U.S. Army Corps of Engineers
Fort Worth District

Final Report

Bosque and Leon River Watersheds Study

February 2004

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US Army Corps of Engineers
Fort Worth District

Brazos River Authority

TIEHH
The Institute of Environmental and Human Health

MWH

Final Report

Bosque and Leon River Watersheds Study

February 2004

Prepared For:

U.S. Army Corps of Engineers
Ft. Worth District

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Executive Summary

Congress directed the U.S. Army Corps of Engineers (USACE) to evaluate the potential impact of perchlorate releases associated with the former Naval Weapons Industrial Reserve Plant in McGregor, Texas (NWIRP McGregor) due to concern that these releases may have impacted water quality and/or the environment in the adjacent Bosque and Leon River watersheds. These watersheds flow into Lake Belton and Lake Waco, which serve as the water supply sources for approximately 500,000 people in the surrounding communities. The primary goal of the USACE and the multi-disciplinary project team they assembled was to evaluate potential human and environmental exposures to perchlorate in the Lake Waco and Lake Belton study area (Figure 2-2). Perchlorate is a contaminant of concern because it interferes with iodide uptake in the thyroid gland. This study was restricted to perchlorate contamination outside of the NWIRP property boundary; it was not intended to characterize perchlorate contamination within the NWIRP boundary, nor was it intended to address contaminants other than perchlorate. This report presents the results of the team's study.

The project team performed a number of investigations to meet the study goal, including extensive sampling and analysis for perchlorate in stream and lake water, stream and lake sediment pore water, and plants and animals from the study area. Additional laboratory and modeling studies provided valuable information on the nature of perchlorate uptake in plants and animals and on the ability of perchlorate to be transformed into less harmful species by natural processes. The project team also collected data to better characterize the surface hydrology and hydrogeological characteristics of the Bosque and Leon River watersheds, as these factors influence how perchlorate moves through the environment. The results from these studies are discussed in detail in Chapter 5.

Based on all of the investigations completed during this study, as well as evidence that the current and historical remediation activities conducted by the U.S. Navy are having a significant and positive effect, the project team concluded that the 500,000 public water supply users in the communities surrounding Lake Belton and Lake Waco are not at risk of exposure to perchlorate from this source. However, certain NWIRP vicinity residents and recreational users of the area could potentially be exposed to perchlorate, as discussed below. An overview map of perchlorate detections in the study area is included on Plate 6. Major findings from this study are summarized below:

<u>Potential for Human Exposure:</u>

- Based on data collected during this study, public water supply use and consumption of beef from cattle raised in the area appear to present no risk of exposure to perchlorate.
- The potential for exposure to perchlorate exists for certain NWIRP vicinity residents who consume vegetation from gardens irrigated with perchlorate-contaminated spring or stream water in the area. An exposure potential also exists for people who consume water from impacted wells or streams or consume wild vegetation collected near impacted streams. Exposure via incidental ingestion during swimming or wading could potentially occur in the watersheds where perchlorate is present at detectable concentrations.

- People who consume fish caught in these watersheds, including Lake Belton and Lake Waco, could potentially be exposed to perchlorate in fish fillets, although the risk of this exposure is thought to be low.

Potential for Ecological Exposure:

- Aquatic and terrestrial plants in the study area do take up perchlorate from streams or groundwater with detectable perchlorate concentrations. Perchlorate is released back into the environment from trees when they lose their leaves.
- Animals in affected areas can be exposed to perchlorate either directly from drinking contaminated stream water or indirectly by consuming plants that have been exposed to perchlorate. Laboratory studies indicate the highest potential exposure levels near NWIRP may be sufficient to cause thyroid histopathological impacts in small mammals. The effects of this exposure on populations of small mammals is unclear. Perchlorate levels in streams are not high enough to affect frog metamorphosis.
- Perchlorate detections in fish were sporadic, occurring in only some fish caught at each location. Unlike other animals, however, some fish with detectable perchlorate concentrations were found even in areas with no detectable concentrations in the water, including Lake Belton and Lake Waco.

Perchlorate Occurrence and Fate and Transport within the Watersheds:

- Concentrations of perchlorate emanating from the NWIRP site in groundwater and surface streams are significantly diluted before reaching either Lake Waco or Lake Belton. No perchlorate was detected during extensive sampling in either the Middle Bosque River or the Leon River, the main rivers flowing into these lakes that receive surface runoff from NWIRP. Likewise, all water and sediment pore water sampling conducted in the lake delta areas, sampling at water intakes, and water sampling along transects throughout Lake Belton showed no detectable concentrations of perchlorate during this study (method detection limits of 1 μg/L).
- Detections of perchlorate in the streams adjacent to NWIRP were sporadic and varied greatly. Even the highest concentrations detected in these relatively low-discharge streams during this study were well below the estimated levels necessary to ultimately cause a concentration at the reporting limit (4 μg/L) in the lakes.
- Based on bench-scale studies, bacteria in the anoxic region of Lake Belton have a demonstrated ability to break down perchlorate to a harmless chemical species.
- Sampling at Cowhouse Creek, which flows into Lake Belton from the Fort Hood area, showed no detectable perchlorate concentrations (method detection limit of 1 μg/L). Based on this limited sampling, surface runoff from Fort Hood is not thought to contribute perchlorate to Lake Belton.

Findings presented on potential exposures to perchlorate are dependent on continued remediation efforts by the U.S. Navy. Chapter 8 includes a more comprehensive list of study findings. All of these findings are supported by study data and discussions included in this report in Chapters 5, 6, and 7.

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Acronyms and Abbreviations

AAES	Anion Atlas Electrolytic Suppressor
ADCP	Acoustic Doppler Current Profile
AF	Acre-feet
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
BCF	Bioconcentration Factor
BRA	Brazos River Authority
cfs	Cubic feet per second
Cl^-	Chlorine
ClO_4^-	Perchlorate Ion
cm	Centimeter
CSM	Conceptual Site Model
DO	Dissolved Oxygen
DQOs	Data Quality Objectives
fbgs	feet below ground surface
FGDC	Federal Geographic Data Committee
FETAX	Frog Embryo Teratogenesis Assay: *Xenopus*
fmsl	feet above mean sea level
GIS	Geographic Information System
gpd/ft	gallons per day per foot
GPS	Global Positioning System
GW	Groundwater
$HCLO_4$	Perchloric acid
IC-50	Inhibitory Concentration in 50% of test subjects
IDW	Investigation Derived Waste
ISM	Interim Stabilization Measures
K	Hydraulic conductivity
K^+	Potassium
LHAAP	Longhorn Army Ammunition Plant
MS/MSD	Matrix Spike/Matrix Spike Duplicate
MDL	Method Detection Limit
mg/kg-day	Milligrams per kilogram per day
mm	Millimeter
m/s	Meters per Second
m^3/s	Cubic Meters per Second
MSDS	Materials Safety Data Sheets
MW	Montgomery Watson
MWH	Montgomery Watson Harza
Na^+	Sodium
NH_4^+	Ammonia
ng/g	Nanograms per gram (equivalent to ppb)
NIS	Sodium (Na+)-Iodide (I-) Symporter
NWIRP	Navel Weapons Industrial Reserve Plant
NRCS	Natural Resources Conservation Service

O	Oxygen
PBPK	Physiologically-Based Pharmacokinetic
ppb	Parts per billion (equivalent to µg/L)
ppm	Parts per million
PQL	Practical Quantitation Limit
PRBs	Permeable Reactive Barriers
psig	Pounds per square inch gage
QAPP	Quality Assurance Project Plan
RAGS	Risk Assessment Guidance for Superfund
RCRA	Resource Conservation and Recovery Act
RL	Reporting Limit
RRR	Risk Reduction Rule
SAV	Secondary Acute Value
SCV	Secondary Chronic Value
SPW	Sediment Pore Water
SPE	Solid Phase Extraction
T_3	Thyroxine (hormone controlled by the thyroid, pituitary gland and hypothalamus)
T_4	Triiodothyronine (hormone controlled by the thyroid, pituitary gland and hypothalamus)
TCEQ	Texas Commission on Environmental Quality
TDS	Total dissolved solids
TIAER	Texas Institute for Applied Environmental Research
TIEHH	The Institute of Environmental and Human Health
TNRCC	Texas Natural Resource Conservation Commission
TOC	top of casing
TPP	Technical project planning
TPWD	Texas Parks and Wildlife Department
TTU	Texas Tech University
TSSWCB	Texas State Soil and Water Conservation Board
TVS	Total Volatile Solids
TWDB	Texas Water Development Board
USACE	United States Army Corps of Engineers
USAF	United States Air Force
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UTM	Universal Transverse Mercator
WWTP	Wastewater Treatment Plant
µg/L	micrograms per liter (equivalent to ppb)

1.0 INTRODUCTION

Congress directed the U.S. Army Corps of Engineers (USACE) to evaluate the potential impact of perchlorate releases associated with the former Naval Weapons Industrial Reserve Plant in McGregor, Texas (NWIRP McGregor). Congress was concerned that the NWIRP McGregor plant may have impacted water quality and/or the environment in the adjacent Bosque River and Leon River watersheds. These watersheds flow into Lake Belton and Lake Waco, two important regional sources of drinking water. The USACE assembled a multi-disciplinary project team to study and report on this issue. This report presents the results of the team's study.

1.1 STUDY HISTORY AND BACKGROUND

The primary source of perchlorate contamination within the Bosque and Leon River watersheds is believed to be a result of former activities at NWIRP McGregor. A brief historical summary of this facility is presented in Section 1.1.1. The goals and activities of the USACE and its project team in evaluating perchlorate releases to the Bosque and Leon River watersheds are described in Section 1.1.2.

1.1.1 NWIRP McGregor History

In 1942, the U.S. Army Ordnance Corps established the Bluebonnet Ordnance Plant on 18,000 acres of land in McGregor, Texas. During this tenure, it included facilities run by National Gypsum of Buffalo, New York, to load explosives into bomb bodies during World War II. Production at this facility ceased at the conclusion of the war and the War Department formally closed the facility in 1946.

After World War II, changes in ownership of the property occurred often and included sales to private parties and to Texas A&M University. In 1952, the U.S. Air Force acquired 11,450 acres of the original 18,000 acres and named the area U.S. Air Force Plant 66. Phillips Petroleum Company oversaw the production of jet-assisted take-off boosters during this time period, until the U.S. Air Force expropriated the property to the U.S. Navy in 1966.

After the transfer from the U.S. Air Force, the U.S. Navy renamed the property the Naval Weapons Industrial Reserve Plant, McGregor, Texas. In 1972, 70 acres were transferred to the McGregor School District, 33 acres surrounding the wastewater treatment plant were transferred to the City of McGregor, and 1,600 acres were sold to private parties. Currently, several thousand acres are leased for agriculture. From 1978 to 1995, the Hercules Corporation produced solid propellant rocket motors at NWIRP McGregor for a variety of missiles. Production of these weapons ceased in 1995, when Hercules Corporation was purchased by Alliant Techsystems, Inc.

In 1998, The City of Waco collected 10 samples from selected surface water locations outside the NWIRP McGregor facility that indicated the presence of perchlorate,

presumably related to activities at the former plant. This presumption resulted from the fact that a major component of the solid propellant of missiles and rockets manufactured at NWIRP McGregor was ammonium perchlorate. Throughout the remainder of this document "perchlorate" will be used to indicate the perchlorate ion, (ClO_4^-).

In 1999, the U.S. Navy collected additional samples from monitoring wells, springs, and Lake Belton. The U.S. Navy detected perchlorate at a total of 19 sites with concentrations in these samples ranging from approximately 2 to 3,300 parts per billion (ppb) (EnSafe, 1999a). The U.S. Navy also detected perchlorate in an isolated sample from Lake Waco in 2000. However, this detection is thought to be an anomaly that cannot be reproduced and does not appear to be representative of conditions in the lake (EnSafe, 1999a).

Investigation and remediation activities are ongoing at the site. As cleanup of NWIRP McGregor proceeds, the U.S. Navy is transferring portions of the property deemed safe for commercial/industrial redevelopment to the City of McGregor by Congressional order. As of September 2003, the U.S. Navy had transferred approximately 6,000 acres to the City of McGregor.

Historical use and summaries of the situation at former NWIRP McGregor are also included in the *Perchlorate Monitoring Plan* (Montgomery Watson, 1999) and *Community Relations Plan* (USACE, 2001a). A complete history of various investigation and remediation activities that have occurred at the former NWIRP McGregor plant is presented in detail in the *Draft-Final Groundwater Investigation Phase III Report* (EnSafe, 2003).

1.1.2 Study Overview

The USACE assembled an integrated, multi-disciplinary project team consisting of the USACE, Brazos River Authority (BRA), The Institute of Environmental and Human Health at Texas Tech University (TIEHH), Montgomery Watson Harza (MWH), the U.S. Environmental Protection Agency (USEPA), the Texas Commission on Environmental Quality (TCEQ), the City of Waco, and the City of Killeen. The primary goal of the USACE and the project team was to evaluate potential human and environmental exposures to perchlorate in the Lake Waco and Lake Belton study area. To meet this goal, the USACE performed a number of investigations, including watershed characterization and analysis to determine how perchlorate migrates through the environment and biological characterization to evaluate potential exposure to humans and the environment. This final report presents the results from all of these investigations.

1.2 STUDY GOAL & OBJECTIVES

1.2.1 Goal

The overall goal of the project team was to evaluate potential human and environmental exposure to perchlorate in the Lake Waco and Lake Belton study area. Perchlorate, because of its high mobility and persistence in the environment, has the potential to migrate far from its source, primarily via water, and to impact not only ecological

systems but also to pose a threat to local drinking water supplies. In the Lake Waco and Lake Belton study area, recognition of this threat resulted from the detection of perchlorate in soils, surface water, and groundwater within the boundaries of NWIRP and the documented migration of this perchlorate via its detection in off site streams, springs, and dug wells (EnSafe, 1999a). This study was not intended to assess certain sites and properties, and findings from this study should not be used for that purpose.

To achieve the study goal, the project team identified and evaluated all of the existing, relevant data, analyzed these data relative to the study objectives, and identified and prioritized the resulting data gaps. This initial phase of the study was documented in the *Final Conceptual Site Model* (MWH, 2002a). The project team then developed and implemented a comprehensive, 12-month field data collection program to fill the most critical data gaps. The team initiated parts of the field data collection program relating to some of the more obvious ecological data gaps prior to completing the conceptual site model. The field data collection efforts for these portions of the study lasted as long as 21 months. The results of this effort are documented in this report, which incorporates available information on sources of perchlorate contamination and release, the surface hydrology and hydrogeological characteristics of the Bosque and Leon River watersheds, the nature of perchlorate fate and transport, potential pathways of perchlorate migration within the study area, and the human receptors and environmental resources that may be exposed to perchlorate.

1.2.2 Objectives

The project team held an initial Technical Project Planning (TPP) Meeting on February 6-7, 2001. At the TPP meeting, existing study area information was discussed, the project scope was defined, and project objectives were outlined. The project objectives are summarized below.

- Develop an effective community relations plan
- Compile existing study area information
- Identify data gaps in existing information
- Define and develop the Conceptual Site Model (CSM)
- Develop Field Sampling Plans based on data gaps
- Implement the Field Investigation to fill the key data gaps
- Evaluate fate and transport pathways
- Evaluate potential environmental exposure to perchlorate in the study area
- Evaluate potential human exposure to perchlorate in the study area
- Assess exposure pathways to vegetation, fish, birds, and mammals
- Assess impact on amphibians, fish, and large and small mammals

A complete set of project documents may be viewed and downloaded at the following web-site: http://www.swf.usace.army.mil/ppmd/Perchlorate/index.html. These documents

include project plans, a bibliography of historical information, the *Final Conceptual Site Model* (MWH, 2002a), team meeting minutes, public meeting minutes, slide presentations, and project generated data.

1.3 REPORT ORGANIZATION

The information presented in the Final Report is organized as follows:

Section 1.0 Introduction. Describes the purpose and scope of the Final Report, and summarizes its organization

Section 2.0 Environmental Setting. Provides a summary of the physical characteristics of the study area.

Section 3.0 Investigation History. Summarizes previous and current investigations of former NWIRP McGregor and the Bosque and Leon River watersheds.

Section 4.0 Study Descriptions. Summarizes the various watershed, ecological, plant and animal investigations conducted as part of this study.

Section 5.0 Data. Presents the various water, sediment, plants and animal data collected as part of this study.

Section 6.0 Updated Hydrologic Conceptual Site Model. Updates the hydrologic conceptual site model originally presented in the *Final Conceptual Site Model* (MWH, 2002a) based on new information gathered.

Section 7.0 Exposure Assessment. Identifies the human receptors and ecological resources within the study area, and describes potential pathways of exposure to perchlorate.

Section 8.0 Conclusions. Summarizes major conclusions of this report.

Section 9.0 References. Lists the sources cited in this report.

2.0 ENVIRONMENTAL SETTING

The following section describes the regional environmental setting of the Bosque and Leon River watersheds study area including the former NWIRP McGregor site.

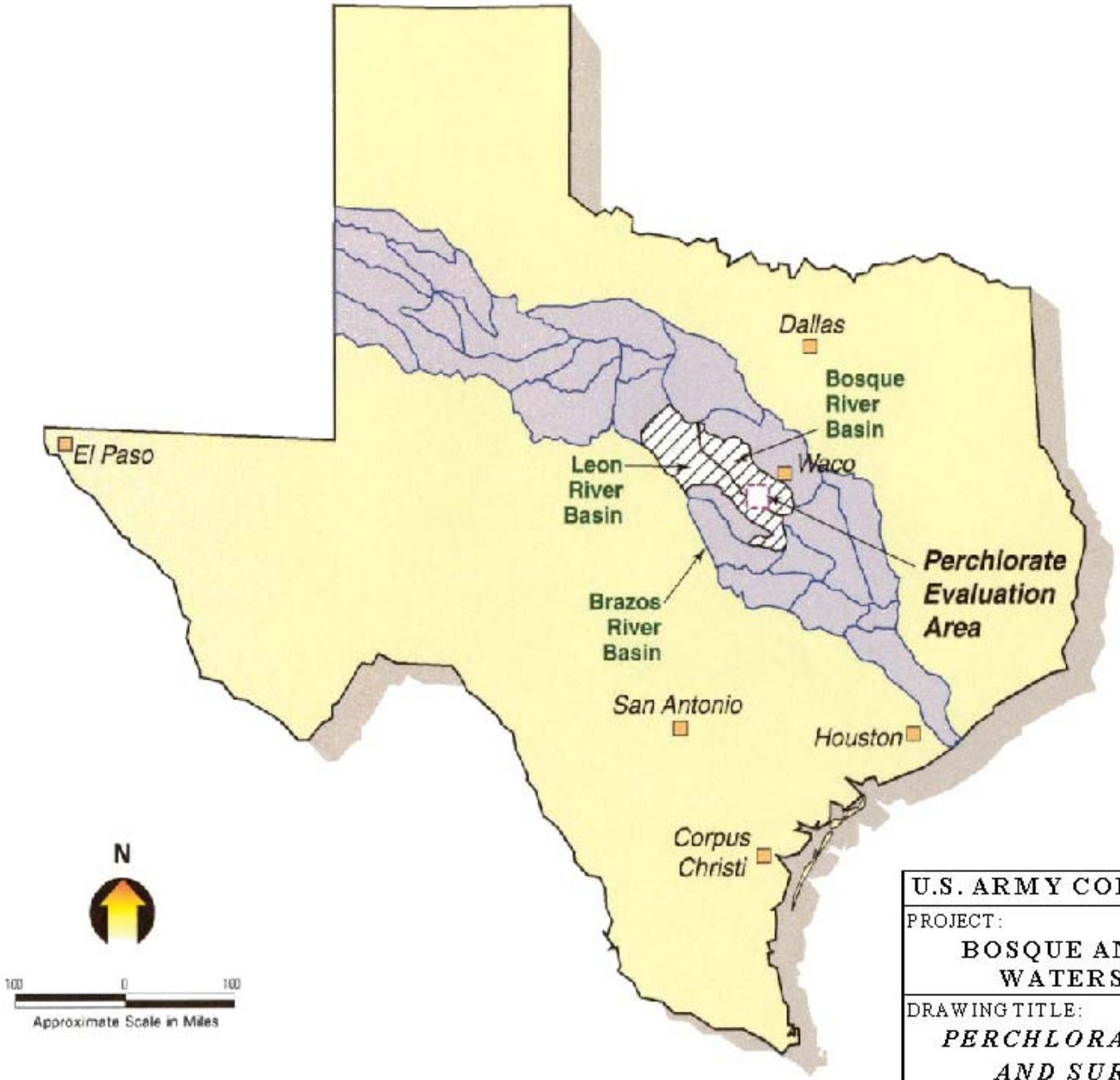
2.1 STUDY AREA LOCATION AND DESCRIPTION

The USACE perchlorate study area comprises portions of the Bosque River watershed and the Leon River watershed, two watersheds that supply surface water to Lake Waco and Lake Belton in Central Texas. This study was restricted to perchlorate contamination outside of the NWIRP property boundary. It was not intended to characterize perchlorate contamination within the NWIRP boundary. The NWIRP boundary was considered to be the boundary at the time this study began. The study area does not include former NWIRP areas that were transferred during the course of this study. **Figure 2-1** shows the overall study area location.

Lake Waco and Lake Belton serve as water supply sources for approximately 500,000 people in the surrounding communities including Waco, Killeen, Belton, and Temple (U.S. Census Bureau, 2001). Former NWIRP McGregor straddles the watershed boundary between Lake Waco and Lake Belton. Thus, stormwater runoff and groundwater flowing from the NWIRP McGregor site are components of these drinking water supplies. **Figure 2-2** shows the location of NWIRP, the study area, and Lakes Belton and Waco.

The city of Temple's intake structure downstream of the Lake Belton dam defines the southern boundary of the study area. The eastern boundary of the study area extends north from the city of Belton, located to the west of U.S. Interstate 35, and continues northeast along U.S. Interstate 35 to the dam at Lake Waco. The dam at Lake Waco defines the northern boundary of the study area. Finally, the western boundary of the study area extends northwest away from Lake Belton along the western edge of the Leon River. The study area does not include environmental investigations within the boundaries of NWIRP McGregor, as other parties are conducting these investigations. In addition to perchlorate contamination originating at NWIRP McGregor, this study also addresses potential perchlorate contamination originating at Fort Hood, which adjoins the western boundary of the study area. However, the USACE study was not intended to address or serve as an environmental investigation within the boundaries of Fort Hood.

NWIRP McGregor is located approximately 20 miles to the southwest of Waco, Texas, and formerly encompassed approximately 9,750 acres of generally flat-lying land at the eastern border of the Texas Hill Country. Based on information from the September 11, 2002 NWIRP Restoration Advisory Board meeting, approximately 6,000 acres have been transferred to the City of McGregor as part of the decommissioning of this facility. State Highway 317 runs along the eastern edge of NWIRP McGregor, and FM 2671 runs along much of its southern boundary. The town of McGregor, Texas is located at the northeast corner of NWIRP McGregor.



U.S. ARMY CORPS OF ENGINEERS

PROJECT:

BOSQUE AND LEON RIVER WATERSHEDS STUDY

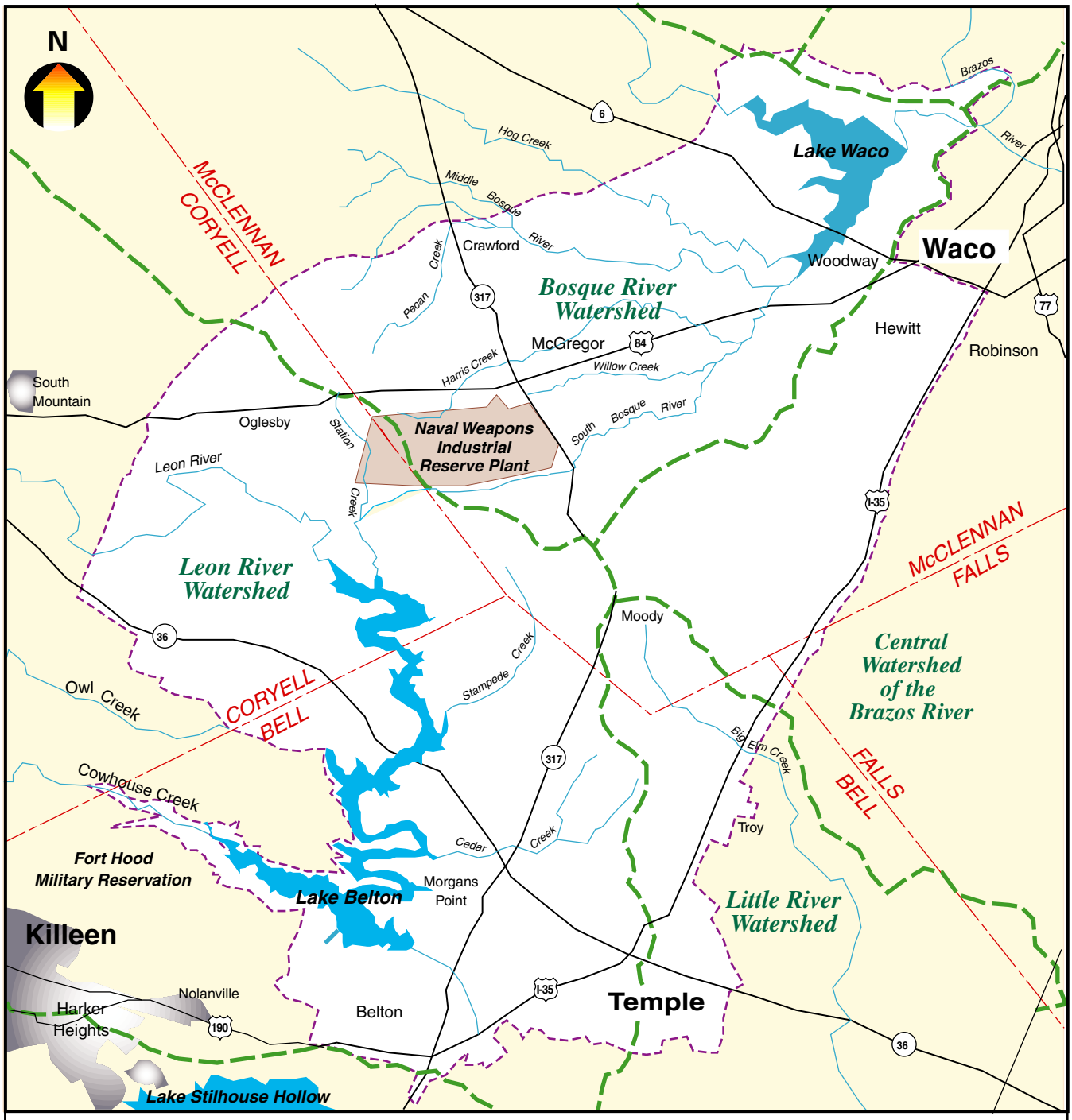
DRAWING TITLE:

PERCHLORATE STUDY AREA AND SURROUNDINGS

MWH MONTGOMERY WATSON HARZA

Sheet 1 of 1 Sheets

SCALE:	FIGURE:
Not to Scale	2-1



Watershed Boundaries

Study Area Boundary

5 0 5

Approximate Scale in Miles

U.S. Army Corps of Engineers

PROJECT:

BOSQUE AND LEON RIVER WATERSHEDS STUDY

DRAWING TITLE:

PERCHLORATE EVALUATION AREA

MWH
MONTGOMERY WATSON HARZA

Sheet 1 of 1 sheets

SCALE: Not to Scale

FIGURE: 2-2

2.2 REGIONAL TOPOGRAPHY

The study area includes portions of McLennan, Coryell and Bell counties and lies in the Washita Prairie, the easternmost part of the Grand Prairie of Texas. The study area is characterized by gently rolling hills and terrain comprised of limestone covered by shallow soil and open land vegetation. Creeks and rivers incise this surface, and cliffs and bluffs develop along these waterways (EnSafe, 1999a).

The land surface in the vicinity of NWIRP McGregor consists of sloping, gently rolling hills and plains underlain by the Georgetown Main Street Limestone. Portions of the facility slope toward the drainage tributaries of the South Bosque River, Harris Creek, and Station Creek (EnSafe, 1999a). Other small drainage ditches and various tributaries contribute to these larger streams. Small bluffs rise above some of the creeks and streams, particularly along tributaries of Harris Creek and the South Bosque River. Elevations at the base range from 840 feet above mean sea level in the northwest corner to 630 feet above mean sea level in the southeast corner of the NWIRP McGregor property. The majority of the property slopes to the southeast, except for the southwest corner where it slopes southwest towards Station Creek.

