatives were available to the painful or distressful procedure. The narrative should be such that the ACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough.

A substantial volume of blood (300-500 ul/animal) and numerous tissues are needed for histopathological and chemical analyses to assess exposure and damage to free ranging populations of small mammals from several locations. There are no alternative methods that can circumvent sacrificing of the small mammals to meet the objectives of this study. This is based upon our experience (B. Rattner serves on the Federal Interagency Testing Committee for Alternative Methods) and knowledge of the literature and litigation process related to damage assessments. The method of sample collection and euthanasia method was reviewed and is believed to be in compliance with AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

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I certify that the above responses are a true and accurate reporting of the animal activities proposed/conducted. I certify that this project plan follows the Animal Welfare Act and applicable guidelines for the care and use of animals.

Signature of Principal Investigator

9/9/13
Date