2.3 REGIONAL GEOLOGY

The Balcones Fault System, a north-northeast trending zone that closely parallels U.S. Interstate 35, is located along the eastern edge of the study area (Hartmann and Scranton, 1992). The system is located at the confluence of two major physiographic systems: the Gulf Coastal Plain and the North Central Plains. Cretaceous rock units dip to the southeast across the Balcones Fault System and into the Gulf Coast Basin. Beds strike northeast to southwest. The Balcones Fault Zone forms a regional boundary that is distinguished by a line of low hills that rise approximately 150 feet above the surrounding plain. However, the line of hills in the study area is erosional and is not directly the result of the faulting in the area (Dr. Joe Yelderman, personal communication, August 1999).

Two normal, northeast trending faults of the Balcones Fault System cross the Leon River Valley, both of which are about 2,000 feet downstream from the Lake Belton dam. Displacements on both faults are about 30 feet and fault blocks are downthrown to the east (USACE, 1998).

Former NWIRP McGregor is situated atop the McGregor High. The McGregor High is a structural feature in the subsurface that caused erosion and nondeposition during the early Cretaceous, but it is questionable whether this has anything to do with the current topographic divide that occurs on the surface in the study area (Dr. Joe Yelderman, personal communication, August 1999).

A thin veneer of soil occurring in two physiographic provinces immediately underlies the study area: Grand Prairie and Blackland Prairie. Soils in the Grand Prairie province form

over areas underlain by marl and limestone, whereas soils in the Blackland Prairie province overlie areas underlain by shale, marl, and chalk.

In general, the soil in the study area is not mature and frequently contains fragments of the limestone parent material. Soil thickness ranges from 0 to 6 feet below ground surface (fbgs) and average two feet in depth. Vertical hydraulic conductivities range from 0.06 to 0.20 inches per hour, except in the presence of vertical desiccation cracks, which presumably increase vertical hydraulic conductivity. Desiccation cracks are a result of high clay content and form during extended periods of dryness (USACE, 1998).

Beneath the soil lies a transgressive-regressive sequence of early to middle Cretaceous fractured limestone of the Washita and Fredericksburg Divisions. These divisions primarily consist of interbedded shale and limestone. The early Cretaceous Trinity Group, also consisting of interbedded shale and limestone, underlies these units (EnSafe, 1998a). In the vicinity of NWIRP McGregor, the Georgetown Formation of the Washita Division is exposed and consists of the following members from approximate 0-100 fbgs:

- Main Street Limestone;
- Pawpaw Shale;
- Weno Limestone; and
- Denton Marl (EnSafe, 1998a).

The Main Street Limestone is characterized as a fine to medium crystalline nodular limestone that has an average thickness of 35 feet. This unit is highly fractured and contains shallow groundwater. The lower portion of the Main Street Limestone was initially characterized as non-water bearing because it is less weathered and contains fewer fractures and porosity features (EnSafe, 1998a). More recently, new data have been utilized to demonstrate that this zone is a zone of lower conductivity that is correlative across the NWIRP McGregor site (Montgomery Watson, 1999; Clark, 2000).

A sharp contact separates the Main Street Limestone from the underlying Pawpaw Shale, which acts as an aquitard and consists of light gray shale that grades to silty shale with depth. The Pawpaw Shale is approximately 5 to 7 feet thick (EnSafe, 1998a).

The Weno Limestone is nodular, bedded limestone with alternating thin marl beds. The unit has a sharp upper contact, gradational lower contact and is approximately 36 feet thick in the McGregor Quadrangle (EnSafe, 1998a).

Finally, the Denton Marl is composed of soft marl with limestone ledges and has an approximate thickness of 7 feet (EnSafe, 1998a).

2.4 REGIONAL HYDROGEOLOGY

As discussed above, the Bosque and Leon River watersheds are comprised primarily of limestone and marl. These rocks primarily belong to the Washita Prairie Edwards Aquifer, the most significant water-bearing formation (Cannata and Yelderman, 1987; Cannata, 1988; Myrick, 1989). Depth to water in the unconfined aquifer is typically less than 10 meters (Cannata, 1988; Collins, 1989). Recharge to the aquifer occurs in the uplands of the watersheds, where thinner soils and exposed bedrock fractures allow downward water percolation (Myrick, 1989). The primary flow through the aquifer is through fractures and bedding plans and is controlled by topography, with flow originating at the hills, and moving down to the valleys (Cannata, 1988; Collins, 1989, Myrick, 1989). Discharge of water is generally to streams and springs. Water quality typically reflects an increase in total dissolved solids (TDS) with depth.

The hydraulic conductivity of the aquifer has been calculated to range from 10^{-3} m/s to 10^{-10} m/s (Clark, 2000). Aquifer heterogeneity is controlled locally by fractures caused by weathering, and regionally due to changes in lithology and tectonics.

Hydrogeology of the study area was described in detail in Section 5.2 of the *Final Conceptual Site Model* (MWH, 2002a).

2.5 REGIONAL HYDROLOGY

Regional drainage and surface water in the study area flow toward the Brazos River, then southeast toward the Gulf of Mexico. The Brazos River Basin drains 15 percent of Texas's land area and eventually empties into the Gulf of Mexico.

In the study area, a number of rivers drain the surrounding watersheds into the Brazos River Basin, including the Bosque, Leon, and Little Rivers. In addition, local creeks and streams contribute to these principal tributaries to the Brazos River.

Former NWIRP McGregor is located on a topographic high near the confluence of four watersheds, two of which occur in the study area, the Leon River watershed and the Bosque River watershed.

Figure 2-2 shows the location of each watershed within the study area. The headwaters of several tributaries within these watersheds originate at NWIRP McGregor. Hydrology of the Bosque and Leon River watersheds is described in detail in Section 5.4 of the *Final Conceptual Site Model* (MWH, 2002a).

3.0 INVESTIGATION HISTORY

Previous investigations have centered primarily on and around the former NWIRP McGregor site. These investigations have ranged from basic baseline surveys of media sampling to investigations aimed at determining the nature, extent, fate, and transport of various identified chemicals at the site. Due to potential contamination at the former NWIRP McGregor site, many of the previous owners have conducted environmental assessments there. A brief description of the previous investigations is presented in the *Final Conceptual Site Model* (MWH, 2002a). A summary of the previous investigations is also outlined in the *Draft-Final Groundwater Investigation Phase III Report* (EnSafe, 2003).

3.1 CONCEPTUAL SITE MODEL DEVELOPMENT

The project team developed a conceptual site model (CSM) for the Bosque and Leon River watersheds, as presented in *Final Conceptual Site Model* (MWH, 2002a), to provide a preliminary conceptual understanding of the relationship between contaminant sources, migration pathways, and exposures to human and ecological receptors. The CSM comprehensively incorporated available information on sources of perchlorate contamination and release, surface hydrology and hydrogeological characteristics of the Bosque and Leon River watersheds, the nature of perchlorate fate and transport, potential pathways of perchlorate migration within the study area, and the human receptors and environmental resources that may receive exposures to perchlorate. As such, the developed CSM combined previous watershed characterization information collected by the U.S. Navy with new watershed characterization information and analyses conducted by the USACE and its project team. The team used this information to identify data gaps in the understanding of perchlorate migration and exposure within the study area and to identify additional investigation activities aimed at filling such data gaps.

3.2 DATA COLLECTION AND REVIEW (BIBLIOGRAPHY)

The project team wanted to provide a detailed and comprehensive compilation and review of available data for the watershed study area coupled with a careful interpretation of relevant data for use during the CSM development. The team acquired data from the following sources during the study:

- EnSafe/U.S. Navy (NWIRP)
- CH2M Hill
- Texas Commission on Environmental Quality (TCEQ)
- Fort Hood
- USACE
- Texas State Soil and Water Conservation Board (TSSWCB)

- Natural Resources Conservation Service (NRCS)
- Black Lands Research Center
- Brazos River Authority
- City of Waco
- Baylor University, including Dr. Yelderman and Dr. Lind
- U.S. Geological Survey (USGS)
- County Appraisal Districts
- Texas Parks and Wildlife
- U.S. Fish and Wildlife Service
- Southern Nevada Water Authority

Various types of data were accumulated as part of this effort. A summary of data types collected is provided below.

- Existing reports
- Electronic databases
- GIS coverage and themes
- Groundwater quality data
- Perchlorate plume delineation
- Soil and sediment data
- Lakes Waco and Belton water quality data
- Intake structure water quality data
- Water level data
- Well location and survey information
- Precipitation data
- Species occurrence lists
- Threatened and endangered species lists
- Geologic and geophysical data
- Toxicology data
- Water budget information
- Lake flow pattern models
- Lake outflow data
- Flow patterns
- Bathymetric surveys

- Topographic information
- Regional groundwater flow data
- Land use data
- Stream gaging

A searchable data repository was established based on the existing study area data reviewed and a bibliographic summary of the compiled data was prepared by the project team. This summary included information regarding the source of the information, the format, the type, and a brief description of the data itself. An overview of the data repository is provided in **Appendix A**. This information was then compiled into a Microsoft Access database to help querying for a particular data set or searching for a particular type of report. This was a prerequisite to the development of a meaningful and representative CSM.

3.3 DATA GAP ANALYSIS

A draft CSM was prepared prior to a meeting among technical members of the USACE project team on February 21, 2002. The primary goal of the draft CSM was to provide a preliminary conceptual understanding of potential human and environmental exposures to perchlorate in the Lake Belton and Lake Waco study area. Based on the draft CSM, the project team concluded that a significant potential exists for human and ecological receptors inhabiting or using the study area to receive exposures to perchlorate through a variety of pathways. The project team also concluded that substantial data gaps exist in the available information regarding the occurrence, migration, and fate of perchlorate within the Bosque and Leon River watersheds. Data gaps as identified in this draft CSM are described in the following subsections.

3.3.1 Fate and Transport of Perchlorate

Chemical-specific characteristics that potentially affect the fate and transport of perchlorate were described in Section 4.0 of the *Final Conceptual Site Model* (MWH, 2002a). Identified data gaps in the understanding of perchlorate fate and transport included the following:

- Perchlorate is denser than water but little information is available regarding the potential for perchlorate to sink in groundwater, or undergo differential stratification in surface waters. Simple bench-top testing, or stratified water column testing in the field, would help to better understand the vertical transport of perchlorate in groundwater and surface water bodies within the watersheds.
- Information regarding the potential for perchlorate to undergo cation exchange with minerals in soil or sediment is not well documented. Such interactions may affect the fate of perchlorate in soil and sediment compartments and could be useful in the development of strategies to mitigate perchlorate transport. Simple bench-top testing, or field evaluation of cation

exchange capacity in watershed soils or sediments, would help to better understand the fate of perchlorate within these media.

- The potential for perchlorate to accumulate in soils following sequential irrigation and evaporation is currently unknown. Laboratory experiments, or sampling investigations in agricultural areas of the watersheds, would help to better evaluate the potential for accumulation of perchlorate in irrigated soils.
- Microbial degradation of perchlorate is an anaerobic process. Assessment of the anaerobic capacity of the watersheds would be helpful in evaluating the potential for perchlorate degradation to less toxic constituents.

3.3.2 Biological Uptake and Transformation

A summary of the biological uptake and transformation processes for perchlorate was presented in Section 4.3.2 of the *Final Conceptual Site Model* (MWH, 2002a). Identified data gaps in the understanding of perchlorate uptake and transformation included the following:

- Potential accumulation of perchlorate in terrestrial vascular plants, including home-grown produce, is unknown. Quantification of plant concentration factors would aid in determining exposure to human and ecological receptors through the food chain pathway.
- Data on accumulation in fish and other aquatic organisms are insufficient to evaluate exposures to organisms that feed on them. Laboratory or field assessments using methods designed to quantify aquatic organism concentration factors would facilitate this evaluation.
- Data on accumulation in litter feeding or herbivorous invertebrates are insufficient to evaluate exposures to mammals or birds that feed on these organisms. Laboratory or field assessments using methods designed to quantify litter feeding or herbivorous invertebrate concentration factors would help to better evaluate potential exposures to these receptors.

3.3.3 Toxicology of Perchlorate

The available toxicological information for perchlorate was briefly described in Section 4.5 of the *Final Conceptual Site Model* (MWH, 2002a). A thorough understanding of perchlorate-related risks requires knowledge of toxicity to all potential ecological receptors. Significant identified data gaps in the toxicological information for perchlorate included the following:

- Effects of perchlorate on algae and aquatic macrophytes are required to estimate risks to aquatic primary producers. Laboratory or field studies could be conducted to quantify effect levels for algae and aquatic macrophytes.
- Toxic effect levels in aquatic invertebrates are limited to daphnids. Laboratory toxicity studies or field assessments across several taxonomic groups would help to reduce the uncertainty in this limited database.
- USEPA's aquatic screening benchmark is based on subchronic fish data and only two taxonomic groups. Uncertainties related to the use of subchronic fish

data in the derivation of the aquatic screening benchmark (i.e., secondary chronic value, SCV) could be addressed through chronic effects testing in either laboratory studies or field assessments across several taxonomic groups.

- Data regarding the dose-response relationship for *Xenopus* are insufficient to properly evaluate potential impacts of perchlorate exposures on amphibians, including frogs. Additional FETAX studies would help to better evaluate the dose-response relationship for amphibians.
- Toxicity data for terrestrial plants are limited to a single study in lettuce. Effects testing for terrestrial plants across additional taxonomic groups and species would help to reduce the uncertainty in this value.
- Toxicity data for soil invertebrates are limited to a single study in the earthworm. Additional acute and/or chronic toxicity studies in soil invertebrates would help to reduce the uncertainty in USEPA's current soil screening benchmark.
- The USEPA's screening toxicity benchmark for herbivorous wildlife is based on laboratory rodent data. Laboratory or field assessments to evaluate the relevance of the rodent study and toxicity endpoints to herbivorous mammals would help to reduce the uncertainty in the current toxicity benchmark for herbivorous wildlife.
- Toxicity data are currently unavailable to evaluate potential impacts of perchlorate on aquatic or terrestrial birds. Laboratory toxicity studies or field assessments conducted in at least two species of birds would allow better evaluation.

3.3.4 Hydrologic Conceptual Model

Identified data gaps in the hydrologic conceptual model for the Bosque and Leon River watersheds included uncertainties in the hydrogeologic features of the watersheds, modeled water budgets, and surface water attributes of Lake Waco and Lake Belton. These data gaps are described in the following subsections.

3.3.4.1 Hydrogeology

A detailed, hydrogeologic conceptual model for the watersheds was developed, as described in Section 5.2 of the *Final Conceptual Site Model* (MWH, 2002a). Data gaps identified while developing this model included the following:

- The only values of hydraulic conductivity (K) for high groundwater levels are estimations used in model calibration runs. Direct measurements of K would be helpful in refining the actual variations. These data should be collected with enough measurements to permit statistical evaluation.
- The chemical fluctuations that result from recharge events need to be better understood. Chemical data should be collected during different recharge events, and at different levels within the aquifer. Tracers could be used to determine the flow paths of contaminants as a result of recharge events.

- Groundwater velocities also need to be measured during recharge events; tracers could be used for these measurements as well.
- Finally, groundwater-stream interactions need to be investigated during recharge events and during different times of the year.

3.3.4.2 Water Budget Modeling

An integrated, groundwater-surface water budget model was used to better understand the inflows to Lakes Waco and Belton within the Bosque and Leon River watersheds as described in Section 5.3 of the *Final Conceptual Site Model* (MWH, 2002a). Several data gaps were found to limit the usefulness of these models for assessing the potential impact of any perchlorate detected in streams on the lakes, as follows:

- Stream gauges at various locations record measurements of rainfall, stream stage (or level), velocity, and flow rate every 15 minutes. Unfortunately, the duration of recorded measurements from these stream gauges is at most eight months long. In order to better quantify flows on these streams, the measurement period should be extended to at least one year.
- Installation of stream gauges and collection of data from additional streams flowing into Lake Waco and Lake Belton would more accurately determine the water budgets for these watersheds. In particular, the contribution of flow from streams originating on NWIRP McGregor may be better understood if permanent stream gauges were established on Harris and Willow Creeks and the South Bosque River in the Lake Waco watershed, and on Station Creek in the Lake Belton watershed.
- Collection of surface flow measurements in conjunction with perchlorate sampling of surface water would allow for correlations between concentrations and flows. At a minimum, such co-located flow and concentration sampling should be conducted at the confluence of tributaries.
- Longitudinal sampling along main tributaries and streams could be conducted to validate results of surface modeling. Comparison of actual concentrations with modeled concentrations can help to identify the relative contributions of groundwater contamination to surface water concentrations of perchlorate.
- Lake elevation measurements are collected once per day and represent a data gap in storage calculations. Collection of more frequent elevation measurements would help to refine the water budget.

3.3.4.3 Surface Water Attributes

Principal surface water attributes that may affect the fate and transport of perchlorate within Lake Waco and Lake Belton were characterized in Sections 5.4.1 and 5.4.2 of the *Final Conceptual Site Model* (MWH, 2002a). Identified data gaps in the understanding of these features limit an accurate assessment of potential fate and transport processes for perchlorate within these watersheds. These data gaps included the following:

- Clay and silt particles are generally charged and are often capable of sorbing charged and/or polar molecules of anthropogenic origin to their surfaces. Such

binding may serve as either a sink or a source of pollutants to biota. Although perchlorate has been measured in surface water samples collected from Lake Waco, the *bioavailability* of perchlorate within this surface water body is currently unknown.

- Lake Waco is classified as moderately eutrophic and, as such, supports seasonal algal blooms. The extent to which these conditions may affect the bioavailability of perchlorate and either hinder or promote transfer to biota is unknown. The potential effects of the diffuser system operated within Lake Waco on the lake's trophic state, flow patterns, or other attributes is also unknown.
- Lake Belton is characterized as a warm monomictic reservoir, and the warm temperature of the bottom is believed to result in high deep-water bacteria metabolism. This phenomenon contributes to oxygen depletion that generally follows a heterograde pattern. As a result, the water column supports a complex redox pattern, potentially affecting both the solubility and metabolism of contaminants. The extent to which these attributes may affect perchlorate solubility and/or metabolism is currently unknown.
- Linear differences in sedimentation, stratification, turbidity and trophic state along the 21-mile length of Lake Belton result in taxonomic differences, as assessed by phytoplankton types. The extent to which such differences may affect surface water concentrations and bioavailability of perchlorate within different portions of this watershed are unknown.
- Evaluation of the bacteria-oxygen relationship of the hypolimnion suggests that the thalweg (old river channel) within the upper portion of Lake Belton may be transporting organic materials of river origin rather than lake origin. Based on water temperature (density), such underflows can transport river-borne materials great distances without mixing with the mass of reservoir water. More research into this phenomenon would allow evaluation of potential impacts on water budgets and potential downstream exposures.

3.3.4.4 Nature and Extent of Perchlorate Contamination

Identified data gaps in the available information regarding the nature and extent of perchlorate contamination and in the migration pathway analysis for the Bosque and Leon River watersheds included the following:

- Portions of the study area with measured or potential concentrations in surface water or groundwater exceeding the 'interim action level' of 4 µg/L require better characterization to evaluate potential impacts to human health and the environment.
- Portions of the study area with measured or potential concentrations in soil or sediment exceeding the screening benchmark of 1 mg/kg perchlorate require further characterization in order to evaluate potential impacts to human health and the environment.

- Dug wells, private groundwater wells, and springs that occur in areas likely to contain greater than the 'interim action level' of 4 µg/L perchlorate require characterization to evaluate potential impacts to human health and the environment.
- Evaluation of the significance of elevated reporting limits during the 1998 and 1999 groundwater sampling events would allow determination of whether or not re-sampling of groundwater would be beneficial at specific locations.
- Evaluation of the significance of elevated reporting limits during collection of the 1998 and 1999 surface water and stock pond grab samples would allow determination of whether or not re-sampling of surface water would be beneficial at specific locations.
- Characterization of tributaries originating on, or in the vicinity of, Fort Hood would allow characterization of potential discharges of perchlorate from this facility to the Lake Belton Watershed.

3.3.5 Exposure Assessment

Information regarding the potential impact of perchlorate releases from the former NWIRP McGregor or other sources on media within the Bosque and Leon River watersheds was used to evaluate potential exposures of human or ecological receptors that may come in contact with these media. The uncertainties and data gaps in the information needed to complete more detailed assessments of exposure and risk to human and ecological receptors are described in the following subsections.

3.3.5.1 Human Health Exposure Analysis

Identified data gaps related to the human exposure assessment included the following:

- Drinking water intake structures located within Lake Belton, downstream of Lake Belton, and within Lake Waco provide the sole-source drinking water supply for nearly 500,000 people in the surrounding communities. Measured or modeled concentrations of perchlorate at these in-take structures would allow quantitative evaluations of exposure and risk to users of public water supplies. Knowledge regarding potential mixing of water sources to achieve contaminant compliance goals, or specific perchlorate monitoring data collected by the utilities, would allow for a refined assessment of public health risks.
- Surface water obtained from springs and some dug wells is also used for human consumption in areas not connected to public water supplies. The extent to which supplies with known or potential contamination are used for potable purposes is unknown.
- Humans may potentially consume home-grown produce or livestock products that are irrigated with contaminated water. Sources of water for irrigation or stock ponds and their relationship to contaminated areas of the watersheds are unknown.

- Recreational users of the Lake Waco and Lake Belton watersheds are susceptible to perchlorate exposures while swimming, fishing, or hunting in contaminated areas. Additional characterization data would allow evaluation of such potential exposures. Data currently being collected by TIEHH should be helpful in an evaluation of recreational exposures through consumption of wildlife.

3.3.5.2 Ecological Exposure Analysis

Identified data gaps in the ecological exposure assessment included the following:

- The ecological exposure analysis included an assessment of the biological resources within the study area. This evaluation resulted in the identification of several protected or special status species. Additional biological assessment including field surveys would allow identification of the co-occurrence of special status species with areas of elevated perchlorate contamination.
- Uptake of perchlorate by aquatic producers (e.g., phytoplankton and algae), submergent macrophytes, and emergent vegetation through foliar transport and/or root uptake is likely to occur within surface water bodies of the Bosque and Leon River watersheds. In addition, benthic invertebrates and omnivorous and carnivorous fish are exposed to these media. Additional characterization data would allow evaluation of such exposures in areas that have not been sampled.
- Little information is available regarding the uptake of perchlorate into phytoplankton and algae. It is possible that serial blooms of such organisms within Lakes Belton and Waco may serve to transfer perchlorate from the water column to planktivorous fish or waterfowl. Measurements of perchlorate uptake by phytoplankton and/or algae would help to identify whether or not this is a potentially significant fate process for perchlorate within these watersheds.
- Once absorbed by producer level receptors (e.g., plants), there is a potential for transfer of perchlorate to consumer level receptors and to higher trophic level receptors through the ecological food chain. The potential for transfer of perchlorate through the ecological food chain is relatively unknown.

3.4 DATA GAP PRIORITIZATION

The technical meeting also resulted in the prioritization of data gaps regarding available information on the occurrence, migration, and fate of perchlorate within the Bosque and Leon River Watersheds.

Table 3-1 provides a comprehensive list of all data gaps identified, general recommendations for resolving each data gap, and a consensus ranking of the importance of resolving each data gap, consistent with the outcome of the February 21, 2002 technical meeting. It also highlights the data gaps which were resolved as a result of this study.

Table 3-1
Data Gaps Summary

Data Gap Identified	Actions Needed	Priority	Work Performed	Data Gap Filled During Study
Fate and Transport of Perchlorate				
The potential for perchlorate to accumulate in soils following sequential irrigation and evaporation is currently unknown.	Conduct laboratory experiments, or sampling investigations in agricultural areas of the watersheds to evaluate the potential for accumulation of perchlorate in irrigated soils.	High	TIEHH Transformation Study (Section 5.2.3)	Partially
The extent to which conditions favorable to anaerobic degradation of perchlorate within portions of the watersheds associated with elevated perchlorate concentrations is currently unknown.	Conduct field assessments of current watershed conditions in areas with elevated perchlorate concentrations.	Medium	TIEHH Transformation Study (Section 5.2.3) and MWH Anoxic Study in Lake Belton (Section 5.2.2)	YES
Hydrologic Conceptual Model				
Groundwater-stream interactions during recharge events and during different times of the year needs to be better understood.	Seasonal measurements of groundwater and stream characteristics should be taken concurrently during known recharge events.	Medium	MWH Longitudinal Stream Sampling Study (Section 5.1.3)	YES
Water Budget Modeling				
The duration of recorded measurements of rainfall, stream stage (or level), velocity, and flow rate is insufficient to quantify flows on the streams.	Collect measurements of rainfall, stream stage (or level), velocity, and flow rate every 15 minutes for at least one year from stream gauges at various locations.	High	MWH Longitudinal Stream Study (Section 5.1.3)	YES
Water budgets for the watersheds are inaccurate due to absence of stream gauges and collection of data from certain streams flowing into Lake Waco and Lake Belton originating on NWIRP McGregor.	Permanent stream gauges should be established on Harris and Willow Creeks and the South Bosque River in the Lake Waco watershed, and on Station Creek in the Lake Belton watershed.	High	MWH Longitudinal Stream Study (Section 5.1.4, Section 6.4)	YES – Station Creek, South Bosque and Harris Creek NO – Willow Creek
A correlation between concentrations and flows is impossible due to the absence of co-located data for surface flow and perchlorate concentrations.	Conduct co-located flow and concentration sampling at the confluence of tributaries.	Medium	MWH Longitudinal Stream Study (Section 5.1.1.2, Section 5.1.4)	YES
It is not currently possible to evaluate the contribution of groundwater contamination to surface water concentrations based on current surface modeling results.	Longitudinal sampling along main tributaries and streams should be conducted to validate results of surface modeling. Compare measured concentrations with modeled concentrations.	Medium	MWH Longitudinal Stream Study (Section 5.1.1.2, Section 5.1.3)	Partially – Sampling was performed but due to concentrations detected, modeling was not conducted.

Data Gap Identified	Actions Needed	Priority	Work Performed	Data Gap Filled During Study
Surface Water Attributes				
The bioavailability of perchlorate within the surface water bodies of Lake Waco and Lake Belton watersheds is currently unknown.	Further studies on the bioavailability of perchlorate in the watersheds are necessary.	Medium	TIEHH Fish and Frog uptake studies (Section 5.4.1, Section 5.4.2)	Partially
The extent to which seasonal algal blooms may affect the bioavailability of perchlorate and either hinder or promote transfer to biota is unknown.	Studies of biota uptake should be conducted to compare uptake rates during and between algal blooms.	Medium	TIEHH Algae Uptake Study and MWH Lake Belton Delta Area Algae Study (Section 5.3.2)	Partially
The extent to which substantial deep-water bacteria metabolism in Lake Belton may affect perchlorate solubility and/or metabolism is currently unknown.	Laboratory tests must be conducted simulating the attributes present in Lake Belton to determine the expected affect on perchlorate.	Low	MWH Anoxic Study of Lake Belton (Section 5.2.2)	YES
Migration Pathway Analysis				
Characterization of perchlorate in surface water and groundwater is insufficient.	Portions of the study area with measured or potential concentrations in surface water or groundwater exceeding the 'interim action level' of 4 µg/L should be further characterized.	High	TIEHH stream and lake water grab sampling and MWH Longitudinal Stream Study and lake water sampling (Section 5.1)	YES – surface water Partially – GW sampling was not performed by the project team, but the U.S. Navy continued to characterize the GW contamination and provided data to the team.
Characterization of perchlorate in soil and sediment is insufficient.	Portions of the study area with measured or potential concentrations in soil or sediment exceeding the screening benchmark of 1 mg/kg perchlorate should be further characterized.	High	TIEHH sediment pore water studies in streams and MWH sediment pore water studies in Lake Belton and Lake Waco. (Section 5.2.1)	YES – Sediment pore water sampling Limited soil testing by TIEHH No sediment testing was performed by team but historical soil and sediment sampling data were provided by the U.S. Navy
Elevated reporting limits during collection of 1998 and 1999 surface water and stock pond grab samples create uncertainty.	The significance of elevated reporting limits should be further evaluated to determine if re-sampling of surface water is required at specific locations.	Medium	MWH Longitudinal Stream Study and lake water sampling (Section 5.1.1, Section 5.1.2)	YES
The flow patterns of streams running into Lake Belton and Lake Waco are poorly understood.	The flow patterns of streams impacted by perchlorate may be better understood by utilizing dye tracers and other related studies.	Medium	MWH Longitudinal Stream Study and ADCP study (Section 5.1.4)	YES

Data Gap Identified	Actions Needed	Priority	Work Performed	Data Gap Filled During Study
Perchlorate detections in the southern portion of Lake Belton suggest potential contamination from another source.	Tributaries in the vicinity of Fort Hood should be characterized to evaluate potential discharges of perchlorate from this facility to Lake Belton.	High	MWH Longitudinal Stream Study and lake water sampling (Section 5.1.1, Section 5.1.2)	Partially – Limited perchlorate testing performed along Cowhouse Creek. Lake Water was also sampled.
Exposure Assessment				
Further knowledge regarding potential mixing of water sources to achieve contaminant compliance goals or specific perchlorate monitoring data collected by the utilities is necessary for assessment of public health risks.	Measured or modeled concentrations of perchlorate at the Lake Belton and Lake Waco in-take structures will be needed to conduct quantitative evaluations of exposure and risk to users of public water supplies.	High	MWH Intake and lake Sampling Studies (Section 5.1.2)	YES
The potential uptake of perchlorate in plants and agricultural products through irrigation of food crops needs to be better understood.	Conduct laboratory or field assessments using methods designed to quantify perchlorate uptake in agricultural products.	High	TIEHH Lab and field plant uptake data (Section 5.3)	Partially – Not all food crops were sampled during the study
Exposure of humans to perchlorate in surface water, sediment and biota associated with recreational uses of the Lake Waco and Lake Belton watersheds is incomplete.	Additional characterization data are needed to in areas that have not been sampled. Data currently being collected by TIEHH should be useful to an evaluation of exposures through consumption of wildlife.	Medium	TIEHH Beef cattle data, market basket survey and catchable fish fillets. MWH Longitudinal Stream Study and lake water sampling (Sections 5.1.1, 5.1.2, 5.3.4, 5.4.1, and 5.5.3)	YES
Measurements of perchlorate uptake by aquatic producers (e.g., phytoplankton and algae), submergent macrophytes, emergent vegetation, benthic invertebrates and omnivorous and carnivorous fish are limited.	Studies of this phenomenon are currently being conducted by TIEHH. This information should be useful to an evaluation of ecological exposures through food chain transfer. Additional biological sampling may be required.	High	TIEHH algae uptake study (Section 5.3.2)	Partially
The extent to which phytoplankton and algae may serve to transfer perchlorate from the water column to planktivorous fish or waterfowl is currently unknown.	Measurements of perchlorate uptake by phytoplankton and/or algae would help to identify whether or not this is a potentially significant fate process for perchlorate within these watersheds.	Medium	TIEHH algae uptake study and MWH Delta Area Algae Study in Lake Belton (Section 5.3.2)	YES
Biological Uptake and Transformation				
Data on perchlorate accumulation in aquatic plants are insufficient to assess exposures to primary consumers (i.e., planktonic and benthic invertebrate communities).	Conduct laboratory or field uptake and accumulation studies using methods designed to quantify aquatic plant concentration factors.	Medium /High	TIEHH aquatic plant study (Section 5.3)	YES

Data Gap Identified	Actions Needed	Priority	Work Performed	Data Gap Filled During Study
Data are insufficient to determine whether perchlorate bioconcentrates in aquatic organisms.	Conduct laboratory or field assessments using methods designed to quantify aquatic organism concentration factors under steady state conditions.	Medium /High	TIEHH aquatic organism study (Section 5.4)	YES
Data on accumulation in fish and other aquatic organisms are insufficient to evaluate exposures to organisms that feed on them.	Conduct laboratory or field uptake and accumulation studies using methods designed to quantify aquatic plant concentration factors across multiple trophic levels.	Medium /High	TIEHH plant uptake and accumulation studies (Section 5.3)	YES
Data on perchlorate accumulation in terrestrial vascular plants are insufficient to evaluate potential exposures to terrestrial herbivores.	Conduct laboratory or field assessments using methods designed to quantify terrestrial herbivore concentration factors.	Medium /High	TIEHH plant studies (Section 5.3)	YES
Data on the fate of perchlorate in irrigated soils and plants are insufficient to evaluate potential exposures to humans through agricultural products.	Conduct laboratory or field assessments to determine effects of perchlorate in irrigated soils and concentration factors in terrestrial plants.	Medium /High	TIEHH terrestrial plant studies and market basket survey (Section 5.3.3, Section 5.3.4)	YES
Ecotoxicology				
USEPA's aquatic screening benchmark is based on subchronic fish data and only two taxonomic groups.	Conduct chronic laboratory toxicity studies or field assessments across several taxonomic groups.	Medium	TIEHH toxicity studies	YES
Data regarding the dose-response relationship for Xenopus are insufficient to properly evaluate potential impacts of perchlorate exposures on amphibians, including frogs.	Conduct additional FETAX studies to better evaluate the dose-response relationship for amphibians.	Medium	TIEHH amphibian studies (Section 5.4.2)	YES
The USEPA's screening toxicity benchmark for herbivorous wildlife is based on laboratory rodent data.	Conduct laboratory or field assessments to evaluate the relevance of the rodent study and toxicity endpoints to herbivorous mammals.	Medium	TIEHH small mammal studies (Section 5.5.1)	YES
Toxicity data are currently unavailable to evaluate potential impacts of perchlorate on aquatic or terrestrial birds.	Conduct laboratory toxicity studies or field assessments in at least two species of birds.	High	TIEHH bird studies (Section 5.5.1)	YES

4.0 STUDY DESCRIPTIONS

The development of the CSM and discussions during the February 21, 2002 technical meeting resulted in the identification of a number of high priority data gaps in the current knowledge regarding perchlorate contamination, migration, and fate within the Bosque and Leon River watersheds. Further investigations to resolve or reduce these high priority data gaps were conducted during this study to provide an accurate assessment of perchlorate exposures and risks to human health and the environment.

This chapter summarizes the studies conducted based on these identified high priority data gaps. Data and conclusions resulting from each of these studies are included in Chapter 5.

4.1 WATERSHED

High priority watershed studies that were identified include the following:

- Longitudinal Stream Sampling Studies
- Groundwater Dye Tracer Studies
- Lake Belton Perchlorate Bioreduction Bench-Scale Study
- Lake Belton Acoustic Doppler Current Profiler (ADCP) Study
- Lake Belton and Lake Waco Delta Areas Study
- Continued Intake Sampling

The objectives and methods of these studies are described further in the following subsections.

4.1.1 Longitudinal Stream Sampling Studies

Fifteen automated monitoring stations were installed along streams that discharge to Lake Waco and Lake Belton. Each monitoring station was programmed to collect surface water samples and to measure and record stream level and rainfall data. The overall rationale for the selected monitoring locations was to provide data that can be used to evaluate the perchlorate concentrations and associated flow rates entering Lake Waco and Lake Belton. The elements of the Longitudinal Stream Sampling Study include:

- Longitudinal surface water level/flow measurements within Harris Creek, Station Creek, Onion Creek, Tributary M, Cowhouse Creek, South Bosque River, Middle Bosque River and the Leon River.
- Longitudinal surface water sampling on Harris Creek, Station Creek, Onion Creek, Tributary M, Cowhouse Creek, South Bosque River, Middle Bosque River and the Leon River.
- Rainfall measurements within the study area.

- Installation of monitoring wells in close proximity to the stream sampling locations in order to collect groundwater elevation (water level) measurements.

4.1.2 Groundwater Dye Tracer Studies

The primary objective of the groundwater tracing activities was to confirm the hydraulic connection between shallow groundwater and the surface water in Station Creek, Onion Creek, Tributary M, Leon River, Harris Creek, South Bosque River, and the Middle Bosque River. The study plan included performing semi-quantitative groundwater dye-tracer tests at selected monitoring stations that were established during the Longitudinal Stream Sampling Study phase of the study.

The groundwater dye-tracer tests were considered to be semi-quantitative given the use of a fluorometer to confirm recovery of the injected dye. A fluorometer is capable of detecting dye concentrations several orders of magnitude lower than what can be detected visually. However, the varying concentrations of dye that pass a specific monitoring site during a given period of time would not have been precisely quantified during this field program.

As with all of the other field program components of this study, the planning phase of the dye tracer investigation involved the establishment of data quality objectives (DQOs). DQOs were developed using the seven-step process as outlined in the *Guidance for the Data Quality Objectives Process* (USEPA, 1994) and additional guidance as provided in *Data Quality Objectives for Hazardous Waste Site Investigations* (USEPA, 2000). After preparing the DQOs and reviewing the initial stream level and groundwater level data from the Longitudinal Stream Sampling monitoring stations, it was determined that the objectives of the groundwater dye tracer study could be substantially met using data from the longitudinal stream sampling monitoring stations. Further, due to uncertainties inherent in conducting dye tracer studies, the resultant data would not significantly improve the ability to meet the objectives of this phase of the study. Consequently, the project team decided not to perform the dye tracer study.

4.1.3 Lake Belton Perchlorate Bioreduction Bench-Scale Study

This study was conducted to develop a better understanding of the anoxic component of Lake Belton. The elements of the Lake Belton Perchlorate Bioreduction Bench-Scale Study included:

- Collection of lake water and sediment samples from the deepest portions of Lake Belton that likely have been under anoxic conditions for the longest period of time.
- Collection of lake water and sediment samples from shallower portions of Lake Belton where the thermocline is close to the water/sediment interface. These sampling locations close to the thermocline were included to assess natural variability within the water column.

To develop a better understanding of the anoxic component of Lake Belton, a series of microecology experiments were performed to determine the potential for natural perchlorate bioreduction in Lake Belton. These tests explored this potential in both the water column and the sediment at the water/sediment interface.

4.1.4 Lake Belton Acoustic Doppler Current Profiler (ADCP) Study

This study was conducted to better understand lake current changes throughout the year and to ascertain what effect changing flow patterns may have on perchlorate fate and transport within Lake Belton. This study was also performed to determine if preferential flow patterns occur in the lake along the thalweg of the old river channel or other submerged features.

For this study, an ADCP was attached to a boat and used to determine current velocity at 21 transects across Lake Belton. A total of four ADCP measurements were obtained at each transect, one during each season of the year. ADCP measurements were repeated on a seasonal basis in order to better understand lake current changes throughout the year and to ascertain what effect changing flow patterns may have on perchlorate fate and transport. The ADCP survey is limited to Lake Belton based on the assumption that Lake Waco is a well-mixed, homogeneous environment, both because of its shallow configuration and the presence of a mechanical aeration system. The recent raising of the Lake Waco pool by five feet may substantially change its flow and mixing characteristics. Because of the timing of this study, it was not possible to account for this change in the project team's assumptions.

4.1.5 Lake Belton and Lake Waco Delta Areas Study

This study was conducted to evaluate the presence and distribution of perchlorate in water and sediment within the delta areas of Lake Belton and Lake Waco. The elements of the study include:

- Establishment of a 20-point sampling grid on both the Lake Belton and Lake Waco delta areas using global positioning system (GPS).
- Collection of surface water and sediment pore water samples at each grid location on Lake Belton and Lake Waco.
- Collection of algae samples at 10 of the grid locations within the Lake Belton delta area.
- Collection of surface water samples at the Heather Run and Ridgewood Golf Course intakes from Lake Waco.

4.1.6 Intake Sampling

The project team collected monthly samples from the potable water and irrigation intakes located within Lake Waco and Lake Belton and downstream of the Lake Belton dam during the study. Potable water intakes sampled include the Bluebonnet intake, the Bell County Water Control and Improvement District #1 intake, the City of Gatesville intake, the City of Temple intake, and the City of Waco intake. Irrigation intakes sampled include Heather Run, Ridgewood Golf, and Wildflower Country Club.

The intake sampling conducted by the project team was a continuation of sampling at these intakes by the U.S. Navy. The U.S. Navy began collecting monthly surface water samples from these water intakes in March 1999 but decided to end this sampling effort in December 2002. The project team agreed to continue collecting samples at these locations through December 2003 because of the importance of these drinking water supplies to the public. Since the completion of this study, the City of Waco has continued sampling from intakes in Lake Waco.

4.2 ECOLOGICAL

High priority ecological studies that were identified include environmental occurrence and effects studies in plants and animals. A general description of these studies is provided below. Chapter 5 describes the ecological studies conducted by the study team in detail.

4.2.1 Plants

Evaluation of the potential movement of contaminants from water and/or soil to vegetation is critical for determining exposure routes to wildlife and humans. The conditions under which contaminants move from soil/sediment to plants are often initially characterized in the laboratory or greenhouse where the environment is more easily controlled and plant species can be selected for comparative purposes. Subsequent studies often utilize samples collected from the field.

Routes of perchlorate exposure in aquatic environments were identified as a data gap related to conditions within the study area. One possible route of uptake in fish is through the food chain, such as through the consumption of periphyton that have taken up and accumulated perchlorate from the water.

Perchlorate uptake by plants can be viewed as both a possible sequestration and perturbation process of perchlorate from sediments and streams. Terrestrial plants are capable of removing perchlorate from sediments and stream water and translocating it to leaves/fruits/nuts. Aquatic vegetation is capable of uptake directly from bulk stream water. Exposure of organisms through ingestion of plant material may depend on the availability of perchlorate to the plant (seasonal and spatial), distribution within the plant (leaves/fruits/nuts), as well as the length of exposure. The fate of perchlorate in the environment can also be influenced by the potential re-release of perchlorate from vegetation after uptake. As plants senesce (the fall leaf drop), vegetative tissues are returned to the soil/sediment. The return of perchlorate to the soil/sediment may be a beneficial process due to the movement of perchlorate from stable environments (aerated stream water or low carbon deeper sediments) to areas where rapid transformation can occur (top organic rich soil layers). On the other hand, uptake of perchlorate can also be viewed as another mechanism of exposure, especially if bioconcentration occurs in plant material.

An important question in the study area is the potential for wildlife and human exposure to perchlorate through the consumption of contaminated food. The use of irrigation water (surface or groundwater), which may be contaminated with perchlorate, to supply water for gardens is a relevant scenario in the study area. Under this scenario, it is critical to establish the relationship between perchlorate concentrations in irrigation water and perchlorate concentrations in edible vegetation. Laboratory and field investigations on perchlorate uptake into vegetation were conducted to address this exposure pathway.

4.2.2 Animals

Ecological investigations included laboratory and field studies on the aquatic and terrestrial components of the ecosystem.

4.2.2.1 Aquatic

Environmental contamination of water sources primarily occurs near military and industrial installations where perchlorate is handled. In contrast to the health effects data on humans and rodents, little data exist on potential ecological effects of environmental perchlorate contamination. Fish and wildlife are also chronically exposed to perchlorate in areas where such contamination exists.

In order to confirm perchlorate exposure, fish were collected from aquatic environments within the study area and analyzed for the presence of the perchlorate anion. The goals of this effort were to assess the exposure (and potential effects) of perchlorate in fish as well as to assess the exposure potential for perchlorate in humans through the consumption of contaminated fish.

Perchlorate is known to affect thyroid function, causing subsequent hormone disruption and potential perturbations of metabolic activities. Because the mechanism of iodide uptake and thyroid hormone synthesis is essentially identical in all vertebrates, the extensive data set on perchlorate mode of action that has been collected over the last 50-60 years can be used to predict potential affects in wildlife. Frogs (amphibians) are one of the groups at greatest risk, as they require normal thyroid hormone secretion to complete the transformation from tadpole to frog. If tadpoles do not complete this transformation they cannot reproduce. If metamorphosis is delayed, tadpoles are at risk of greater exposure to predators and pond drying. Finally, because metamorphosis is such a robust and dramatic morphological change, the effects of perchlorate can be observed using non-invasive endpoints such as limb emergence, tail resorption, or limb length.

In teleost fish, thyroid hormones promote growth, stimulate early gonadal development, stimulate steroidogenesis, and initiate metamorphosis in many fish. Because of the involvement of thyroid hormone in fitness components such as reproduction, growth, and development, thyroid endocrine disruption could ultimately be manifested at the population, community, or ecosystem level of biological organization. Although the mechanisms by which perchlorate acts on the thyroid have been well established, there is little information on the potential impact of perchlorate in ecologically relevant species. Uptake and depuration kinetics of perchlorate may provide a better understanding of perchlorate fate and effects in fishes, as well as providing information on the dose-

response of perchlorate in the environment. Also, there is little information on tissue distribution of perchlorate in fish.

4.2.2.2 Terrestrial

Small mammals and birds are routinely used as sentinel species to assess environmental exposure to chemicals (movement of chemicals from the environment into organisms) and the possible effects resulting from any exposure. Their utility is often tied directly to their diet, relative small home ranges or foraging areas, and position in the food chain. Thus, many small mammals and birds collected from a specific location can be viewed with confidence as living and feeding in the area of collection. In addition, their position near the bottom of most food chains makes them excellent conduits for chemical movement into top-level mammalian, reptile, and avian predators.

Perchlorate exerts its primary effect on animals by inhibiting uptake of iodide into the thyroid gland. This inhibition of iodide uptake in turn inhibits the production of thyroid hormones that are subsequently sent into the peripheral blood. Therefore, analyzing the concentration of thyroid hormones in blood (specifically plasma) samples from native animals is one effect measurement indicative of exposure to perchlorate in individual animals. Modeling can then be used to determine the impact, if any, on a population of individuals.

Perchlorate can be detected in soils at many contaminated sites, but it is most often associated with ground and surface water bodies. Therefore, aquatic organisms including fish, amphibians, and some reptiles are considered to be the most likely ecological receptors of perchlorate exposure. However, perchlorate has been detected in terrestrial organisms as well, indicating that exposure pathways to terrestrial organisms exist. Domestic animals inhabiting a pasture near perchlorate-contaminated water bodies may be at risk for exposure through drinking water or consumption of plant matter grown in the presence of perchlorate. For example, cattle are obligate herbivores. As such, they may be at risk for perchlorate exposure and subsequent effects since perchlorate can accumulate in plants. Beef and dairy cattle may represent a direct exposure pathway for perchlorate to humans.

4.3 DATA MANAGEMENT (GIS)

All digital data collected or generated and validated throughout the study are stored and managed in a centralized Geographic Information System (GIS) database system. The GIS was developed to facilitate storage of both spatial and non-geographic data. The GIS was developed in ArcSDE using an Oracle 9i spatial database. The GIS was used for data analysis, data conversion, data validation, data management and creation of maps and animations for the study. This system contains relevant spatial and tabular information for use during the study and possible use in future investigations. The information stored within the database includes topographical, hydrological, geological, ecological, stream height, groundwater elevation and analytical GIS layers. The system is used to organize, store, manage, analyze, and present pertinent study data. Additional tools were also developed for the import of data, data management and export into various formats for

use in analysis, reports and presentations. These tools include a data import wizard, data importer, document viewer and graph manager. The GIS database system and developed import/export tools and graph manager are described in detail in **Appendix B**.

5.0 DATA

5.1 SURFACE WATER

5.1.1 Perchlorate Occurrence in Streams

5.1.1.1 Stream Water Grab Samples

5.1.1.1.1 Introduction

In order to better characterize the potential points of exposure within the study area, grab samples of surface water were collected periodically and specifically to coincide with sampling of biota. Samples were collected in order to evaluate perchlorate exposure to mammals, birds, fish, and amphibians; however, these samples were not meant to characterize the spatial and temporal variation in perchlorate concentrations within surface water in the Lake Belton and Lake Waco watersheds. In addition, grab samples provided a mechanism to evaluate the potential movement of perchlorate from surface water to terrestrial environments through vegetation and/or other routes of trophic transfer.

5.1.1.1.2 Methodology

Stream water grab samples were collected from various locations within the study area (35 locations). Sample locations were chosen to include both "reference" areas (no perchlorate contamination suspected) and "contaminated" areas (suspect areas based on historical data and watershed hydrology). The 35 locations from which surface water samples were collected are identified in **Figure 5-1** and described in **Table 5-1**.

Water samples were collected in pre-cleaned glass vials (20 mL) from the surface of the water body. GPS coordinates of sample locations were recorded at the time of sampling. Water samples were placed on ice during transport back to the laboratory and stored at 4 °C until analysis by ion chromatography (**Appendix X**). Within this section and throughout the entire document, all perchlorate concentrations are expressed as the concentration of the perchlorate anion (ClO_4^-) only; the ammonium counterion is not included in the concentration expression.

5.1.1.1.3 Data

Analytical results of surface water grab samples collected beginning in March 2001 are included in **Appendix C**. Perchlorate residue data for selected sites are also presented graphically in **Appendix C**. Several exposure points within the study area were identified through this sampling effort including (1) the spring on Oglesby Road, (2) Station Creek south of the NWIRP property, (3) S Creek at Highway 317, and (4) the Unidentified tributary near the wastewater treatment plant (WWTP) at Highway 317.

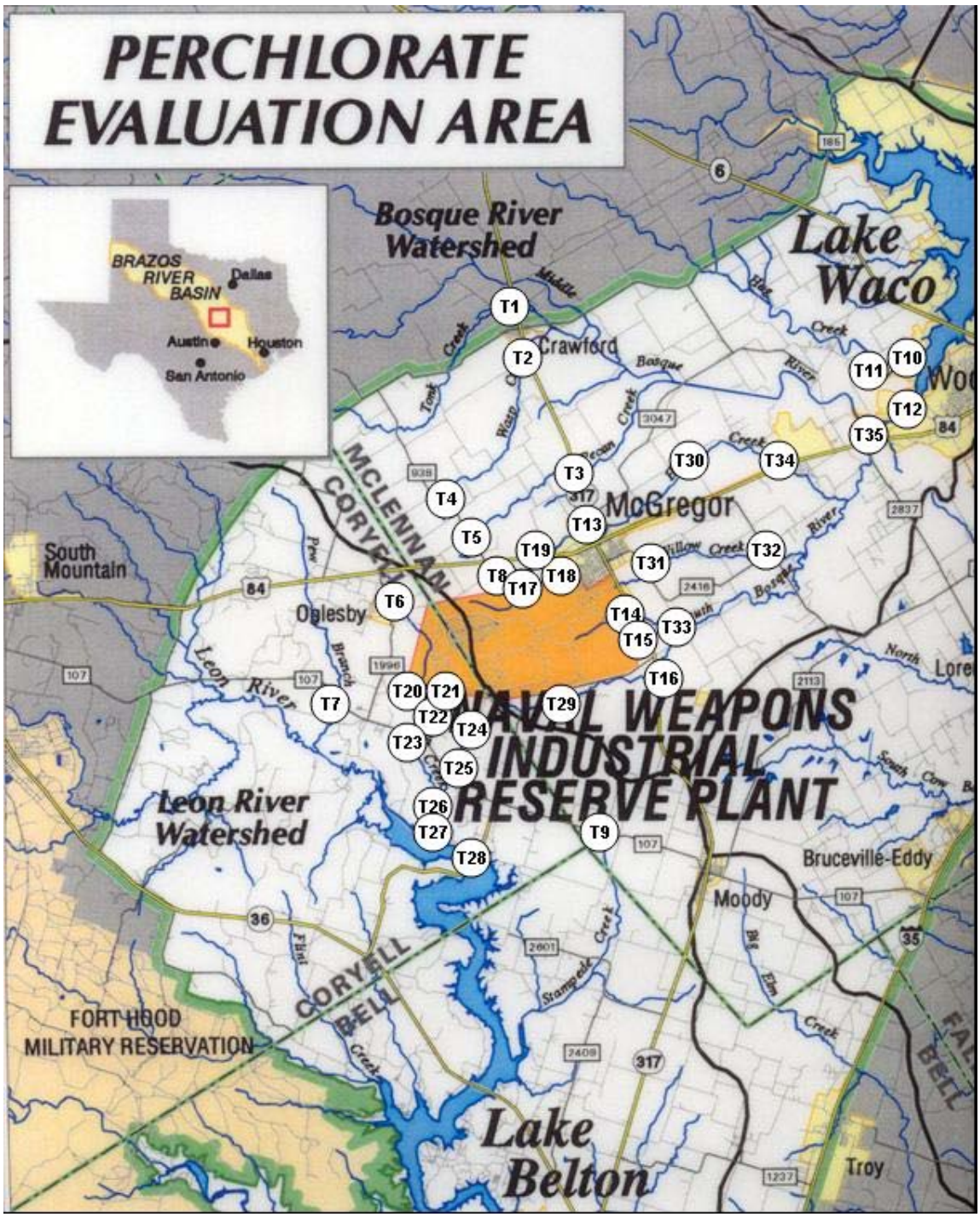


Figure 5-1
Map of Study Area Illustrating the Approximate Locations Where Surface Water Grab Samples Were Collected

Table 5-1
Locations of Stream Water Grab Samples

Location Type	ID[a]	Location Description	UTM
Reference Sites	T1	Tank Creek at Highway 317	647618E 3490671N
	T2	Wasp Creek at Highway 317	648033E 3488680N
	T3	Pecan Creek at Highway 317	650195E 3483242N
	T4	Wasp Creek at FM 938	644411E 3482032N
	T5	Pecan Creek at FM 938	645673E 3479993N
	T6	Station Creek at Oglesby Road	642344E 3477044N
	T7	Pew Branch at Highway 107	639323E 3472938N
	T8	Harris Creek at Oglesby Road North	647790E 3478770N
	T9	Stampede Creek at Highway 107	650859E 3466885N
	T10	Hog Creek	664928E 3487889N
	T11	Hog Creek 2	664146E 3487661N
	T12	Middle Bosque near Highway 84	664464E 3485354N
Sites down-gradient from NWIRP	T13	Harris Creek at Highway 317	651046E 3480160N
	T14	Unnamed Tributary near WWTP at Highway 317	652853E 3476589N
	T15	S Creek at Highway 317	653646E 3474993N
	T16	South Bosque at Highway 317	653810E 3473908N
	T17	Harris Creek at Oglesby Road	648178E 3478942N
	T18	Spring on Oglesby Road	649218E 3479243N
	T19	Harris Creek at Highway 84 West of McGregor	649100E 3479383N
	T20	Station Creek near the NWIRP Boundary	643318E 3472885N
	T21	Station Creek near the NWIRP Boundary 2	643351E 3472742N
	T22	Tributary Feeding Station Creek near NWIRP Boundary	643736E 3472922N
	T23	Station Creek at Highway 107 (T23)	642988E 3471304N
	T24	Onion Creek North of Highway 107	645534E 3472063N
	T25	Onion Creek at Highway 107	644703E 3469668N
	T26	Station Creek at Old River Road	643925E 3466457N
	T27	Station Creek at Leon River	644516E 3466167N
	T28	Leon River at Mother Neff Park	645312E 3465366N
	T29	South Bosque West of Highway 317	650074E 3473895N
	T30	Harris Creek at Windsor Road	653243E 3401660N
	T31	Willow Creek at Highway 2416	653479E 3478494N
	T32	Willow Creek 2 at Highway 2416	655924E 3478058N
	T33	South Bosque at Indian Trail	655034E 3476011N
	T34	Harris Creek at Highway 84 near the Executive Airport	659529E 3483444N
	T35	South Bosque at Highway 84	664177E 3484767N

[a]see **Figure 5-1** for approximate locations.

5.1.1.1.4 Discussion

Historical data and several months of periodic sampling of surface water in and around the study area provided some details as to where perchlorate exposures had the highest potential to occur:

- Station Creek south of NWIRP (T20-T23)
- The spring on Oglesby Road (T18)
- Harris Creek from Highway 84 to areas east of McGregor (T19, T13)

- An unnamed tributary near the wastewater treatment plant at Highway 317 (T14)
- S Creek at Highway 317 (T15)

The highest perchlorate concentrations (range = 67 - 540 ppb) were consistently observed in S Creek near Highway 317 (T15). Overall, perchlorate contamination of surface water within the study area varies with location, season, and rainfall conditions, with the exception of the spring on Oglesby Road (T18) (range = 26 - 67 ppb). Most of these surface water bodies were surrounded by vegetation (likely exposure pathway to terrestrial animals) and supported aquatic life (potential exposure pathway to humans). The spring on Oglesby Road (T18) had surface water flow most of the year, serving as a source of exposure even during drought periods. Property access indicated that larger mammals (beef cattle) also used the spring water on this site, as well as areas surrounding Station Creek near the NWIRP property (T20-T22).

5.1.1.2 Auto Samples

5.1.1.2.1 Introduction

Fifteen automated surface water monitoring stations were installed along streams that discharge to Lake Waco and Lake Belton as described in Section 4.1.1. Each monitoring station was programmed to collect surface water samples and to measure and record stream level and rainfall data. These monitoring stations are listed in **Table 5-2** and their locations are shown on **Plate 1**.

Table 5-2
Longitudinal Stream Monitoring Station Locations

Station ID	Location	Lake discharged to by Stream
SC1	Station Creek at A&M Property	Lake Belton
TRM1	Tributary M at A&M Property	Lake Belton
SC3	Station Creek at 107	Lake Belton
OC1	Onion Creek at 107	Lake Belton
SC5	Station Creek at Oglesby Neff Park Road	Lake Belton
LR1	Leon River at 236	Lake Belton
HC1	Harris Creek at Middle Windsor Road	Lake Waco
HC2	Harris Creek near Val Verde Road	Lake Waco
SBR3	South Bosque River at Church Road	Lake Waco
SBR1	South Bosque River at Indian Trail	Lake Waco
SBR2	South Bosque River near Cottonbelt Parkway	Lake Waco
SBR4	South Bosque River near Ruff Road	Lake Waco
SBR5	South Bosque River at 84	Lake Waco
MBR1	Middle Bosque River, downstream of intersection of South Bosque River	Lake Waco
CHC1	Cowhouse Creek at Tank Destroyer Road	Lake Belton

The monitoring stations were installed to collect perchlorate concentration data and associated stream level data at various points along each stream to determine what concentrations and flow may be entering Lake Waco and Lake Belton from each of the streams. The Cowhouse Creek monitoring station was used to help determine if runoff from Fort Hood contributes perchlorate to Lake Belton. All sampling methodologies and protocols followed during the Study are detailed in the *Final Longitudinal Stream Sampling Study Field Sampling Plan* (MWH, 2002b). Deviations from the Field Sampling Plan are discussed further below.

5.1.1.2.2 Methodology

Each auto sampler was programmed to collect periodic grab surface water samples for perchlorate analysis. Samples were collected at the same time during most sampling events. However, to evaluate a variety of hydrologic scenarios, grab sampling at different times as well as sampling in conjunction with rainfall was also performed. The surface water samples were collected in plastic sample bottles and were stored within the auto sampler until retrieved by the field crew. In addition to the grab samples, stream level and rainfall data were collected every 15 minutes. Level and rainfall data are discussed in later sections.

Equipment. The following equipment was installed at each of the 15 automated monitoring station locations, except as noted below:

- ISCO 4230 Flow Meter (equipped with a bubbler and a rain gauge). No rain gauge was installed in station SBR5 (South Bosque River at US 84) because the station was located under a bridge.
- ISCO 6712 Auto Sampler.
- PVC pipe to run sample tubing and bubbler to the center of the stream.
- 12-volt battery and solar panel to recharge the battery.
- An additional data logger to store groundwater-elevation data from nearby monitoring well (monitoring well information is presented in Section 5.1.3).
- A protective portable steel enclosure to house the flow meter, auto sampler, battery, and data logger.

Photographs of typical automated stream sampling stations are shown in **Figure 5-2**, **Figure 5-3**, and **Figure 5-4**.

Figure 5-2
Typical Longitudinal Stream Sampling Station with Monitoring Well

Figure 5-3
Rain Gauge and Flow Meter at a Station

Figure 5-4
PVC Pipe Containing Bubbler for Level and Strainer for Stream Sampling

Standard Sampling. The ISCO sampler was programmed to collect data at the following frequencies under standard sampling conditions:

- Once daily for the first 15 days.
- Once weekly for the next three weeks
- Once every two weeks for the remainder of the study (i.e., 12 months).

During standard bi-weekly surface water sampling, the field crew visited the sampling stations at least every 14 days to collect samples, reload the auto sampler, download data from the data loggers, check the operation of the metering station and equipment, and perform any needed maintenance activities. The field crew attempted to pick up and ship the samples within 24 hours of sample collection.

Storm Event Sampling. Storm sampling was necessary to determine how perchlorate concentrations in the study area streams may vary based on increased surface water and "first flush" groundwater flows that occur immediately after a storm event. Factors that contribute to defining a significant storm event included rainfall (total and duration), and change in stream level.

During storm event sampling, the automated monitoring stations were taken out of standard sampling mode and were reprogrammed to detect rainfall in the rain gauge and automatically start sampling during a significant storm event. The standard sampling during this time was conducted by utilizing the "manual" function on the autosampler.

After evaluating the various types of storms that occur in the study area and gathering stream flow, groundwater level and rainfall data for a period of time to better understand the interaction of rainfall, stream height, and depth to groundwater, a significant storm criteria was determined as 0.1 inches of rain within a 15-minute time period. Discussions with ISCO, the equipment vendor, verified this determination as a typical criterion used for determining storm events in Texas. Each sampler was programmed to collect samples every four hours for 14 days. The samples were picked up as frequently as possible, at a minimum of every three days. Two storm events were studied during the course of the study: May 1 through May 16, 2003 and September 11 through October 2, 2003.

Duplicate Samples. Blind duplicate samples were collected at all sampling locations at a frequency to represent 10% of the environmental samples collected and MS/MSD samples were collected at a frequency to represent 5% of the environmental samples collected.

Sample Designation. Each surface water sample was designated with an alphanumeric character string set apart by hyphens. The designation began with the stream name abbreviation and monitoring station number (e.g., "SBR1" for South Bosque River monitoring station 1, "HC1" for Harris Creek monitoring station 1, etc.), followed by "SW" to indicate a surface water sample, and finally by the date and military time the sample was collected. For example, the surface water sample collected from Station Creek monitoring station 1 at 16:15 on November 18, 2002 was designated "SC1- SW-11-18-1615".

Blind duplicate samples were designated with a fictitious number so that the laboratory was unaware of where the sample was taken. For example, the blind duplicate sample collected with environmental sample "SC1-SW-11-18-1615" was designated "SW-1001". The field crew kept careful records of the designations given to the blind duplicate samples and their corresponding environmental sample so that the analytical results could be correlated when they were received. Each MS/MSD sample had the same designation as its associated environmental sample except that "MS" or "MSD" followed the sample designation (e.g., "SC1-SW-11-18-1615 MS" and "SC1-SW-11-18-1615 MSD").

Sampling Comments. If a sample was not collected because the sample intake was above the water level, the sample was designated as "Dry". If a sample was not collected due to equipment error, the sample was designated as "Equipment Error". The holding time for perchlorate is 28 days, but most samples were collected within 24 hours. When samples were not picked up within 24 hours, the samples were marked as "> 24 Hours" for quality tracking purposes.

Sample Analysis. All surface water samples were analyzed for perchlorate by USEPA Method 314.0 at the USACE Engineer Research and Development Center Environmental Laboratory at the Environmental Chemistry Branch in Omaha, Nebraska. (See **Appendix V**). The data verification report for the samples analyzed is included in **Appendix W**.

Field Observations. The field crew also collected the following field observations during each visit to each sampling station.

- Cloud cover
- Wind velocity
- Secchi Disk transparency
- Water color
- Aquatic vegetation in percent cover
- Instantaneous flow rate.

In addition, the following field measurements were obtained through the use of a Hydrolab Mini Sonde 4a (multi-parameter instrument):

- Temperature
- Dissolved oxygen
- Specific conductance
- pH
- Salinity
- Dissolved oxygen percent saturation.

The field observations and Hydrolab water quality data collected as part of this study are presented in **Appendix D**.

5.1.1.2.3 Data

This section summarizes the perchlorate analytical data collected over the course of the study at each automated monitoring station location. As previously discussed, the former NWIRP McGregor plant is located on a topographic high near the confluence of four watersheds. The divide for two of these watersheds, the Leon River watershed and the Bosque River watershed is located on the plant site. Based on this watershed divide, contaminants located in the southwest section of the plant drain into local streams within the Leon River watershed, to the Leon River and finally into Lake Belton. Surface and groundwater from all other plant locations drain into local streams that flow into the Middle Bosque River and then into Lake Waco. For ease of discussion and understanding, the stream network has been divided into two stream segment study areas (NWIRP to Lake Belton and NWIRP to Lake Waco) and will be discussed moving from upstream to downstream in the following sections. An additional tributary to Lake Belton, Cowhouse Creek, was also evaluated during this study to determine if runoff from Fort Hood might be contributing detectable concentrations of perchlorate to Lake Belton.

Bi-weekly and storm samples from streams were collected throughout the study from October 17, 2002 to October 2, 2003. Bi-weekly samples were collected from October 17, 2002 to August 28, 2003. Data were also collected during two storm events during

this study. The first set of storm sampling data were collected between May 1, 2003 and May 16, 2003, and the second set of storm sampling data were collected between September 11, 2003 and October 2, 2003.

5.1.1.2.3.1 Biweekly Sampling

This section summarizes data from the biweekly sampling conducted at all the automated monitoring stations. Samples that had perchlorate concentrations below the MDL (<1 µg/L) are reported as "<1 µg/L" in the data tables and are plotted at 0.5 µg/L on the data plots. Similarly, the average perchlorate concentration presented for each station was calculated using 0.5 µg/L for samples that were below the MDL (<1 µg/L). Refer to **Appendix E** for tables and plots of bi-weekly sampling data at all of the stations.

5.1.1.2.3.1.1 NWIRP to Lake Belton

Station Creek at A&M property (SC1)

Monitoring station SC1 is located on Station Creek, approximately 0.4 mile downstream of the NWIRP boundary, on the Texas A & M property. The project team attempted to collect 48 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, one sample could not be collected due to the site being dry. Perchlorate was not detected in any of the 47 samples (100 percent) collected. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

Tributary M at A&M property (TRM1)

Monitoring station TRM1 is located on Tributary M (informal name) on the Texas A& M property just off the NWIRP property. The project team attempted to collect 52 samples at this location as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, five samples could not be collected because the site was dry, and two samples could not be collected due to equipment error. Perchlorate was not detected in 10 of the samples collected (22.2 percent). Perchlorate concentrations in three of the samples collected (6.7 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and were flagged as estimated values. Perchlorate concentrations in 32 samples ranged between the RL (4 µg/L) and 111 µg/L. The average perchlorate concentration at this site was 10.1 µg/L and the maximum perchlorate concentration was 111 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

Station Creek at 107 (SC3)

Monitoring station SC3 is located where State Highway 107 crosses Station Creek. This location is approximately one mile downstream of the intersection of Tributary M and Station Creek and is approximately 1.5 miles downstream from the NWIRP boundary along Station Creek. The project team attempted to collect a total of 42 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, one sample could not be collected due to the site being dry, and four samples could not be collected due to equipment error. Perchlorate was not detected in seven samples (18.9 percent) of the samples collected. Perchlorate concentrations in 14 of the samples collected (37.8 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. Perchlorate concentrations in 16 samples ranged between the RL (4 µg/L) and 38 µg/L. The average perchlorate concentration at this site was 5.11 µg/L, and

the maximum perchlorate concentration was 38 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

Onion Creek at 107 (OC1)

Monitoring station OC1 is located where Highway 107 crosses Onion Creek, approximately 2.2 miles downstream of the NWIRP boundary. The project team attempted to collect a total of 47 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, three samples could not be collected due to the site being dry. Perchlorate was not detected in 43 samples (97.7 percent) of the samples collected. The perchlorate concentration in one sample (2.3 percent) was between the MDL (1 µg/L) and the RL (4 µg/L) and is flagged as estimated. No samples had perchlorate concentrations above the RL (4 µg/L). The average perchlorate concentration at this site was 0.56 µg/L, and the maximum perchlorate concentration at this site was 3 µg/L. The maximum perchlorate concentration was detected in a duplicate sample collected on October 30, 2002. No perchlorate was detected in the original sample. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

Station Creek at Oglesby Neff Park Road (SC5)

Monitoring station SC5 is located on Station Creek near Oglesby Neff Park Road. This station is near the confluence of Station Creek and the Leon River, approximately 5.2 miles downstream of the NWIRP boundary and 1.4 miles downstream of the intersection of Onion Creek and Station Creek. This stream discharges into the Leon River. The project team attempted to collect a total of 39 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, 14 samples could not be collected due to the site being dry, and three samples could not be collected due to equipment error. Perchlorate was not detected in two (9.1 percent) of the samples collected. Perchlorate concentrations in 14 samples (63.6 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. Perchlorate concentrations in six samples ranged between the RL (4 µg/L) and 7.6 µg/L. The average perchlorate concentration at this site was 2.73 µg/L, and the maximum perchlorate concentration was 7.6 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

Leon River at 236 (LR1)

Monitoring station LR1 is located at the intersection of the Leon River and State Highway 236, approximately 6.5 miles downstream of the NWIRP boundary along Station Creek. The Leon River flows directly into Lake Belton. The project team attempted to collect a total of 37 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these, one sample could not be collected due to equipment error. Perchlorate was not detected in any of the 36 samples (100 percent) collected. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

5.1.1.2.3.1.2 NWIRP to Lake Waco

Harris Creek at Middle Windsor Road (HC1)

Monitoring station HC1 is located at the intersection of Harris Creek and Middle Windsor Road just outside the city boundary of McGregor, Texas, approximately 3.6 miles downstream of the NWIRP boundary along Harris Creek. The project team collected a total of 43 samples as part of the bi-weekly sampling from October 17, 2002 to August 17, 2003. Perchlorate was not detected in 21 (48.8 percent) of the samples collected. Perchlorate concentrations in 15 samples (34.9 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. Perchlorate concentrations in seven samples ranged between the RL (4 µg/L) and 6.5 µg/L. The average perchlorate concentration at this site was 1.81 µg/L and the maximum concentration was 6.5 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

Harris Creek near Val Verde Road (HC2)

Monitoring station HC2 is located on Harris Creek, approximately 7.2 miles downstream of the NWIRP boundary along Harris Creek, near Val Verde Road. The project team attempted to collect a total of 42 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, one sample could not be collected due to the site being dry, and one sample could not be collected due to equipment error. Perchlorate was not detected in 22 (55.0 percent) of the samples collected. Perchlorate concentrations in 16 samples (40.0 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. Two samples collected had perchlorate concentrations between the RL (4 µg/L) and 4.8 µg/L. The average perchlorate concentration at this site was 1.25 µg/L and the maximum perchlorate concentration was 4.8 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

South Bosque River at Church Road (SBR3)

Monitoring station SBR3 is located on the South Bosque River at its intersection with Church Road, approximately 12 miles downstream of the NWIRP boundary. The project team attempted to collect a total of 36 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these, six samples could not be collected due to equipment error. Perchlorate was not detected in 21 (70.0 percent) of the samples collected. Perchlorate concentrations in nine samples (30.0 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL (4 µg/L). The average perchlorate concentration at this site was 0.88 µg/L, and the maximum perchlorate concentration was 2 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

South Bosque River at Indian Trail (SBR1)

Monitoring station SBR1 is located at the intersection of the South Bosque River and Indian Trail Road, approximately 1.6 miles downstream of the NWIRP boundary. The project team attempted to collect a total of 38 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these, three samples could not be collected due to equipment error. Perchlorate was not detected in seven samples (20.0 percent) of

the samples collected. Perchlorate concentrations in 16 samples (45.7 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. Perchlorate concentrations in twelve samples collected ranged between the RL (4 µg/L) and 4.7 µg/L. The average perchlorate concentration at this site was 2.73 µg/L, and the maximum perchlorate concentration was 4.7 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

South Bosque River near Cotton Belt Parkway (SBR2)

Monitoring station SBR2 is located on the South Bosque River near Cotton Belt Parkway, approximately 6.4 miles downstream of the NWIRP boundary. The project team attempted to collect a total of 36 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, one sample could not be collected due to equipment error. Perchlorate was not detected in 16 (45.7 percent) of the samples collected. Perchlorate concentrations in 19 samples of the samples collected (54.3 percent) were between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL (4 µg/L). The average perchlorate concentration at this site was 1.51 µg/L, and the maximum perchlorate concentration was 3 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

South Bosque River near Ruff Road (SBR4)

Monitoring station SBR4 is located on the South Bosque River near Ruff Road, approximately 10 miles downstream of the NWIRP boundary. The project team attempted to collect a total of 41 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, four samples could not be collected due to equipment error and one sample was broken in shipment. Perchlorate was not detected in 23 (63.9 percent) of the samples collected. Perchlorate concentrations in 13 of the samples collected (36.1 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL (4 µg/L). The average perchlorate concentration at this site was 1.10 µg/L, and the maximum perchlorate concentration was 3 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

South Bosque River at 84 (SBR5)

This monitoring station is located on the South Bosque River at its intersection with Highway 84, approximately 13.2 miles downstream of the NWIRP boundary along the South Bosque. This stream discharges into the Middle Bosque River. The project team attempted to collect a total of 38 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, three samples could not be collected due to equipment error. Perchlorate was not detected in 25 (71.4 percent) of the samples collected. Perchlorate concentrations in 10 of the samples collected (28.6 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL (4 µg/L). The average perchlorate concentration at this site was 0.81 µg/L and the maximum perchlorate concentration was 2 µg/L. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

Middle Bosque River (MBR1)
Monitoring station MBR1 is located on the Middle Bosque River approximately 14 miles downstream of the NWIRP boundary along the South Bosque. It is downstream of the intersection of South Bosque River and Highway 84. The Middle Bosque River discharges directly into Lake Waco. The project team attempted to collect a total of 37 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these, two samples could not be collected due to equipment error. Perchlorate was not detected in 35 (100 percent) of the samples collected. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

5.1.1.2.3.1.3 Cowhouse Creek at Tank Destroyer Road (CHC1)

Monitoring station CHC1 is located on Cowhouse Creek at the intersection of Tank Destroyer Road, before Cowhouse Creek discharges into Lake Belton. The project team collected a total of 38 samples as part of the bi-weekly sampling from October 17, 2002 to August 28, 2003. Of these samples, one sample was broken in shipment. Perchlorate was not detected in 37 (100 percent) of the samples collected. The bi-weekly perchlorate data table and plot for this station are included in **Appendix E**.

5.1.1.2.3.2 Storm Sampling

The first storm-sampling event was started on May 1, 2003 (summer season) when most of the streams were flowing and groundwater levels were high. The second storm-sampling event was started on September 11, 2003 (fall season), when most of the streams were dry and the groundwater level was among the lowest encountered in the year. The exact time storm sampling was triggered at each station depended on the rainfall at that location. Some stations did not trigger automatically due to tree cover or due to being located under a bridge and were manually started by the project team. Even during a storm event, there were samples that had to be designated as "dry" because there was insufficient water to collect a sample. The following is a summary of the storm sampling collected at each of the automated monitoring stations. As with preceding sections, the monitoring station results are discussed in order from upstream to downstream. The average perchlorate concentration reported for each station was calculated assuming a value of 0.5 µg/L for all samples below the MDL (<1 µg/L). Refer to **Appendix F** for tables and plots of perchlorate data during the first storm-sampling event and to **Appendix G** for tables and plots of perchlorate data during the second storm-sampling event. Samples that had perchlorate concentrations below the MDL (<1 µg/L) are listed as "<1 µg/L" in the data table and are plotted at 0.5 µg/L on the graphs.

5.1.1.2.3.2.1 NWIRP to Lake Belton

Station Creek at A&M property (SC1)
The project team collected a total of 93 samples as part of the storm sampling from May 1, 2003 to May 16, 2003. Perchlorate was not detected in any of the samples collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 116 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. None of these samples could be collected because the site was dry. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

Tributary M at A&M property (TRM1)
The project team collected a total of 92 samples as part of the storm sampling from May 1, 2003 to May 16, 2003. Six of these samples could not be analyzed because the samples were broken during shipment. Perchlorate was not detected in 86 samples (100 percent) of the samples analyzed. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 122 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, 110 samples could not be collected due to the site being dry. Perchlorate was not detected in eight samples (66.7 percent) of the samples collected. Perchlorate concentrations in four of the samples collected (33.3 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the Reporting Limit (4 µg/L). During this storm event, the average perchlorate concentration at this site was 1.25 µg/L, and the maximum perchlorate concentration at this site was 3 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

Station Creek at 107 (SC3)
The project team collected a total of 93 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in 32 (34.4 percent) of the samples collected. Perchlorate concentrations in 29 of the samples collected (31.2 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. A total of 32 samples had perchlorate concentrations between the RL(4 µg/L) and 11 µg/L. During this storm, the average perchlorate concentration at this site was 2.87 µg/L and the maximum perchlorate concentration was 11 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 132 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Two of these samples could not be collected because the site was dry, and three samples could not be collected due to equipment error. Perchlorate was not detected in 127 samples (100 percent) of the samples collected during this storm. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

Onion Creek at 107 (OC1)
The project team collected a total of 93 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in any of the 93 samples collected (100 percent). The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 120 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, 115 samples could not be collected because the site was dry. Perchlorate was not detected in 5 samples (100 percent) of the samples collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

Station Creek at Oglesby Neff Park Road (SC5)
The project team attempted to collect a total of 84 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. None of these samples could be collected because the site was dry. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 121 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. None of these samples could be collected because the site was dry. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

Leon River at 236 (LR1)
The project team collected a total of 90 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in 90 (100 percent) of the samples collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 132 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, 28 samples could not be collected due to equipment error. Perchlorate was not detected in 104 samples (100 percent) of the samples collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

5.1.1.2.3.2.2 NWIRP to Lake Waco

Harris Creek at Middle Windsor Road (HC1)
The project team collected a total of 95 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in 44 (46.3 percent) of the samples collected. Perchlorate concentrations in 51 of the samples collected (53.7 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL. During this storm, the average perchlorate concentration at this site was 0.96 µg/L and the maximum perchlorate concentration was 2 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 128 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, one sample could not be collected due to equipment error. Perchlorate was not detected in 125 (98.4 percent) of the samples collected. Perchlorate concentrations in two of the samples collected (1.6 percent) were at the MDL (1 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL. During this storm, the average perchlorate

concentration at this site was 0.51 µg/L, and the maximum perchlorate concentration was 1 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

Harris Creek near Val Verde Road (HC2)
The project team collected a total of 98 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in 95 (96.9 percent) of the samples collected. Perchlorate concentrations in three of the samples collected (3.1 percent) were between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL. During this storm, the average perchlorate concentration at this site was 0.54 µg/L, and the maximum perchlorate concentration was 2 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 126 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, one sample could not be collected because the site was dry, and three samples could not be collected due to equipment error. Perchlorate was not detected in 122 samples (100 percent) of the samples collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

South Bosque River at Church Road (SBR3)
The project team attempted to collect a total of 90 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Of these samples, 52 samples could not be collected due to equipment errors resulting from high sedimentation on the strainer. Perchlorate was not detected in any of the 38 samples (100 percent) collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 112 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. One of these samples could not be collected because the site was dry, and 41 samples could not be collected due to equipment errors resulting from high sedimentation on the strainer. One sample was broken in shipment. Perchlorate was not detected in any of the 69 samples (100 percent) collected and analyzed. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

South Bosque River at Indian Trail (SBR1)
The project team collected a total of 92 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in 69 (75.0 percent) of the samples collected. Perchlorate concentrations in 22 of the samples collected (23.9 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. One sample collected had a perchlorate concentration at the RL (4 µg/L). During this storm, the average perchlorate concentration at this site was 0.94 µg/L, and the maximum perchlorate concentration was 4 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 125 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, 47 samples could not be collected due to equipment error. Perchlorate was not detected in 57 (73.1 percent) of the samples collected. Perchlorate concentrations in 21 of the samples collected (26.9 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL. During this storm, the average perchlorate concentration at this site was 0.71 µg/L and the maximum perchlorate concentration was 2 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

South Bosque River near Cotton Belt Parkway (SBR2)
The project team collected a total of 102 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in 88 (86.3 percent) of the samples collected. Perchlorate concentrations in 14 of the samples collected (13.7 percent) ranged between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL. The average perchlorate concentration at this site was 0.63 µg/L, and the maximum perchlorate concentration was 2 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 133 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, seven samples could not be collected because the site was dry, three samples could not be collected due to equipment error, and one sample bottle was broken in shipment. Perchlorate was not detected in any of the 122 samples collected (100 percent). The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

South Bosque River near Ruff Road (SBR4)
The project team collected a total of 94 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in 90 (95.7 percent) of the samples collected. Perchlorate concentrations in four of the samples collected (4.3 percent) were at the MDL (1 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL. The average perchlorate concentration at this site was 0.52 µg/L and the maximum perchlorate concentration was 1 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 130 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, one sample could not be collected because the site was dry, and one sample could not be collected due to equipment error. Perchlorate was not detected in any of the 128 samples (100 percent) collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

South Bosque River at 84 (SBR5)
The project team collected a total of 93 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in 90 (96.8 percent) of the samples

collected. Perchlorate concentrations in 3 of the samples collected (3.2 percent) were between the MDL (1 µg/L) and the RL (4 µg/L) and are flagged as estimated. No samples had perchlorate concentrations above the RL. The average perchlorate concentration at this site was 0.53 µg/L, and the maximum perchlorate concentration was 2 µg/L. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 120 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Of these samples, 68 samples could not be collected because the site was dry, and seven samples could not be collected due to equipment error. Perchlorate was not detected in any of the 45 samples (100 percent) collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

Middle Bosque River (MBR1)

The project team collected a total of 88 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in any of the 88 samples (100 percent) collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 101 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Six of these samples could not be collected due to equipment error. Perchlorate was not detected in any of the 95 samples (100 percent) collected. The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

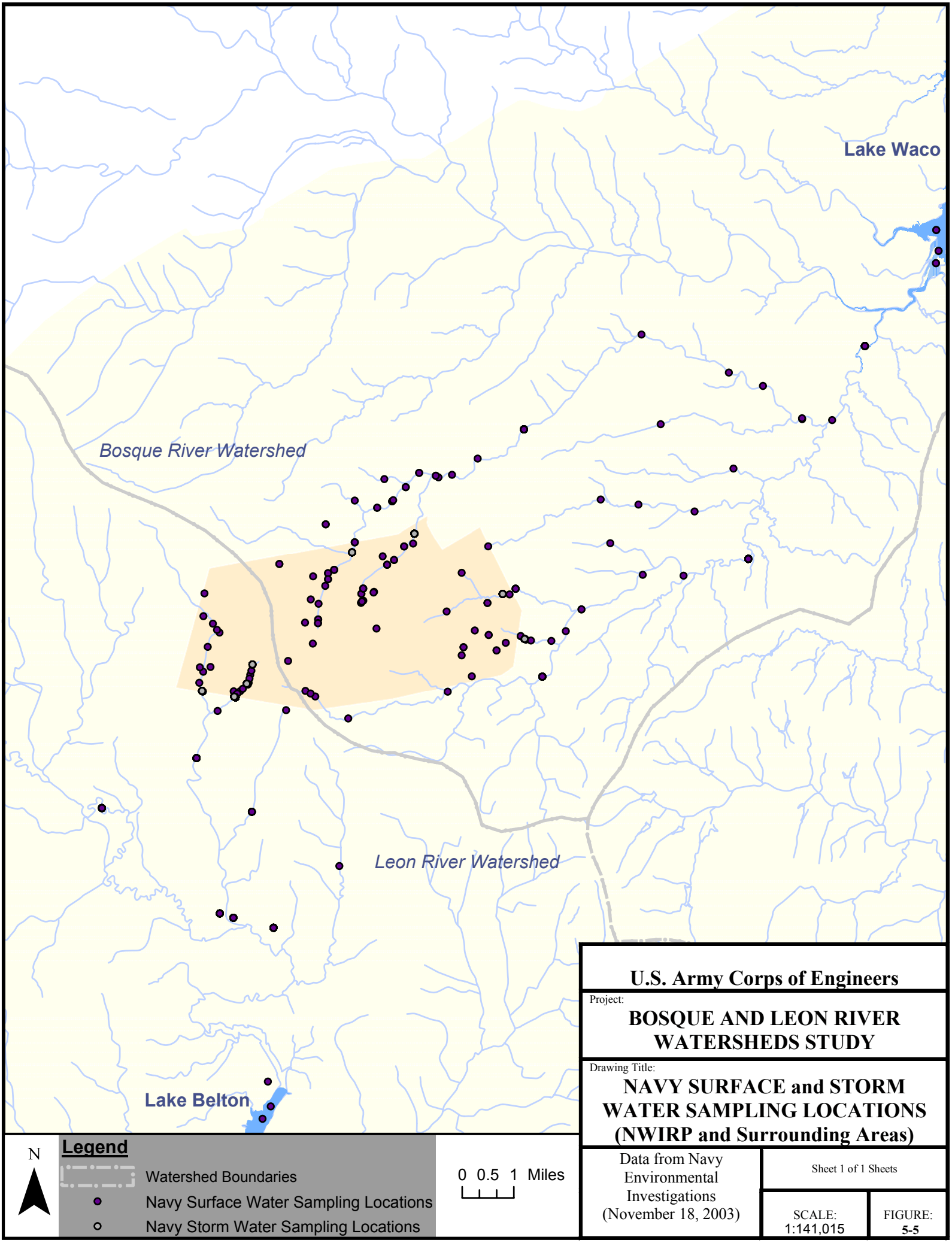
5.1.1.2.3.2.3 Cowhouse Creek at Tank Destroyer Road (CHC1)

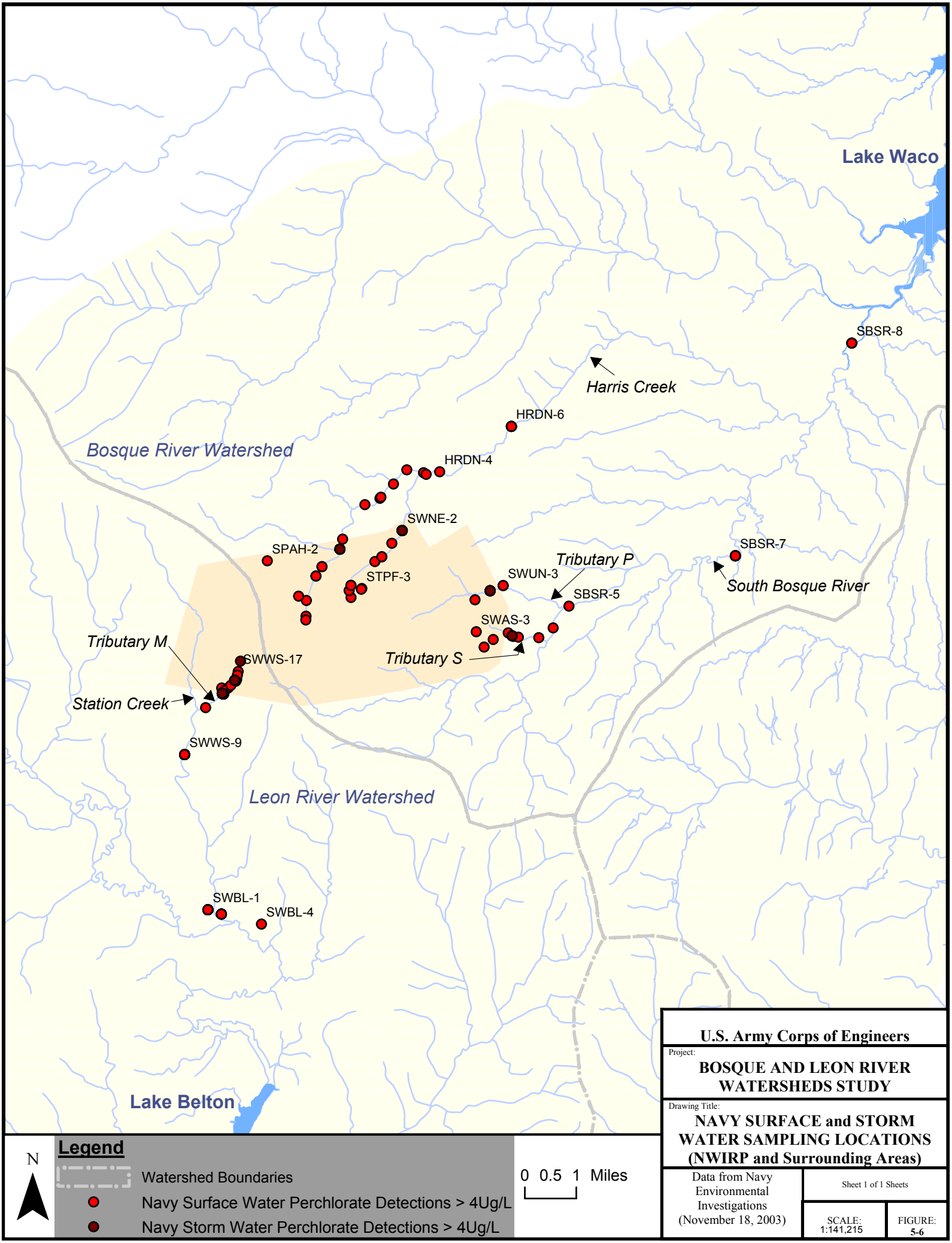
The project team collected a total of 95 samples as part of the storm sampling from May 2, 2003 to May 16, 2003. Perchlorate was not detected in any of the 95 samples collected (100 percent). The perchlorate data table and plot for this station during this storm event are included in **Appendix F**.

The project team attempted to collect a total of 132 samples as part of the storm sampling from September 11, 2003 to October 2, 2003. Two of these samples could not be collected due to equipment error. Perchlorate was not detected in any of the 130 samples collected (100 percent). The perchlorate data table and plot for this station during this storm event are included in **Appendix G**.

5.1.1.2.4 Historical Data

The U.S. Navy conducted surface water sampling for perchlorate between 1998 and 2003 and storm water sampling at the perimeter of NWIRP McGregor in 2000 and 2001. The locations of the surface water and storm water sampling points are shown in **Figure 5-5**. All locations that had a detection greater than 4 µg/L at any time are shown on **Figure 5-6**. Detailed results of all sampling conducted by the Navy are presented in the *Draft-Final Groundwater Investigation Phase III Report* (EnSafe, 2003). A summary of the historical perchlorate analytical results from this report is presented below.


Lake Waco
Bosque River Watershed
Leon River Watershed
Lake Belton
N
Legend
Watershed Boundaries
Navy Surface Water Sampling Locations
Navy Storm Water Sampling Locations
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SURFACE and STORM WATER SAMPLING LOCATIONS (NWIRP and Surrounding Areas)
Data from Navy Environmental Investigations (November 18, 2003)
Sheet 1 of 1 Sheets
SCALE: 1:141,015
FIGURE: 5-5


Lake Waco
SBSR-8
Harris Creek
HRDN-6
Bosque River Watershed
HRDN-4
SWNE-2
SPAH-2
STPF-3
SBSR-7
Tributary P
SWUN-3
SBSR-5
South Bosque River
SWAS-3
Tributary M
SWWS-17
Tributary S
Station Creek
SWWS-9
Leon River Watershed
SWBL-1
SWBL-4
Lake Belton
N
Legend
Watershed Boundaries
Navy Surface Water Perchlorate Detections > 4Ug/L
Navy Storm Water Perchlorate Detections > 4Ug/L
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SURFACE and STORM WATER SAMPLING LOCATIONS (NWIRP and Surrounding Areas)
Data from Navy Environmental Investigations (November 18, 2003)
Sheet 1 of 1 Sheets
SCALE: 1:141,215
FIGURE: 5-6

5.1.1.2.4.1 NWIRP To Lake Belton

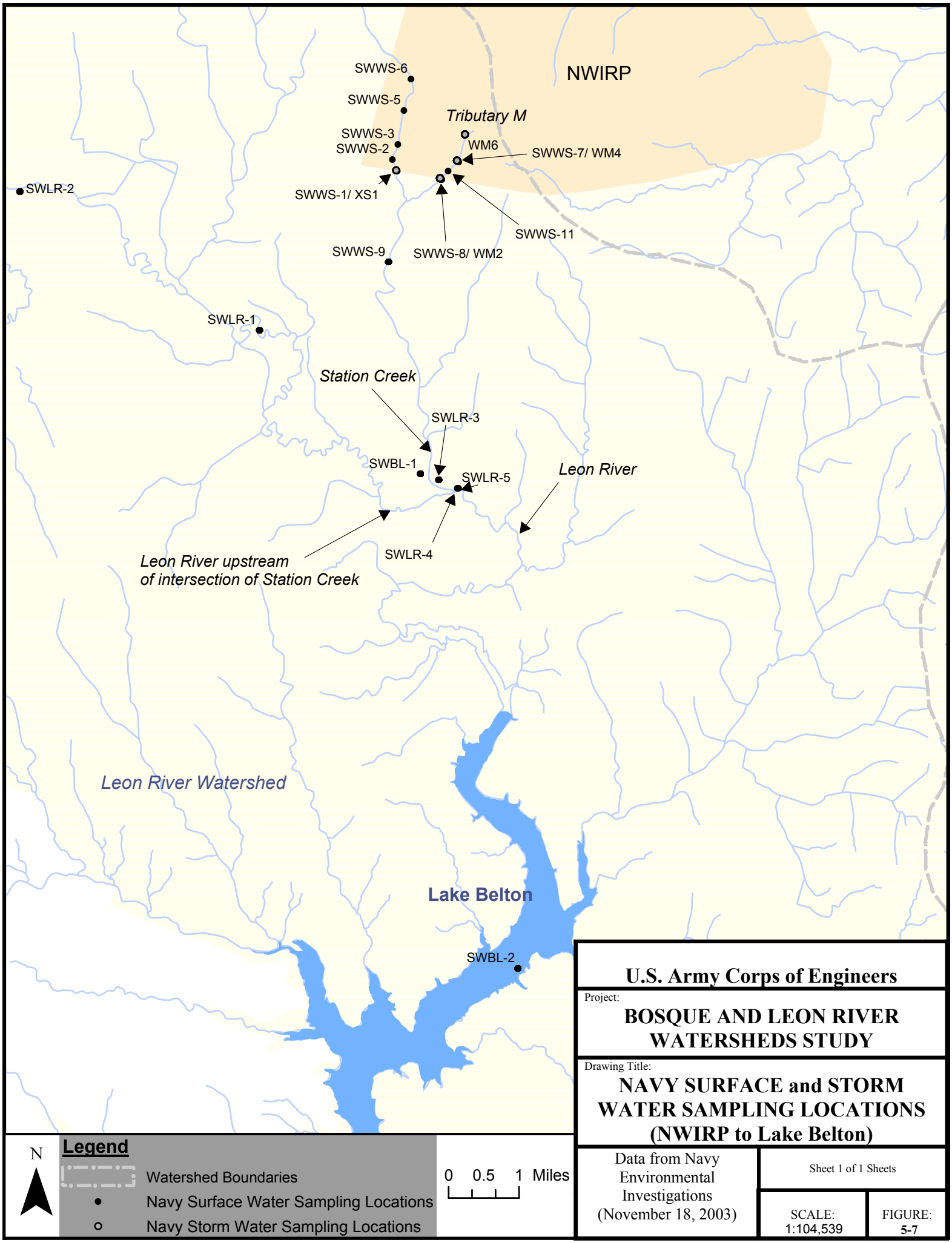
This section discusses the history of U.S. Navy perchlorate sampling conducted from NWIRP to Lake Belton. The locations where perchlorate was detected are discussed further below and are shown in **Figure 5-7**.

Station Creek Upstream of Intersection of Tributary M

In 1998, the surface water quality of Station Creek was initially evaluated by collecting five grab samples (SWWS-2, SWWS-3, SGAM-1 (*not in map*), SWWS-5 and SWWS-6) which were all non-detect for perchlorate. Eighteen additional surface water samples were collected from Station Creek (SWWS-1) upstream of the intersection of Tributary M between January 8, 1999 and September 1, 2002 and analyzed for perchlorate. Of these samples, two samples had perchlorate detections of 3.8 J µg/L (March 2000) and 12 µg/L (October 2001). Storm water samples were collected from March to June 2000. Ten rain events were recorded, and 40 grab and composite samples were collected from this stream section. All were non-detect for perchlorate except for one composite sample which had a detection of 3.8 J µg/L. Storm sampling was also conducted in November 2000, and January, May, and September 2001 at XS1 (Station Creek and the NWIRP South Property line). All the samples collected were non-detect for perchlorate during each event.

Tributary M

Surface water in Tributary M has been closely monitored since January 1999 to evaluate the effectiveness of the Interim Stabilization Measures (ISM) system. This system was installed in 1999 along the southern boundary of NWIRP near Tributary M to capture contaminated groundwater before it flows offsite. SWWS-8 is a monthly sampling location where Tributary M exits the property. From January to June 1999, samples were analyzed for perchlorate at this location. Before the ISM was in place, perchlorate was detected four times in SWWS-8 samples: 5,500 µg/L (1/11/99), 3,600 µg/L (4/19/99), 4,700 µg/L (5/11/99), and 3,100 µg/L (7/16/99). Surface water samples were then collected along Tributary M at various time intervals and distances to better define perchlorate contamination in surface water and groundwater so that an interim response action plan could be implemented. In January 1999, SWWS-7 was collected at Tributary M at the perimeter road and was analyzed for perchlorate. Perchlorate was detected at 2,300 µg/L. In February 1999, SWWS-11 was collected 800 feet north of SWWS-8 and analyzed for perchlorate. Perchlorate was detected at 1,800 µg/L. Eight surface water samples were collected on April 19, 1999 at different locations along Tributary M and analyzed for perchlorate. Based on this information and corresponding data, the U.S. Navy established that the groundwater-to-surface water pathway was the main mechanism for contaminant transfer along the southern boundary at NWIRP. As a result, in late summer and early fall of 1999, ISM trenches were put in place to intercept the groundwater-to-surface water pathway and to abate the offsite migration of perchlorate and other contaminants. The trenches were designed to capture, collect and treat groundwater that would normally be discharged to Tributary M under natural processes. These trenches were slightly modified to support an in-situ pilot test as in-situ permeable reactive barriers (PRBs) were shown to viable alternatives to pump-and-treat.


NWIRP
SWWS-6
SWWS-5
Tributary M
SWWS-3
SWWS-2
WM6
SWWS-7/ WM4
SWLR-2
SWWS-1/ XS1
SWWS-11
SWWS-9
SWWS-8/ WM2
SWLR-1
Station Creek
SWLR-3
SWBL-1
SWLR-5
Leon River
SWLR-4
Leon River upstream
of intersection of Station Creek
Leon River Watershed
Lake Belton
SWBL-2
N
Legend
Watershed Boundaries
Navy Surface Water Sampling Locations
Navy Storm Water Sampling Locations
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SURFACE and STORM WATER SAMPLING LOCATIONS (NWIRP to Lake Belton)
Data from Navy Environmental Investigations (November 18, 2003)
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SCALE: 1:104,539
FIGURE: 5-7

Initial results showed perchlorate could be reduced to non-detect levels in the PRB's. However, aquifer water levels rose in March 2000, and a seep developed at the intersection of the property line trench and Tributary M. Eighty-one samples were collected from the seep from March 6, 2000 and July 24, 2000, when the seep flow ceased. Fifty-six (56) samples were non-detect for perchlorate and the remaining 25 samples ranged from 5.2 µg/L to 4,200 µg/L. From July to September 2000, additional water storage capacity (8 million gallons) was constructed to help store additional water and control the seep during the next wet season. However, beginning in late October 2000, heavy rains began (200% above normal) and continued into November overwhelming the available storage capacity. Additional rainfall in late December caused the water level in the trench to intersect the Tributary M streambed and another seep began to flow. From January 5 to May 9, 2001, 123 grab samples were collected from the seep. Perchlorate was detected in all except two samples and ranged from non-detect to 5,300 µg/L and average 2,200 µg/L. Because water levels in the trench system have been pumped down since May 2001 to control the aquifer water level, Tributary M remains dry for most of the year, except during storm events. Four samples have been collected since May 2001 and perchlorate concentrations range from 4.1 µg/L (4/10/2002) to 480 µg/L (1/8/2002). Storm sampling was also conducted in 2000 at various points along Tributary M to access possible soil-surface water cross media contamination (WM2, WM4, WM6). WM2 recorded seven storm events and collected twenty-three grab samples which ranged from non-detect to 500 µg/L. WM4 recorded four storm events and collected 12 grab samples which ranged from non-detect to 26 µg/L. WM6 recorded two storm events and collected eight grab samples which were non-detect for perchlorate. Tributary M flows into a stock pond in the Texas A&M property after leaving the NWIRP plant. This stock pond was sampled three times and analyzed for perchlorate in March 2000 (7.3 µg/L), September 2000 (non-detect) and December 2000 (370 µg/L).

Station Creek Downstream of Intersection of Tributary M

An in-situ pilot system is installed downstream of the intersection of Station Creek and Tributary M. It begins at the Texas A&M south property line and extends 1,700 feet to the northeast. The system and pilot study results are presented in detail in the *Offsite In Situ Bioremediation (Bio-Borings) Pilot Study Report* (EnSafe, 2002).

Samples were collected from SWWS-9 (Station Creek at 107) whenever the stream was flowing from September 18, 1998 to September 1, 2002. Thirty-one (31) samples were collected which ranged from non-detect to 540 µg/L and averaged 88.5 µg/L. Before May 2001, when an emergency discharge order was in effect, the average perchlorate concentration was 144.5 µg/L with two non-detect values recorded at this time. The U.S. Navy findings indicate that between May 2001 and September 2002 the average concentration dropped to 20.4 µg/L (an 86% reduction).

Samples were collected from SWBL-1 (Station Creek under Park Road) between February 1999 and September 1, 2002. Results ranged from non-detect (4 µg/L) to 210 µg/L and averaged 36 µg/L. The average pre-May 2001 concentration was 59 µg/L. The U.S. Navy findings indicate that between May 2001 and September 2002 the average concentration dropped to 11 µg/L (an 82% reduction).

Leon River upstream of Intersection of Station Creek
Samples were collected upstream of the confluence of Station Creek and Leon River at SWLR-1 (Leon River at County Road 344 (3.4 miles upstream)) and SWLR-2 (Leon River at County Road 315 (6.5 miles upstream)) from July 1999 to September 1, 2002. Thirty-six samples were collected from each station and all were non-detect for perchlorate.

Leon River downstream of Intersection of Station Creek
Samples were also collected at different points downstream of the confluence of Station Creek and the Leon River to understand the mixing dynamics of the two bodies.

Twenty samples were collected from SWLR-3 (150 feet downstream) and ranged from non-detect (< 2 µg/L) to 27 µg/L. Nine of these samples were non-detect for perchlorate. The U.S. Navy found the perchlorate concentration at this location comparable to concentrations at Station Creek and Park Road (SWBL-1). Based on these results and a detailed flow and mixing analysis, the U.S. Navy concluded that SWBL-1 was considered to lie in a backwater area of Station Creek in the Leon River, and thus, not representative of Leon River water quality.

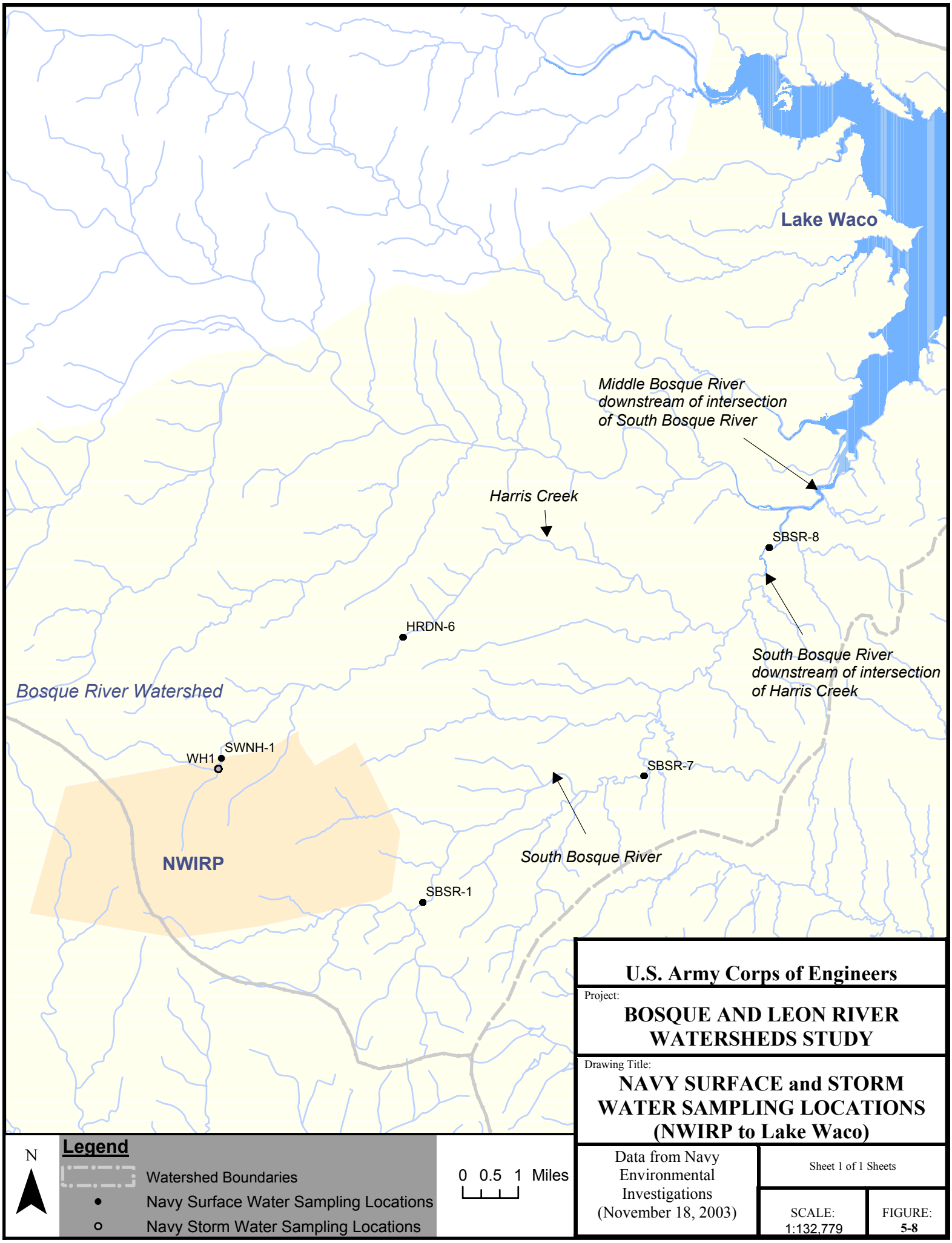
Six surface water samples were collected from two additional locations downstream of the confluence from SWLR-4 (250 feet downstream) and SWLR-5 (350 feet downstream), respectively from March 2002 to September 1, 2002. All samples collected from SWLR-4 and SWLR-5 were non-detect for perchlorate. The U.S. Navy has determined that perchlorate results from these locations are representative of the Leon River water quality.

Forty-two surface water samples were collected from SWBL-2 (Leon River at Highway 236). This location is upstream of where the Leon River enters Lake Belton and is 3,600 feet downstream of the confluence of Station Creek and the Leon River. Perchlorate results were non-detect except for April 8, 1999 when it was detected at 2.6 µg/L.

5.1.1.2.4.2 NWIRP to Lake Waco

This section discusses the history of the U.S. Navy sampling conducted from NWIRP to Lake Waco. The locations where perchlorate was detected are discussed further below in **Figure 5-8**.

Harris Creek
Twenty-one surface water samples were collected from Harris Creek at SWNH-1 between January 10, 1999 and September 1, 2002. Perchlorate concentrations ranged from non-detect to 1,600 µg/L. The 1,600 µg/L sample was collected on April 10, 2002, but the perchlorate concentration at the same location collected a week later was 7.1 µg/L. Excluding the 1,600 µg/L sample, perchlorate concentrations ranged from 1 µg/L to 15 µg/L in 12 samples with an average of 11.3 µg/L. Perchlorate was not detected in the remaining nine samples. Storm water samples were collected from March to June 2000. WH1 recorded five rain events and collected 17 grab samples. 11 were non-detect


Lake Waco
Middle Bosque River
downstream of intersection
of South Bosque River
Harris Creek
SBSR-8
HRDN-6
South Bosque River
downstream of intersection
of Harris Creek
Bosque River Watershed
SWNH-1
WH1
SBSR-7
NWIRP
South Bosque River
SBSR-1
N
Legend
Watershed Boundaries
Navy Surface Water Sampling Locations
Navy Storm Water Sampling Locations
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SURFACE and STORM WATER SAMPLING LOCATIONS (NWIRP to Lake Waco)
Data from Navy Environmental Investigations (November 18, 2003)
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SCALE: 1:132,779
FIGURE: 5-8

for perchlorate and six had concentrations ranging from 3.3 J µg/L to 24 µg/L. Monthly surface water samples were collected at HRDN-6 (Harris Creek and Windsor Road) when Harris Creek was flowing. Thirty-nine samples were collected from February 1999 to September 2002. Perchlorate was detected in 12 samples with concentrations ranging from 3.2 µg/L to 9.6 µg/L with an average of 5.2 µg/L.

South Bosque River

Monthly surface water samples have been collected from three locations along the South Bosque River since 1999. These are SBSR-1 (South Bosque River at Highway 317 and upstream of the confluence of Tributary S and Tributary P with the South Bosque River), SBSR-7 (South Bosque River at Cotton Belt Parkway and downstream of the confluence) and SBSR-8 (South Bosque Road at Old Lorena Road and downstream of the confluence).

Thirty monthly surface water samples were collected at SBSR-1 from July 1998 to September 1, 2002 when the stream was flowing. Perchlorate was detected at 0.45 µg/L in one of the samples collected. Thirty-six surface water samples were collected at SBSR-7 from February 1999 to September 1, 2002 when the river was flowing. Perchlorate was detected in 17 samples at an average of 6 µg/L and ranged from 4 µg/L to 9.9 µg/L. Thirty-nine surface water samples were collected further downstream at SBSR-8 from February 1999 to September 1, 2002 when the river was flowing. Perchlorate was detected in 10 samples at an average of 4.5 µg/L and ranged from 1.2 µg/L to 13 µg/L.

Based on the results of the stream monitoring program and the lake studies described in Section 5.1.2.4, the U.S. Navy concluded that perchlorate concentrations have, in general decreased from 1999. Perchlorate concentrations in surface water decreases away from the facility and is not present above the drinking water standard in area drinking water reservoirs. The U.S. Navy attributed the general decrease in perchlorate concentration reduction to various factors including absence of continued source loading (the facility ceased operation in 1996), ongoing introduction of clean water through precipitation, and the result of interim remedial actions completed to mitigate offsite migration of perchlorate in groundwater and surface water along the impacted tributaries; Tributary M (Fall 1999) and Tributary S (October 2002). The U.S. Navy also plans further remedial actions for the offsite property south of Area M in early summer of 2004.

5.1.1.2.5 Discussion

As in previous sections, the stream network has been divided into two stream segment study areas (NWIRP to Lake Belton and NWIRP to Lake Waco) and data are discussed moving from upstream to downstream in the following sections. An additional tributary to Lake Belton, Cowhouse Creek, was also evaluated during this study to determine if runoff from Fort Hood might be contributing detectable concentrations of perchlorate to Lake Belton. The following discussion summarizes all of the biweekly perchlorate data gathered for surface water as a part of this project. Average perchlorate concentrations reported here for each station are based on the bi-weekly samples.

5.1.1.2.5.1 NWIRP to Lake Belton

This section summarizes findings from perchlorate sampling in streams along the various surface drainage routes to Lake Belton collected during this study.

Tributary M - Station Creek - Leon River (TRM1, SC3, SC5, LR1)

Tributary M to Station Creek to the Leon River is one potential route for perchlorate contamination from NWIRP runoff to reach the Leon River and Lake Belton. As shown on **Figure 5-9**, perchlorate concentrations in Tributary M at TRM1 are at a maximum of 111 µg/L and at an average of 10.1 µg/L. This decreases to a maximum perchlorate concentration of 38 µg/L and an average perchlorate concentration of 5.11 µg/L in Station Creek at SC3 beyond the intersection of Tributary M and Station Creek. This further decreases to a maximum perchlorate concentration of 7.6 µg/L and an average perchlorate concentration of 2.73 µg/L in Station Creek at SC5 beyond the intersection of Station Creek and Onion Creek. Finally, there are no perchlorate detections in the Leon River at LR1 beyond its intersection with Station Creek.

Station Creek - Leon River (SC1, SC3, SC5, LR1)

Station Creek to the Leon River is a second potential route for perchlorate from NWIRP to reach Lake Belton via surface flow. As shown on **Figure 5-10**, there have been no perchlorate concentrations detected in Station Creek at monitoring station SC1. Since perchlorate concentration increases to a maximum of 38 µg/L and an average of 5.11 µg/L in Station Creek at SC3 beyond the intersection of Tributary M and Station Creek, Station Creek (SC1) is likely not a significant contributor of perchlorate to Station Creek (SC3). The perchlorate in Station Creek at SC3 is likely originating from Tributary M (TRM1).

Onion Creek - Station Creek - Leon River (OC1, SC3, SC5, LR1)

Onion Creek is the third route that surface runoff from NWIRP may follow to Lake Belton. As shown on **Figure 5-11**, perchlorate concentrations in Onion Creek at OC1 are at a maximum of 3 µg/L and at an average of 0.56 µg/L. Onion Creek (OC1) has only had one perchlorate detection in all the perchlorate sampling conducted as part of this project. This detection occurred in a duplicate sample and perchlorate was not detected in the original sample. Since perchlorate concentration increases to a maximum of 7.6 µg/L and an average of 2.73 µg/L in Station Creek at SC5 beyond the intersection of Onion Creek and Station Creek, Onion Creek (OC1) is not likely to be a significant contributor of perchlorate to Station Creek (SC5). The perchlorate is more likely migrating from Station Creek (SC3).

Figure 5-9

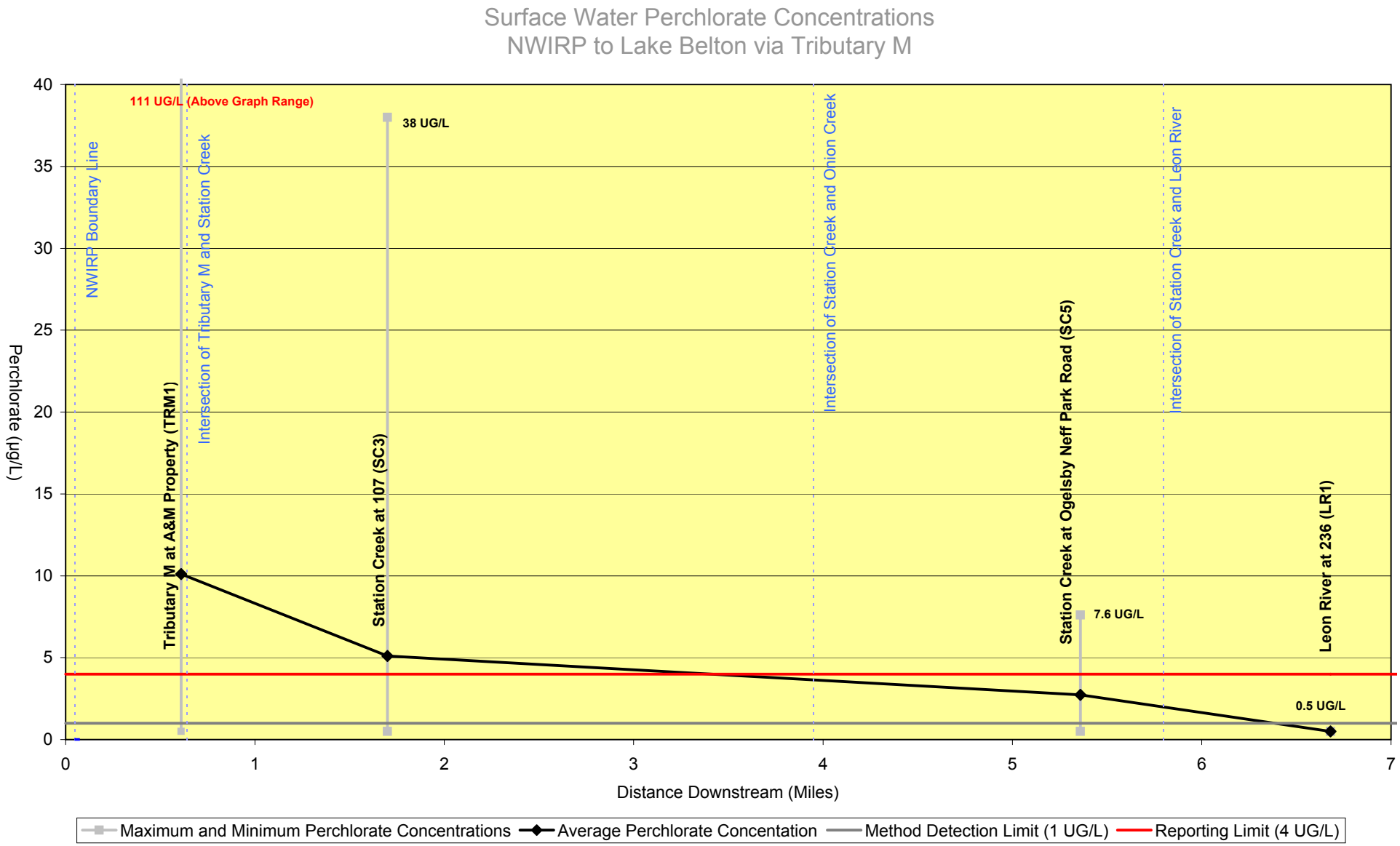

Surface Water Perchlorate Concentrations
NWIRP to Lake Belton via Tributary M
Perchlorate (µg/L)
Distance Downstream (Miles)
111 UG/L (Above Graph Range)
38 UG/L
7.6 UG/L
0.5 UG/L
NWIRP Boundary Line
Intersection of Tributary M and Station Creek
Intersection of Station Creek and Onion Creek
Intersection of Station Creek and Leon River
Tributary M at A&M Property (TRM1)
Station Creek at 107 (SC3)
Station Creek at Ogelsby Neff Park Road (SC5)
Leon River at 236 (LR1)
Maximum and Minimum Perchlorate Concentrations
Average Perchlorate Concentation
Method Detection Limit (1 UG/L)
Reporting Limit (4 UG/L)

Figure 5-10

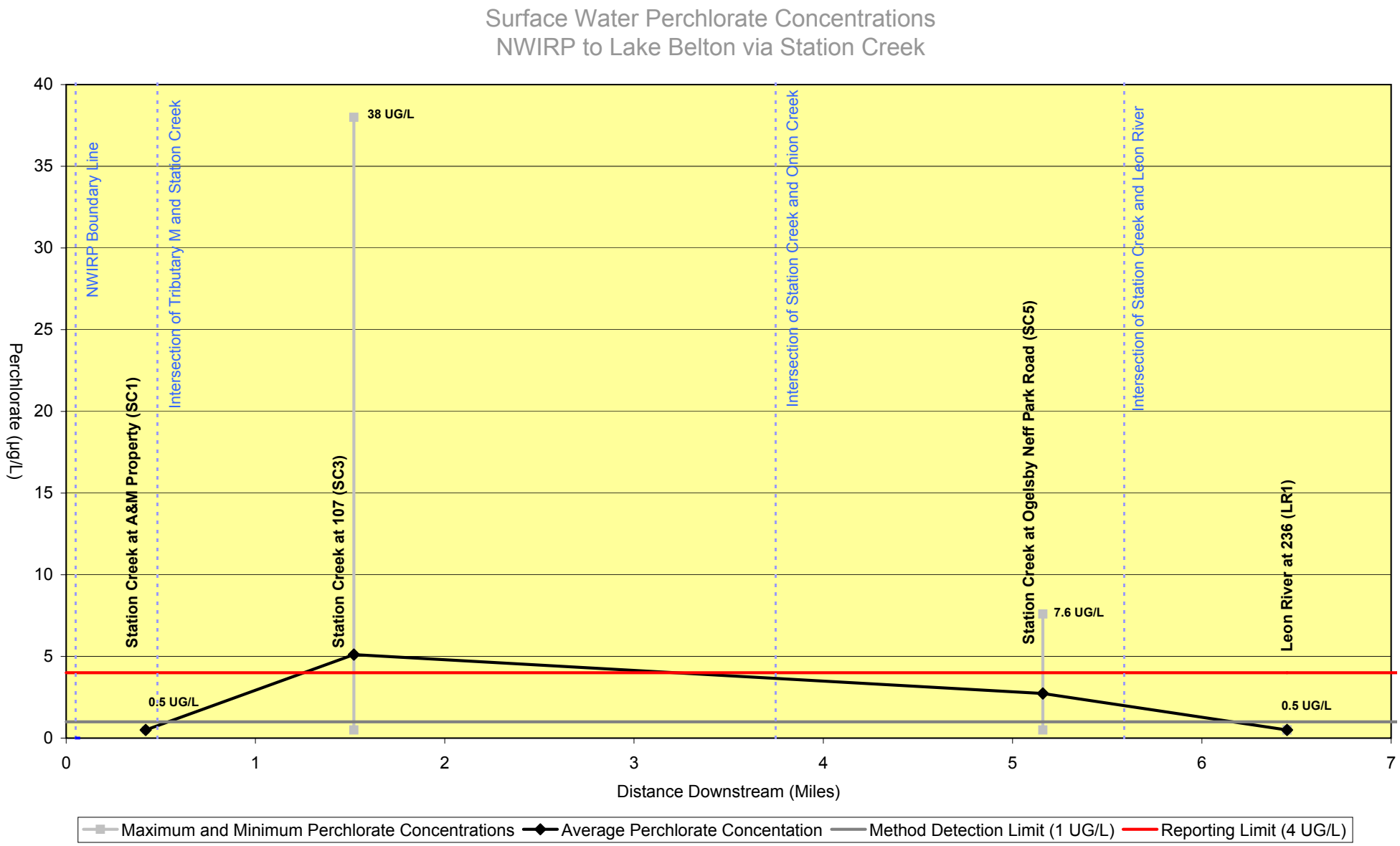
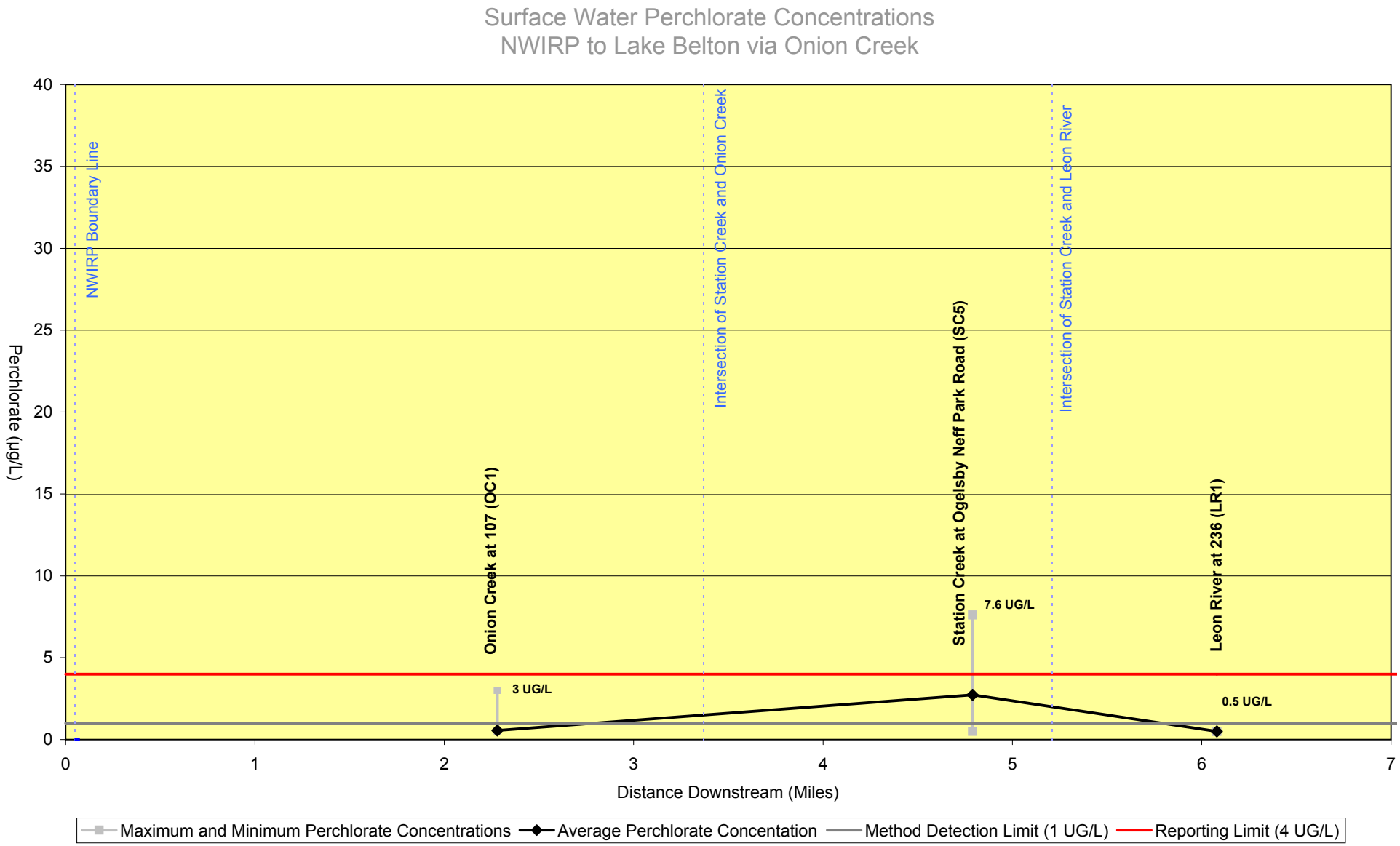

Surface Water Perchlorate Concentrations
NWIRP to Lake Belton via Station Creek
Perchlorate (µg/L)
Distance Downstream (Miles)
NWIRP Boundary Line
Intersection of Tributary M and Station Creek
Intersection of Station Creek and Onion Creek
Intersection of Station Creek and Leon River
Station Creek at A&M Property (SC1)
Station Creek at 107 (SC3)
Station Creek at Ogelsby Neff Park Road (SC5)
Leon River at 236 (LR1)
38 UG/L
7.6 UG/L
0.5 UG/L
0.5 UG/L
Maximum and Minimum Perchlorate Concentrations
Average Perchlorate Concentation
Method Detection Limit (1 UG/L)
Reporting Limit (4 UG/L)

Figure 5-11


Surface Water Perchlorate Concentrations
NWIRP to Lake Belton via Onion Creek
Perchlorate (µg/L)
40
35
30
25
20
15
10
5
0
NWIRP Boundary Line
Intersection of Station Creek and Onion Creek
Intersection of Station Creek and Leon River
Onion Creek at 107 (OC1)
Station Creek at Ogelsby Neff Park Road (SC5)
Leon River at 236 (LR1)
7.6 UG/L
3 UG/L
0.5 UG/L
0
1
2
3
4
5
6
7
Distance Downstream (Miles)
Maximum and Minimum Perchlorate Concentrations
Average Perchlorate Concentation
Method Detection Limit (1 UG/L)
Reporting Limit (4 UG/L)

5.1.1.2.5.2 NWIRP to Lake Waco

This section summarizes findings from perchlorate sampling in streams along the various surface drainage routes to Lake Waco.

Harris Creek - South Bosque River - Middle Bosque River (HC1, HC2, SBR3, SBR5, MBR1)

Harris Creek to the South Bosque River to the Middle Bosque River is one potential route for perchlorate from NWIRP to reach Lake Waco via surface flow. As shown on **Figure 5-12**, perchlorate concentrations in Harris Creek at HC1 are at a maximum of 6.5 µg/L and at an average of 1.81 µg/L. This decreases to a maximum perchlorate concentration of 4.8 µg/L and an average perchlorate concentration of 1.25 µg/L in Harris Creek (HC2) and then to a maximum perchlorate concentration of 2 µg/L and an average perchlorate concentration of 0.88 µg/L in the South Bosque River (SBR3). This remains consistent with a maximum perchlorate concentration of 2 µg/L and an average perchlorate concentration of 0.81 µg/L in South Bosque River (SBR5) beyond the intersection of Harris Creek and South Bosque River. Finally, there are no perchlorate detections in the Middle Bosque River at MBR1 beyond its intersection with the South Bosque River.

South Bosque River - Middle Bosque River (SBR1, SBR2, SBR4, SBR5, MBR1)

The South Bosque River to the Middle Bosque River is a second route that surface runoff from NWIRP could follow to Lake Waco. As shown on **Figure 5-13**, perchlorate concentrations in the South Bosque River at SBR1 reach a maximum of 4.7 µg/L and average 2.73 µg/L. This concentration decreases to a maximum perchlorate concentration of 3 µg/L and an average perchlorate concentration of 1.51 µg/L in the South Bosque River (SBR2). The maximum perchlorate concentration detected further downstream in the South Bosque River (SBR4) is 3 µg/L, and the average perchlorate concentration is 1.10 µg/L. This concentration further decreases to a maximum of 2 µg/L and an average of 0.81 µg/L in South Bosque River (SBR5) beyond the intersection of Harris Creek and South Bosque River. Finally, there are no perchlorate detections in the Middle Bosque River at MBR1 beyond the intersection of South Bosque River and the Middle Bosque River.

5.1.1.2.5.3 Cowhouse Creek at Tank Destroyer Road (CHC1)

We found no evidence of perchlorate contributions to Lake Belton from the Fort Hood drainage of Cowhouse Creek, as no perchlorate was detected at monitoring station CHC1 during this project.

Figure 5-12

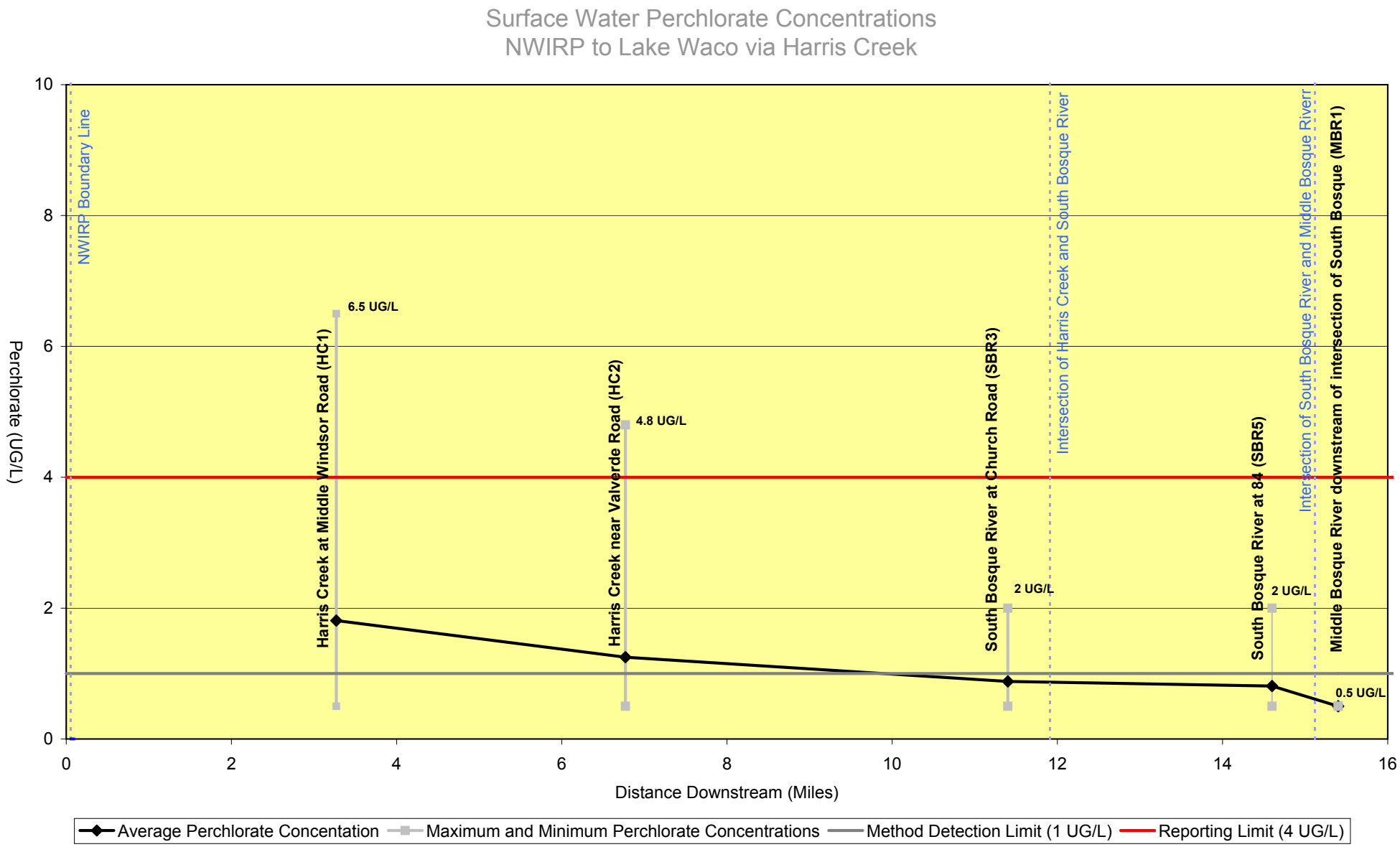
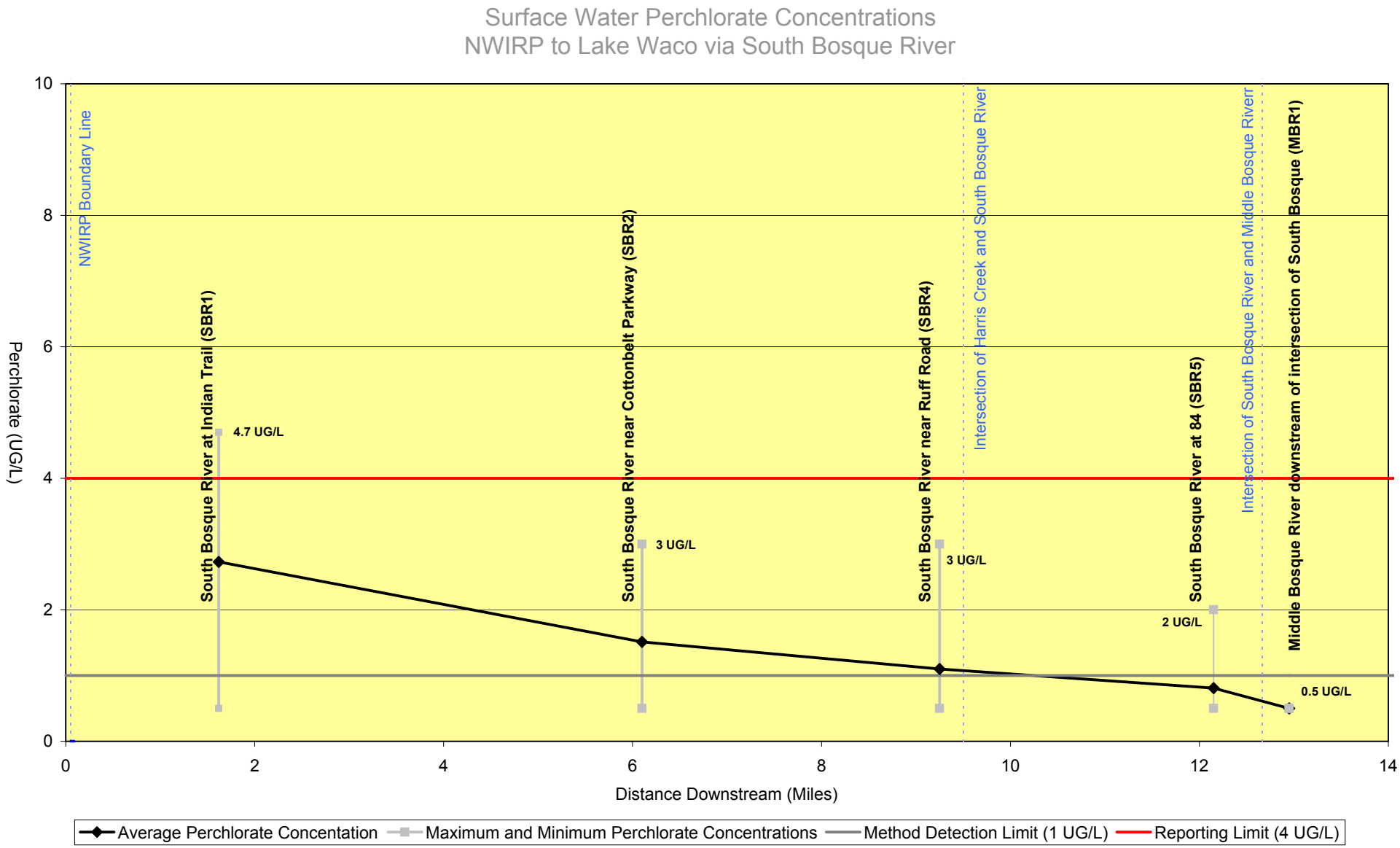

Surface Water Perchlorate Concentrations
NWIRP to Lake Waco via Harris Creek
Perchlorate (UG/L)
Distance Downstream (Miles)
NWIRP Boundary Line
Harris Creek at Middle Windsor Road (HC1)
6.5 UG/L
Harris Creek near Valverde Road (HC2)
4.8 UG/L
South Bosque River at Church Road (SBR3)
2 UG/L
Intersection of Harris Creek and South Bosque River
South Bosque River at 84 (SBR5)
2 UG/L
Intersection of South Bosque River and Middle Bosque Riverr
Middle Bosque River downstream of intersection of South Bosque (MBR1)
0.5 UG/L
Average Perchlorate Concentation
Maximum and Minimum Perchlorate Concentrations
Method Detection Limit (1 UG/L)
Reporting Limit (4 UG/L)

Figure 5-13


Surface Water Perchlorate Concentrations
NWIRP to Lake Waco via South Bosque River
Perchlorate (UG/L)
Distance Downstream (Miles)
NWIRP Boundary Line
South Bosque River at Indian Trail (SBR1)
South Bosque River near Cottonbelt Parkway (SBR2)
South Bosque River near Ruff Road (SBR4)
Intersection of Harris Creek and South Bosque River
South Bosque River at 84 (SBR5)
Intersection of South Bosque River and Middle Bosque Riverr
Middle Bosque River downstream of intersection of South Bosque (MBR1)
4.7 UG/L
3 UG/L
3 UG/L
2 UG/L
0.5 UG/L
Average Perchlorate Concentation
Maximum and Minimum Perchlorate Concentrations
Method Detection Limit (1 UG/L)
Reporting Limit (4 UG/L)

5.1.1.2.5.4 Overall Evaluation

Based on the historical data presented and the bi-weekly and storm sampling data collected during this study, the following observations have been made regarding perchlorate in streams.

(1) Perchlorate has been detected consistently in the South Bosque, Harris Creek and Station Creek streams since 1998. However, concentrations have decreased significantly over time in each of these locations as follows:

- Concentrations in Tributary M ranged from a minimum of 3,100 µg/L to a maximum of 5,500 in the upper stream reaches (Tributary M where it crosses the NWIRP boundary) based on the data collected by the U.S. Navy from January 1999 to July 1999. Perchlorate concentrations were found to be much lower during this study in Tributary M just south of the NWIRP boundary (TRM1) and ranged from non-detect (< 1 µg/L) to 111 µg/L. These decreases in perchlorate concentrations appear to be the result of significant source removal, groundwater collection, and groundwater remediation efforts at NWIRP by the U.S. Navy. These remedial activities have significantly reduced the quantities and concentrations of impacted groundwater leaving this site and entering adjoining streams.
- Concentrations in Station Creek at 107 (SWWS-9) ranged from a minimum of non-detect to a maximum of 540 µg/L based on the data collected by the U.S. Navy from September 1998 to September 2002. Perchlorate concentrations were found to be much lower during this study in Station Creek at 107 (SC3) and ranged from non-detect (< 1 µg/L) to 38 µg/L.
- Concentrations in Harris Creek ranged from a minimum of 3.2 µg/L to a maximum of 9.6 µg/L based on the data collected by the U.S. Navy at HRDN-6 from February 1999 to September 2002. Perchlorate concentrations were found to be lower during this study in Harris Creek (HC1) and ranged from non-detect (<1 µg/L) to 6.5 µg/L.
- Concentrations in the South Bosque River upper reaches (SBSR-7) ranged from a minimum of 4 µg/L to a maximum of 9.9 µg/L based on the data collected by the U.S. Navy from February 1999 to September 1, 2002. Perchlorate concentrations were found to be lower during this study in the South Bosque River upper reaches (SBR2) and ranged from non-detect (<1 µg/L) to 3 µg/L. Concentrations in the South Bosque River lower reaches (SBSR-8) ranged from a minimum of 1.2 µg/L to a maximum of 13 µg/L based on the data collected by the U.S. Navy from February 1999 to September 1, 2002. Perchlorate concentrations were found to be lower during this study in the South Bosque River lower reaches (SBR5) and ranged from non-detect (<1 µg/L) to 2 µg/L.

(2) Perchlorate concentrations decrease the further you move downstream from the NWIRP property. Dilution is the likely cause of these decreased concentrations.

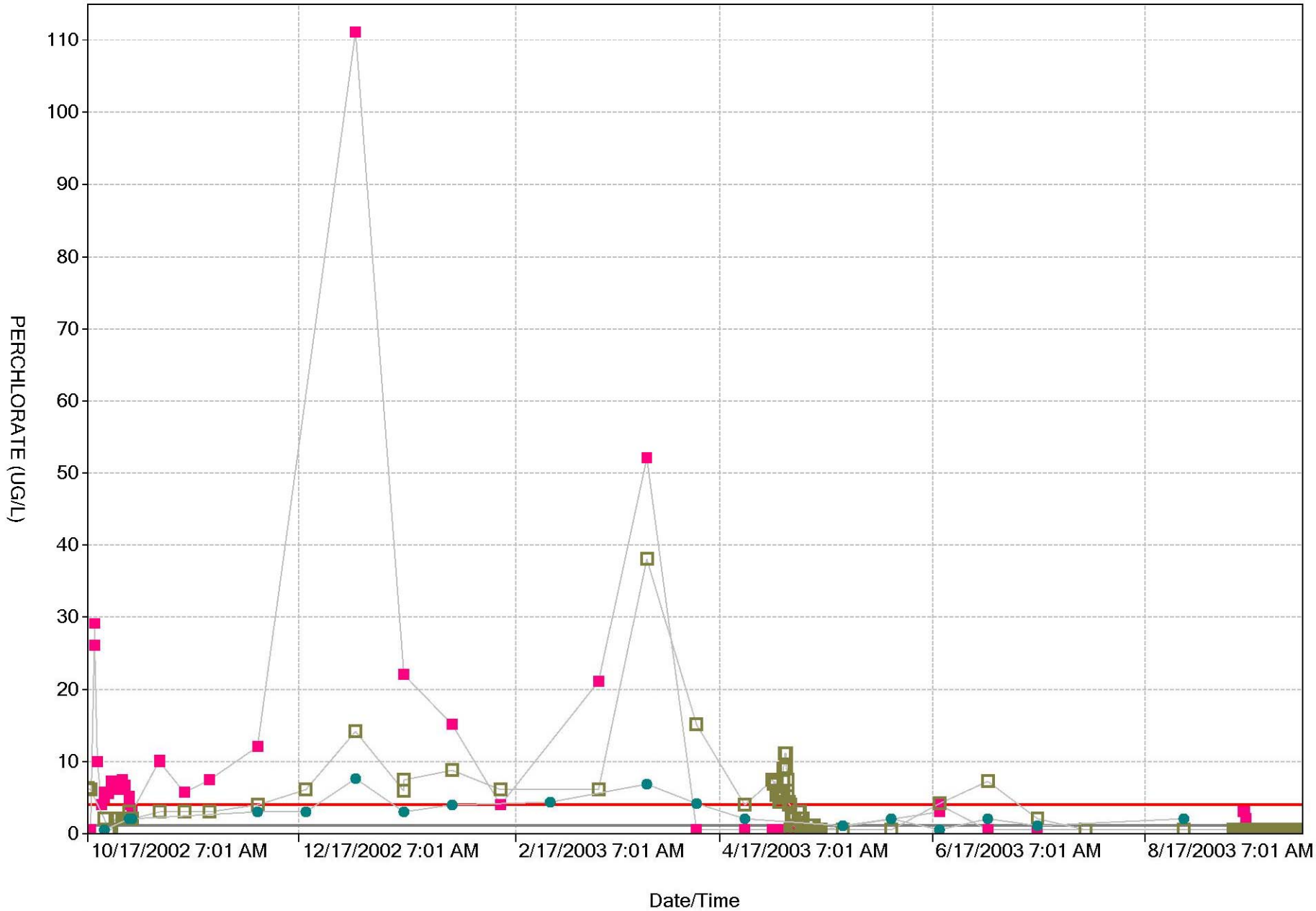
(3) All samples collected from stations SC1, OC1, SBR2, SBR3, SBR4, and SBR5 during all bi-weekly and storm sampling events have been below the interim action level for perchlorate (4 μg/L).

(4) All samples collected from the major rivers during bi-weekly and storm sampling events, including Cowhouse Creek (262 samples), the Middle Bosque River (218 samples) and the Leon River (230 samples) have all been below detectable levels (<1 μg/L) for perchlorate.

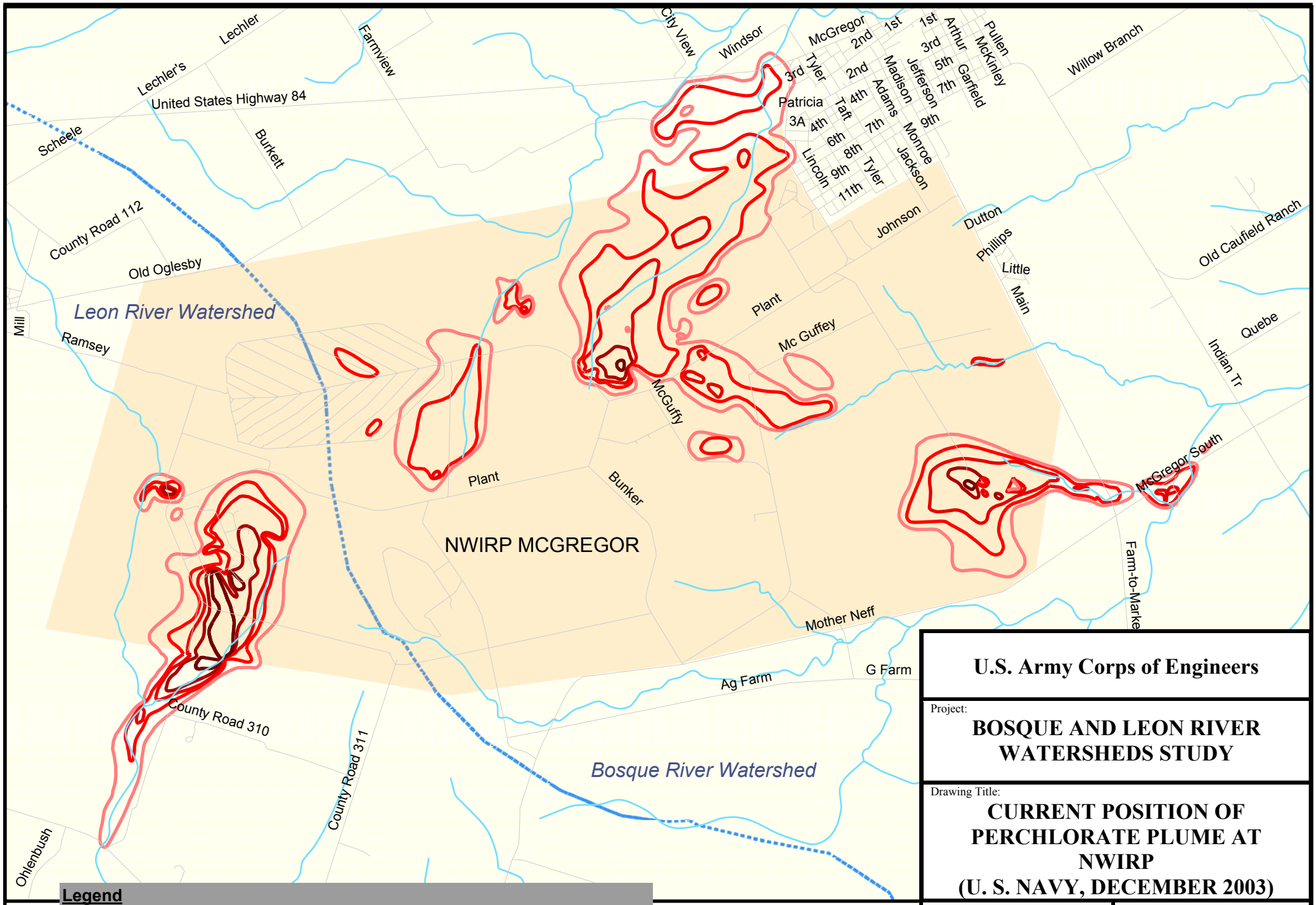
(5) Detected concentrations are still subject to spikes that may occur as shown on **Figure 5-14**.

(6) In addition to all the surface water sampling discussed above, many groundwater sample locations have been sampled multiple times by the U.S. Navy since the Groundwater Investigation began in 1998. The current position of the plume is shown in **Figure 5-15**. The plume size has been decreasing at a rate of 5 to 7% per year, according to the U.S. Navy.

Figure 5-14: NWIRP to Lake Belton
Spikes in Perchlorate Data



Legend:	Stats:		
REPORTING LIMIT	Min: 4.0	Max: 4.0	Avg: 4.00
METHOD DETECTION LIMIT	Min: 1.0	Max: 1.0	Avg: 1.00
TRM1	Min: 0.5	Max: 111.0	Avg: 3.58
SC3	Min: 0.5	Max: 38.0	Avg: 2.02
SC5	Min: 0.5	Max: 7.6	Avg: 2.73



U.S. Army Corps of Engineers

Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY

Drawing Title:
CURRENT POSITION OF PERCHLORATE PLUME AT NWIRP (U. S. NAVY, DECEMBER 2003)

Sheet 1 of 1 Sheets

SCALE: 1:50,993

FIGURE: 5-15

5.1.2 Perchlorate Occurrence in Lakes

To assist in determining if perchlorate is present within Lake Waco and Lake Belton, the project team collected surface water samples from the lakes during separate field-sampling events. These sampling events included collecting several samples during delta area grid sampling, intake sampling (potable water and irrigation), and transect sampling as part of the Acoustic Doppler Current Profiler Study (ADCP) Study (See Section 5.1.4.2). Complete descriptions of the work completed during each of these studies are included below.

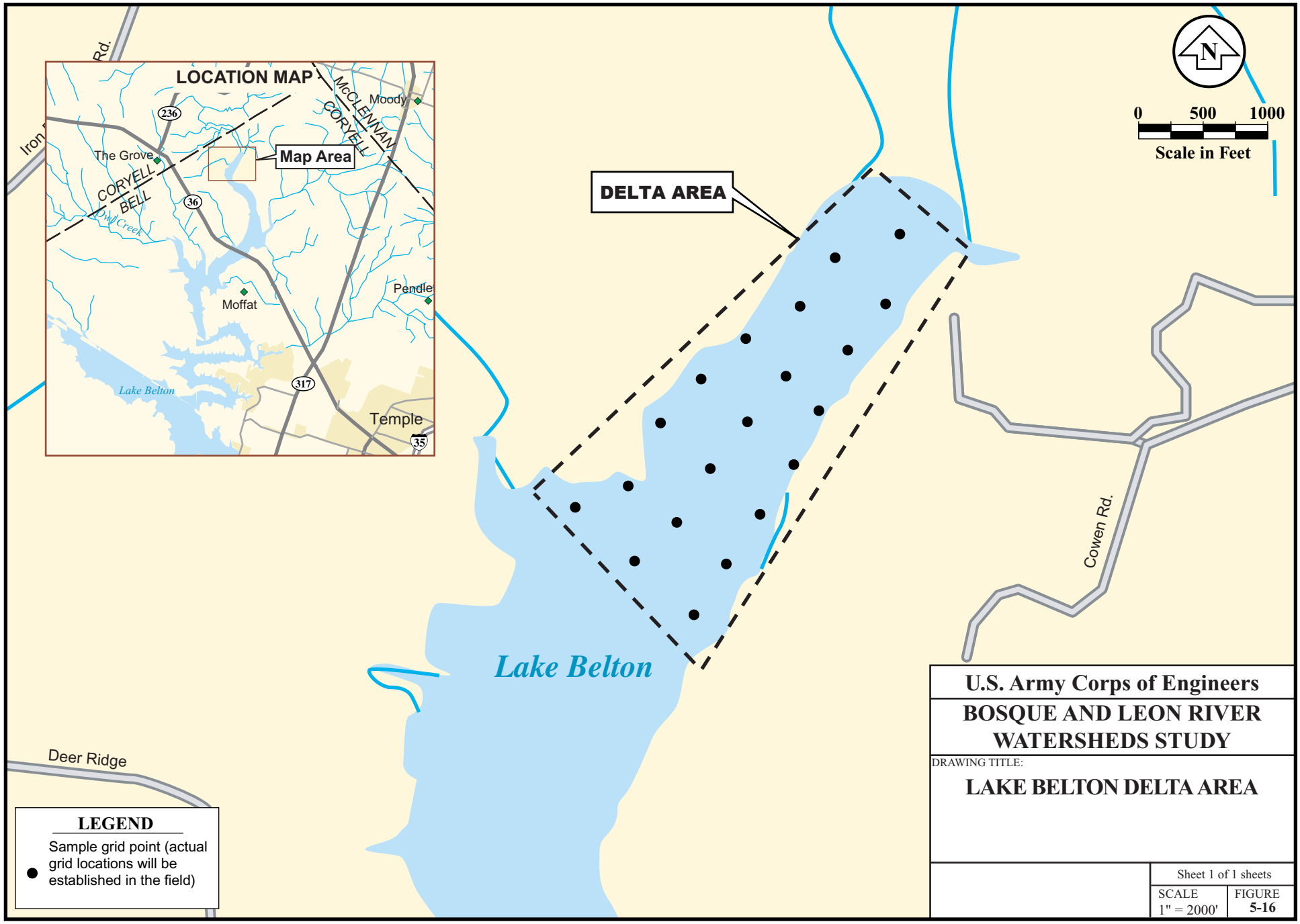
5.1.2.1 Delta Area Grid Sampling

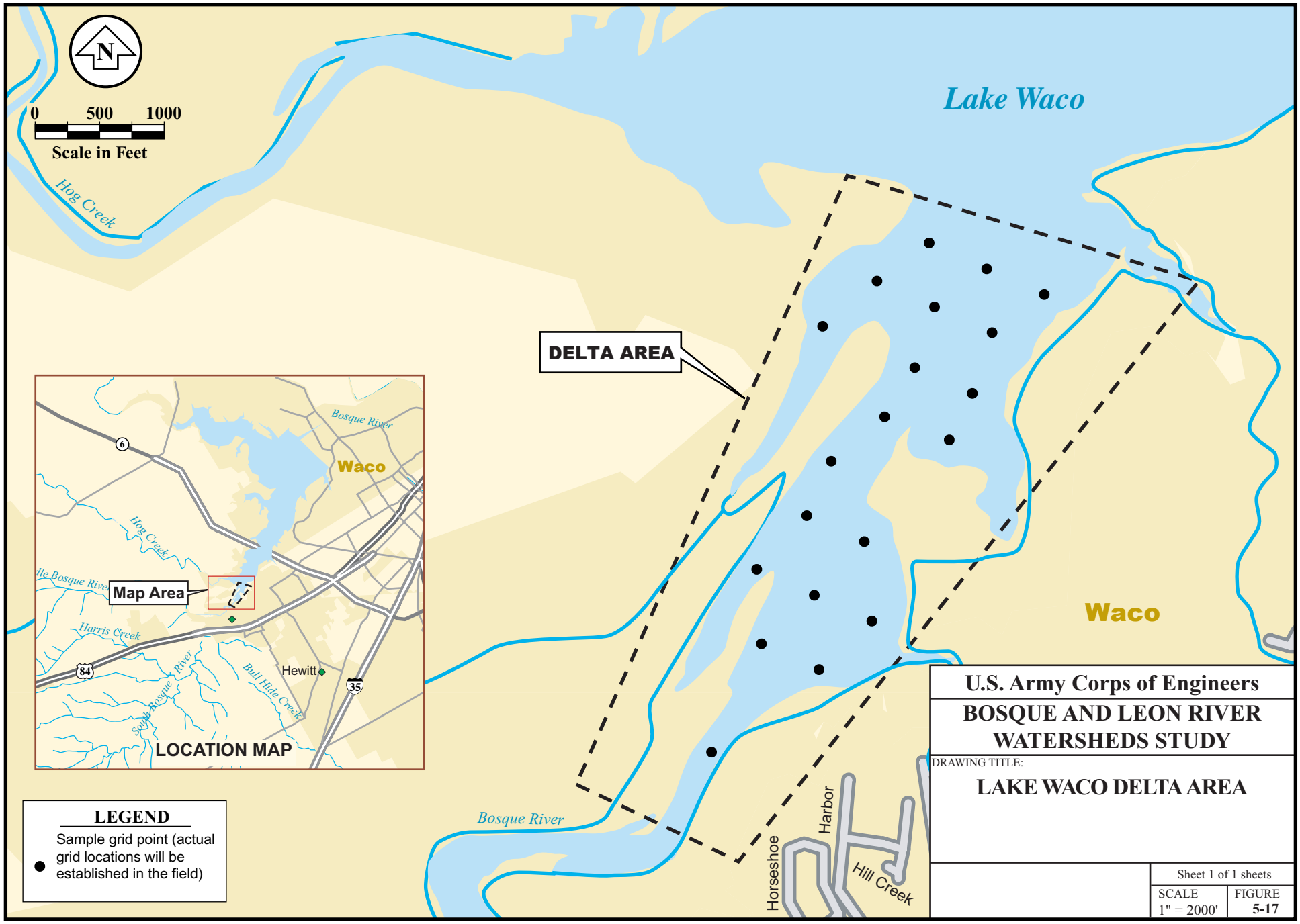
5.1.2.1.1 Introduction

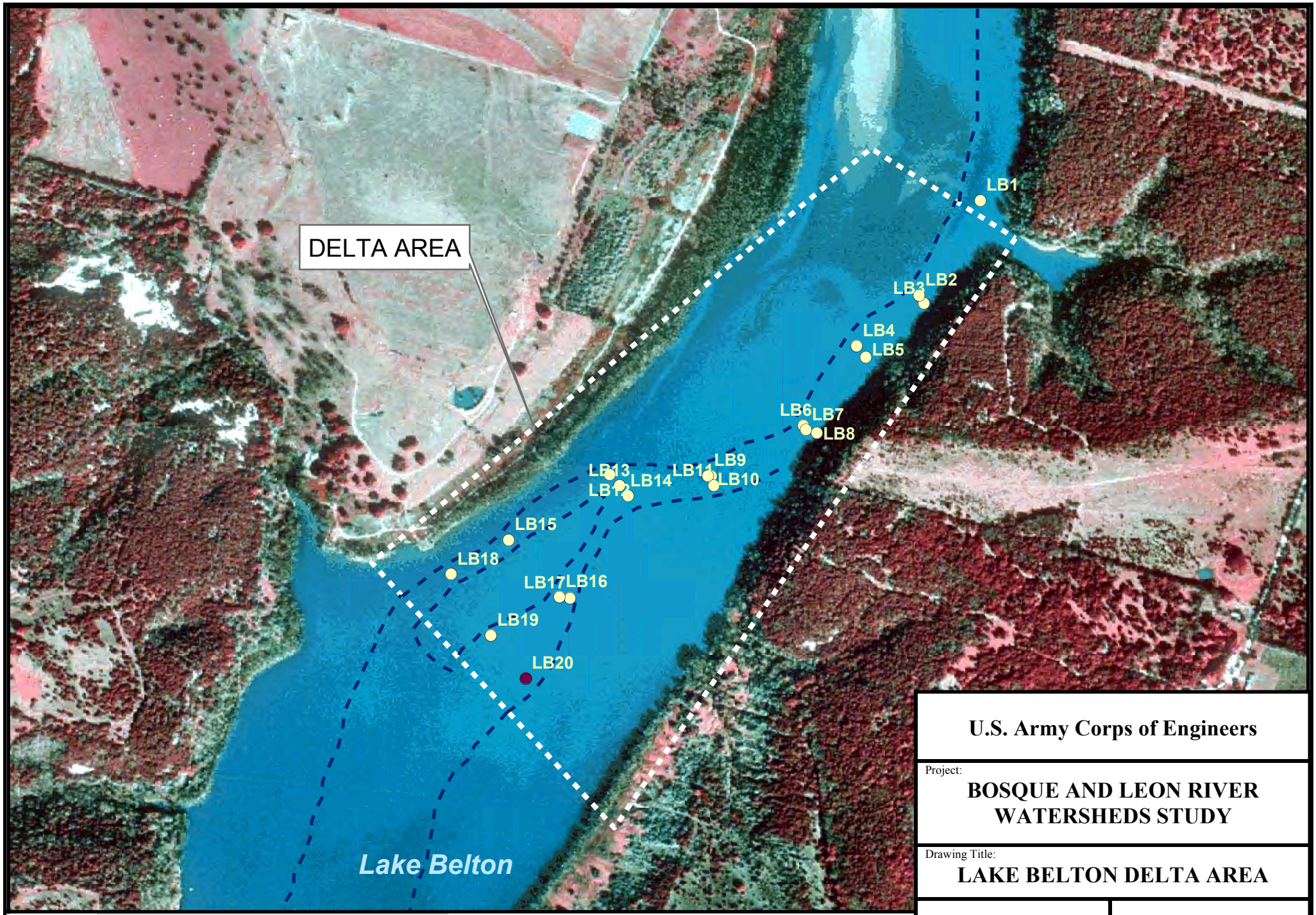
The USACE project team collected surface water samples from grid locations within the delta areas of both Lake Belton and Lake Waco. This sampling was performed to evaluate the presence and distribution of perchlorate, if any, within these areas because they receive direct discharge from the Bosque and Leon River watersheds and they are the areas where the greatest sediment deposition is expected to occur in each lake. In addition to surface water sampling, sediment pore water sampling was also conducted within the delta area locations. The results of the surface water sampling activities are discussed within this section of the report and the sediment pore water results are presented in Section 5.2.1.2. This portion of the study was conducted as part of the Delta Areas Study. All the methodologies and protocol followed are detailed in the *Final Lake Belton and Lake Waco Delta Area Field Sampling Plan* (MWH, 2002c). Any deviations from the Field Sampling Plan are discussed further below.

5.1.2.1.2 Methodology

The sampling of the delta areas of Lake Belton and Lake Waco was initially planned to be performed along a 20-point sample grid as shown in **Figure 5-16** and **Figure 5-17**, respectively. Due to the low lake water levels at the time of sampling and limited accessibility to various portions of the lakes, sampling was performed along transects. This longitudinal grid sampling pattern was chosen to minimize sample bias and to provide adequate coverage of the relatively large delta areas. Sample points were located using a boat and a Garmin GPS 76 instrument. The GPS information recorded at each grid point included latitude (degrees and minutes) and longitude (degrees and minutes) and are documented in **Table 5-3**. The locations of each sampling point and are shown in **Figure 5-18** and **Figure 5-19**.


LOCATION MAP
Map Area
Moody
McCLENNAN
CORYELL
The Grove
236
CORYELL
BELL
36
Owl Creek
Moffat
Pendle
Lake Belton
317
Temple
35
Iron
Rd.
DELTA AREA
N
0
500
1000
Scale in Feet
Lake Belton
Cowen Rd.
Deer Ridge
LEGEND
Sample grid point (actual grid locations will be established in the field)
U.S. Army Corps of Engineers
BOSQUE AND LEON RIVER WATERSHEDS STUDY
DRAWING TITLE:
LAKE BELTON DELTA AREA
Sheet 1 of 1 sheets
SCALE
1" = 2000'
FIGURE
5-16


N
0
500
1000
Scale in Feet
Lake Waco
Hog Creek
DELTA AREA
Waco
Bosque River
Horseshoe
Harbor
Hill Creek
Bosque River
Waco
Hog Creek
Map Area
Harris Creek
South Bosque River
Bull Hide Creek
Hewitt
6
84
35
LOCATION MAP
LEGEND
Sample grid point (actual grid locations will be established in the field)
U.S. Army Corps of Engineers
BOSQUE AND LEON RIVER WATERSHEDS STUDY
DRAWING TITLE:
LAKE WACO DELTA AREA
Sheet 1 of 1 sheets
SCALE
1" = 2000'
FIGURE
5-17



N

Legend

- SURFACE WATER AND SEDIMENT PORE WATER SAMPLING LOCATIONS
- SURFACE WATER SAMPLING LOCATION
- ACTUAL BOUNDARY OF LAKE DELTA AREA AT TIME OF SAMPLING

0 0.15 0.3 Miles

U.S. Army Corps of Engineers

Project:

BOSQUE AND LEON RIVER WATERSHEDS STUDY

Drawing Title:

LAKE BELTON DELTA AREA

Sheet 1 of 1 Sheets

SCALE: 1:8,808

FIGURE: **5-18**

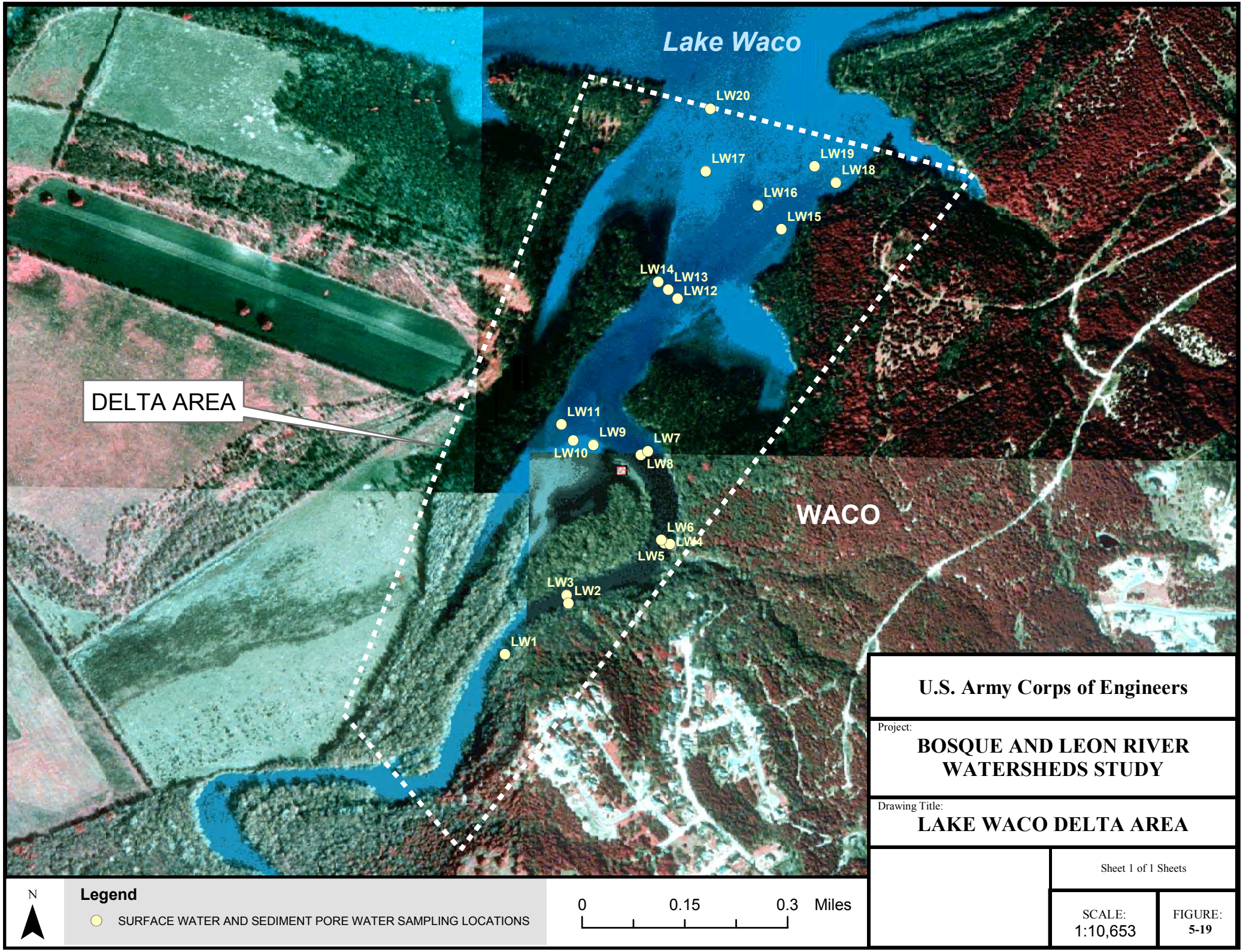

Lake Waco
LW20
LW17
LW19
LW18
LW16
LW15
LW14
LW13
LW12
DELTA AREA
LW11
LW9
LW7
LW10
LW8
WACO
LW6
LW4
LW5
LW3
LW2
LW1
N
Legend
SURFACE WATER AND SEDIMENT PORE WATER SAMPLING LOCATIONS
0
0.15
0.3
Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
LAKE WACO DELTA AREA
Sheet 1 of 1 Sheets
SCALE:
1:10,653
FIGURE:
5-19

Table 5-3
Lake Belton and Lake Waco Delta Sampling Locations

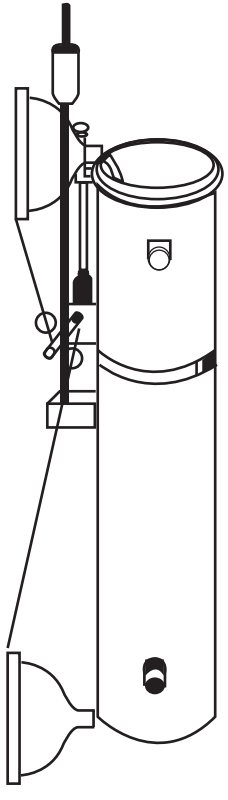
Location	Lake	Latitude	Longitude
LB1	Lake Belton	31.27453	-97.46893
LB2	Lake Belton	31.27298	-97.46993
LB3	Lake Belton	31.27285	-97.46985
LB4	Lake Belton	31.27215	-97.47095
LB5	Lake Belton	31.27197	-97.47080
LB6	Lake Belton	31.27085	-97.47182
LB7	Lake Belton	31.27078	-97.47178
LB8	Lake Belton	31.27073	-97.47160
LB9	Lake Belton	31.27003	-97.47338
LB10	Lake Belton	31.27002	-97.47332
LB11	Lake Belton	31.26987	-97.47328
LB12	Lake Belton	31.27005	-97.47498
LB13	Lake Belton	31.26987	-97.47482
LB14	Lake Belton	31.26970	-97.47468
LB15	Lake Belton	31.26898	-97.47663
LB16	Lake Belton	31.26805	-97.47580
LB17	Lake Belton	31.26803	-97.47563
LB18	Lake Belton	31.26842	-97.47757
LB19	Lake Belton	31.26742	-97.47692
LB20	Lake Belton	31.26672	-97.47635
LW1	Lake Waco	31.49950	-97.25392
LW2	Lake Waco	31.50058	-97.25258
LW3	Lake Waco	31.50075	-97.25262
LW4	Lake Waco	31.50183	-97.25043
LW5	Lake Waco	31.50185	-97.25055
LW6	Lake Waco	31.50192	-97.25062
LW7	Lake Waco	31.50380	-97.25090
LW8	Lake Waco	31.50372	-97.25105
LW9	Lake Waco	31.50393	-97.25205
LW10	Lake Waco	31.50403	-97.25248
LW11	Lake Waco	31.50437	-97.25273
LW12	Lake Waco	31.50703	-97.25027
LW13	Lake Waco	31.50722	-97.25047
LW14	Lake Waco	31.50738	-97.25068
LW15	Lake Waco	31.50850	-97.24808
LW16	Lake Waco	31.50900	-97.24858
LW17	Lake Waco	31.50972	-97.24968
LW18	Lake Waco	31.50948	-97.24693
LW19	Lake Waco	31.50983	-97.24738
LW20	Lake Waco	31.51105	-97.24958

Once the sampling grid was established, surface water samples were collected from each of the grid points during a single sampling event on June 12, 2003 for Lake Belton and June 26, 2003 for Lake Waco. Each surface water sample was a discrete grab sample collected with an Alpha thief sampler. A photograph of this process is shown in **Figure 5-20**. Thief samplers consist of a cylinder with stoppers or check valves on each end. The samplers allow water to pass through the sample cylinder as it is lowered to the desired sampling depth. The stoppers are then activated to retain the sample prior to retrieval. The alpha thief sampler is shown on **Figure 5-21** (B. Van Dorn type).

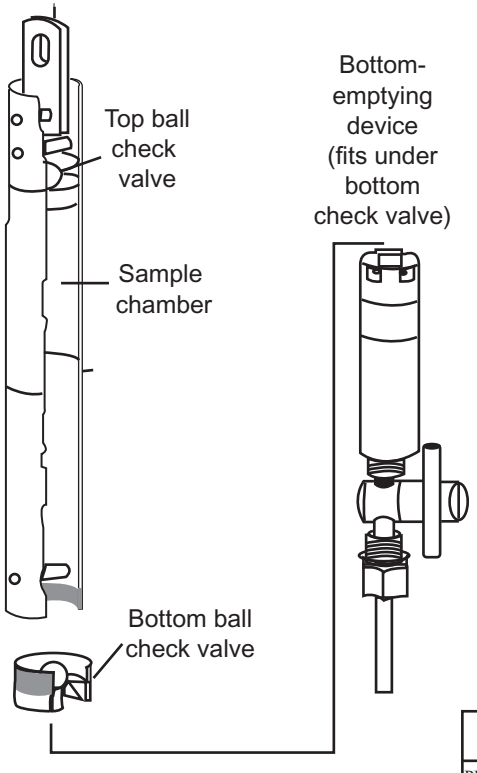
Figure 5-20
Surface Water Sampling

Actual sample depths depended on whether the lake was thermally stratified at that sample location. Temperature profiles were initially established prior to collecting the surface water samples. At each grid location, a multi-parameter instrument (Hydrolab Mini Sonde 4a) was lowered through the water column in order to develop a vertical temperature profile. The temperature data were collected at 5-foot increments from the lake surface to the lake bottom and were recorded directly onto a copy of the Thermal Profile Graph. The completed graph was compared with **Figure 5-22**, which shows a thermal profile typical of a thermally stratified lake. If the thermal profile indicated the lake was thermally stratified (i.e., clear evidence of an epilimnion, thermocline, and hypolimnion as shown on **Figure 5-22**), then one surface water sample was collected from mid-depth in the epilimnion (upper layer) and another from mid-depth of the hypolimnion (lower layer). If the thermal profile indicated the lake was not stratified, one surface water sample was collected midway between the lake surface and the lake bottom.

A. Kemmerer sampler

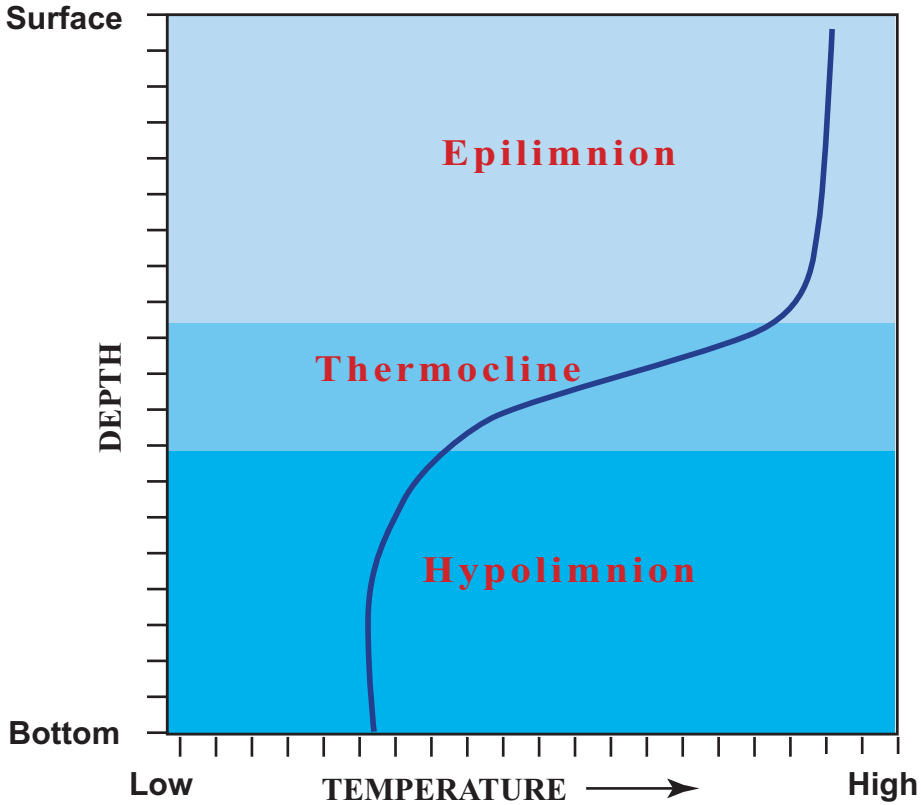


B. Van Dorn sampler



C. Doublecheck-valve bailer and bottom-empyting device

U.S. Army Corps of Engineers		
PROJECT: BOSQUE AND LEON RIVER WATERSHEDS STUDY		
DRAWING TITLE: THIEF SAMPLERS		
	Sheet 1 of 1 sheets	
	SCALE No Scale	FIGURE 5-21



U.S. Army Corps of Engineers

BOSQUE AND LEON RIVER WATERSHEDS STUDY

DRAWING TITLE:

TYPICAL THERMAL PROFILE OF A THERMALLY STRATIFIED LAKE

Sheet 1 of 1 sheets

SCALE	FIGURE
1" = 2000'	5-22

Upon retrieval of the sampler, the sample was transferred to the appropriate sample containers. Since an Alpha sampler was used, one of the stoppers was opened and the sample carefully poured into the sample container. Sample containers and preservation requirements for the surface water samples are presented in the *Final Lake Belton and Lake Waco Delta Area Field Sampling Plan* (MWH, 2002c).

Field Observations. The following field observations and water quality measurements also were collected at each sampling location.

Field Observations:

- Cloud cover
- Wind velocity
- Secchi Disk transparency
- Water color
- Aquatic vegetation in percent cover (qualitative)

Water Quality Measurements (at each sampled location and depth):

- Temperature
- Dissolved oxygen
- Specific conductance
- pH
- Salinity
- Dissolved oxygen percent saturation

The water quality measurements were obtained by lowering the probe of the multi-parameter instrument to the sampled depth after the surface water samples were collected. All water quality and field observation data collected as part of this study are included for reference in **Appendix H**.

Duplicate Samples. At all sampling locations, blind duplicate and equipment blank samples were collected at a frequency to represent 10 percent of the environmental samples collected, and MS/MSD samples were collected at a frequency to represent 5 percent of the environmental samples collected.

Sample Designation. Each surface water sample was designated with an alphanumeric character string set apart by hyphens. For the samples collected from the delta area grid points, the designation began with the lake name abbreviation and grid number (e.g., "LB1" for Lake Belton grid point number 1, "LW1" for Lake Waco grid point number 1, etc.), followed by "SW" to indicate a surface water sample, and finally by the depth the sample was collected. For example, the surface water sample collected from Lake Belton grid point number 1 from 15 feet deep was designated "LB1-SW-15' ".

Blind duplicate surface water samples were designated with a fictitious number so the laboratory would not know where the sample was taken. For example, the first blind duplicate sample was designated "SW-1001". The field crew kept careful records of the designations given to the blind duplicate samples and their corresponding environmental sample so that the analytical results could be correlated with the sample locations. Each MS/MSD sample had the same designation as its associated environmental sample except that "MS" or "MSD" followed the sample designation (e.g., "LB1-SW-15' MS" and "LB1-SW-15' MSD"). Each equipment blank sample had the same designation as its associated sampling location except that "EB" followed the sample designation (e.g., "LB1-SW-15'-EB").

Sample Analysis. All surface water samples were analyzed for perchlorate by USEPA Method 314.0 at the USACE Engineer Research and Development Center Environmental Laboratory at the Environmental Chemistry Branch in Omaha, Nebraska. (See **Appendix V**). The USACE laboratory conformed to the analytical method requirements, analytical quality control requirements, instrument calibration frequency, and the laboratory quality control requirements presented in the QAPP (MWH, 2002e). A discussion of sample labeling, chain-of-custody, handling and shipping is presented in *Final Lake Belton and Lake Waco Delta Areas Field Sampling Plan* (MWH, 2002c). The data verification report for samples analyzed by the USACE laboratory is included in **Appendix W**.

5.1.2.1.3 Data

A total of 20 water samples were collected from 20 sample locations within both Lake Belton and Lake Waco. Two duplicate samples were also collected from each lake during sampling activities. No perchlorate was detected in any of the 44 surface water samples collected from either Lake Belton or Lake Waco. The surface water sampling results for Lake Belton and Lake Waco are included in **Appendix I**. Perchlorate was also not detected in any of the equipment blank and investigations derived waste (IDW) samples collected during field activities. These results are also included in **Appendix I**.

5.1.2.2 Intake Sampling

5.1.2.2.1 Introduction

Surface water samples were collected from many of the drinking water and irrigation intakes located within Lake Waco and Lake Belton and downstream of the Lake Belton dam during the study. The names of the intakes, their physical locations and the type of intake are shown in **Figure 5-23** and documented in **Table 5-4**.

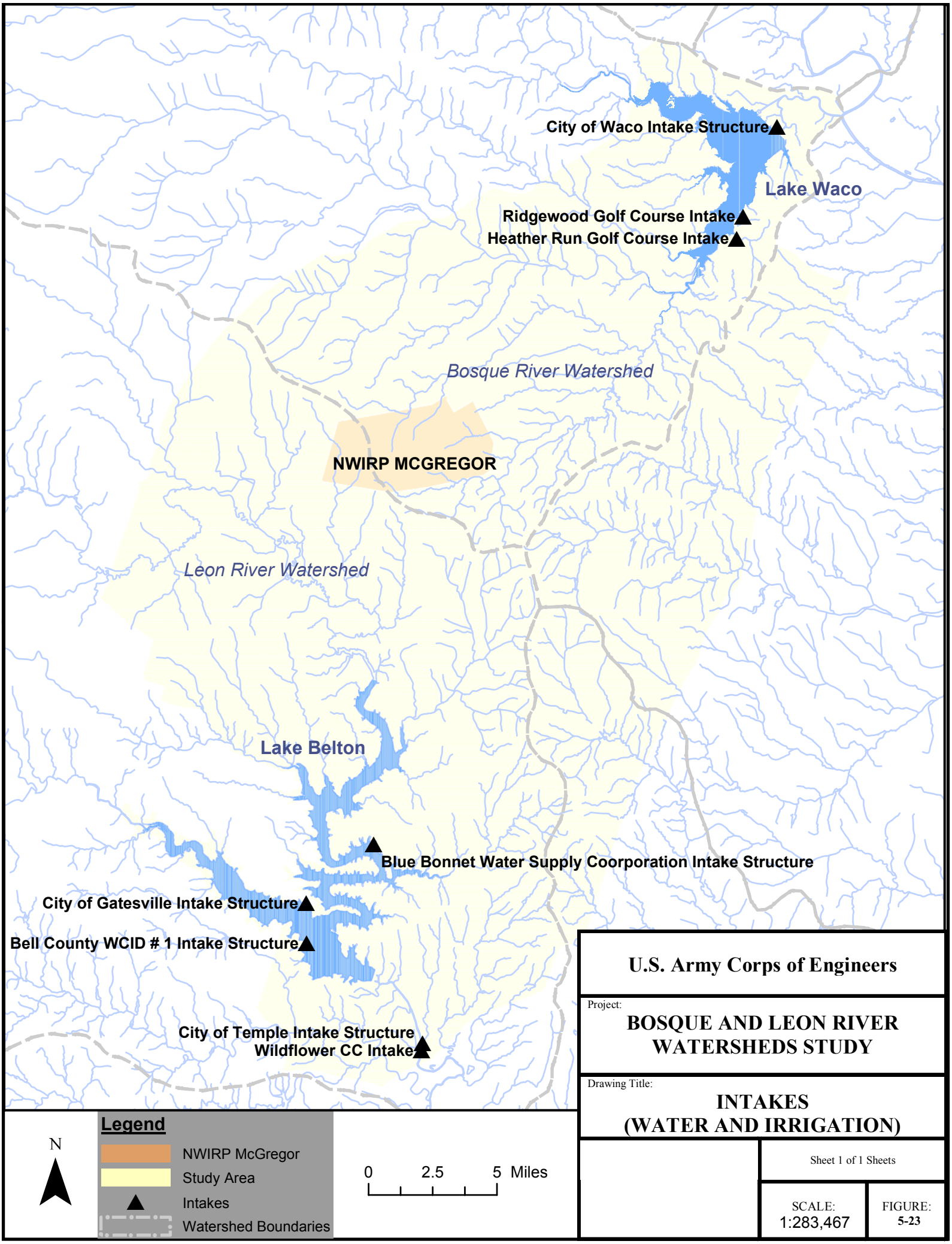

City of Waco Intake Structure
Lake Waco
Ridgewood Golf Course Intake
Heather Run Golf Course Intake
Bosque River Watershed
NWIRP MCGREGOR
Leon River Watershed
Lake Belton
Blue Bonnet Water Supply Coorporation Intake Structure
City of Gatesville Intake Structure
Bell County WCID # 1 Intake Structure
City of Temple Intake Structure
Wildflower CC Intake
N
Legend
NWIRP McGregor
Study Area
Intakes
Watershed Boundaries
0 2.5 5 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
INTAKES (WATER AND IRRIGATION)
Sheet 1 of 1 Sheets
SCALE: 1:283,467
FIGURE: 5-23

Table 5-4
Intake Types and Locations

Intake Name	ID	Type	Location	Latitude	Longitude	Method of Collection
Blue Bonnet Water Supply Corporation Intake Structure	BB	Drinking	Lake Belton	31.1826000	-97.4717000	Samples were collected from a hose bib at the raw water intake.
Bell County WCID # 1 Intake Structure	BC	Drinking	Lake Belton	31.1271000	-97.5168000	Samples were collected from a hose bib at the raw water intake location.
City of Gatesville Intake Structure	GV	Drinking	Lake Belton	31.1500000	-97.5167000	Samples were collected from a hose bib at the raw water intake.
Heather Run Golf Course Intake	HR	Irrigation	Lake Waco	31.5213100	-97.2264300	Grab Sample
Ridgewood Golf Course Intake	RW	Irrigation	Lake Waco	31.5338100	-97.2219700	Grab Sample
City of Temple Intake Structure	TP	Drinking	Downstream of Belton Dam	31.0700400	-97.4414500	Samples were collected directly from the lake using an Alpha thief sampler.
City of Waco Intake Structure	WA	Drinking	Lake Waco	31.5838890	-97.1986110	Collected by City of Waco
Wildflower CC Intake	WF	Irrigation	Downstream of Belton Dam	31.0659600	-97.4422100	Samples were collected directly from the surface water as a grab sample.

5.1.2.2.1.1 Potable Water

The U.S. Navy began collecting monthly surface water samples from all of the potable water intakes in both Lake Waco and Lake Belton and one location downstream of the Lake Belton dam in March 1999. The various intakes that were sampled in Lake Belton include the Bluebonnet intake, Bell County Water Control and Improvement District Number 1 intake, and the City of Gatesville intake. The location sampled downstream of the Lake Belton dam is the City of Temple intake. The water intake sampled in Lake Waco was the City of Waco intake located near the dam. The U.S. Navy decided to end this sampling effort in December 2002 since they had collected around 202 samples over a 45 month period and perchlorate concentrations in nearly all the samples were below detectable levels (See Section 5.1.2.4). Because of the importance of these drinking water supplies to the public, the project team agreed to continue collecting samples at each of these locations through December 2003 to complement the various other data being collected in the lakes by the project team. Because this sampling effort was not part of the original study scope, a Field Sampling Plan specific to this effort was not developed. Rather, all of the sampling methodologies and protocol followed during these sampling activities were derived from the *Final Lake Belton and Lake Waco Delta Areas Field*

Sampling Plan (MWH, 2002c). Any deviations from the Field Sampling Plan are discussed further below.

5.1.2.2.1.2 Irrigation

Surface water samples were collected from irrigation water intakes located in Lake Waco. These irrigation water intakes serve the Heather Run and Ridgewood Golf Courses. Surface water samples were also collected from the Wildflower Country Club irrigation intake downstream of Belton Dam. This portion of the Study was conducted as part of the Delta Areas Study. All of the methodologies and protocol followed are detailed in the *Final Lake Belton and Lake Waco Delta Areas Field Sampling Plan* (MWH, 2002c). Any deviations from the Field Sampling Plan are discussed further below.

5.1.2.2.2 Methodology

5.1.2.2.2.1 Potable

The various intakes from which samples were collected and methods of collection are described above in **Table 5-4**.

These water samples were collected in accordance with the thief sampling methodologies as discussed in Section 5.1.2.1.2 or direct sampling into appropriate containers from hose bib locations. Sample designation and analysis were performed as described for irrigation water intakes.

5.1.2.2.2.2 Irrigation

Surface water samples were collected within Lake Waco near the location of the irrigation water pump intakes serving the Heather Run (HR) and Ridgewood (RW) Golf Courses. In addition, surface water samples were collected from the Wildflower Country Club Irrigation Intake, which was also sampled previously by the U.S. Navy.

One surface water sample was collected from each golf course intake structure on a monthly basis for a period of 12 months. The locations of the irrigation pump intakes were documented using a Garmin GPS 76 instrument and included latitude (degrees and minutes) and longitude (degrees and minutes). At all sampling locations, blind duplicate and equipment blank samples were collected at a frequency to represent 10 percent of the environmental samples collected, and MS/MSD samples were collected at a frequency to represent 5 percent of the environmental samples collected.

Sample Designation. At the golf course intake structures, the sample designations were the golf course intake number (e.g., “HR” for the Heather Run intake structure and “RW” for the Ridgewood intake structure), followed by “SW” to indicate a surface water sample, and finally by the date and military time the sample was collected. For example, the surface water sample collected from the Heather Run Golf Course intake structure at 16:15 hours on November 18, 2002 would be designated “HR- SW-11-18-1615”.

Blind duplicate surface water samples were designated with a fictitious number so the laboratory would not know where the sample was collected. For example, the first blind duplicate sample was designated "SW-1001". The field crew kept careful records of the designations given to the blind duplicate samples and their corresponding environmental sample so that the analytical results could be correlated with the sample locations. Each MS/MSD sample had the same designation as its associated environmental sample except that "MS" or "MSD" followed the sample designation (e.g., "LB1-SW-15' MS" and "LB1-SW-15' MSD"). Each equipment blank sample had the same designation as its associated sampling location except that "EB" followed the sample designation (e.g., "LB1-SW-15'-EB").

Sample Analysis. All surface water samples were analyzed for perchlorate by USEPA Method 314.0 at the USACE Engineer Research and Development Center Environmental Laboratory at the Environmental Chemistry Branch in Omaha, Nebraska. (See **Appendix V**). The USACE laboratory conformed to the analytical method requirements, analytical quality control requirements, instrument calibration frequency, and the laboratory quality control requirements presented in the QAPP (MWH, 2002e). The data verification report for samples analyzed by the USACE laboratory is included in **Appendix W**.

5.1.2.2.3 Data

A total of 77 water samples, including three duplicates, were collected from the five potable water intakes over the course of this study. A total of 41 water samples, including nine duplicates, were collected from the two irrigation intakes in Lake Waco and the Wildflower Country Club irrigation intake over the course of this study. No perchlorate was detected in any of the samples collected from the intakes (potable or irrigation). The analytical results for both the irrigation water and potable water samples results are included in **Appendix J**.

5.1.2.3 Acoustic Doppler Current Profiler (ADCP) Sampling

5.1.2.3.1 Introduction

To assist in obtaining additional information regarding perchlorate concentrations within Lake Belton, additional surface water samples were collected from each of the 23 ADCP transect locations surveyed during the fall 2003 and winter 2003 events (see Section 5.1.4.2). This sampling was performed to collect data from identified preferential flow pathways encountered along each transect and to provide more focused sampling coverage within Lake Belton. Samples were collected along each transect in two locations: 1) above and below the thermocline at the deepest point along the transect, and 2) within preferential flow pathways that were identified along the transect. All of the methodologies and protocols followed during this sampling were derived from the *Final Lake Belton and Lake Waco Delta Areas Study Field Sampling Plan* (MWH, 2002c). Any deviations from the Field Sampling Plan are discussed further below.

5.1.2.3.2 Methodology

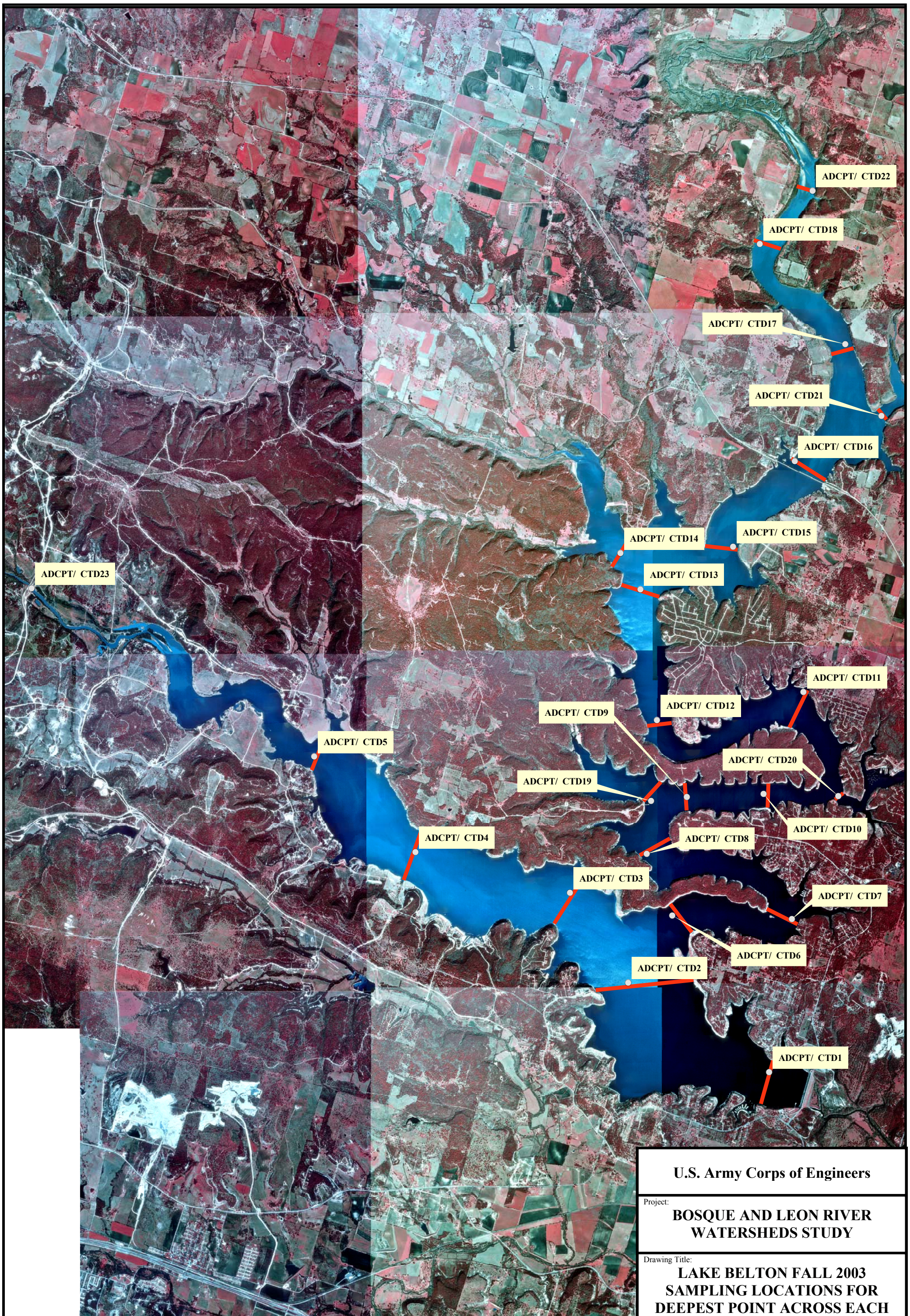
Initially, two surface water samples were collected at the deepest point along each transect. The first sample was collected from the epilimnion (upper layer) 5 feet below

the surface and the second sample was collected in the hypolimnion (lower layer) 5 feet above the water/ sediment interface. If the depth was less than 10 feet, one surface water sample was collected from mid-depth. If there was significant preferential flow detected across the transect, a water sample was also collected at the location depth of the identified preferential flow. The sample collection locations were documented using a Garmin GPS 76 instrument and included latitude (degrees and minutes) and longitude (degrees and minutes). The actual GPS coordinates of the sampling locations for the deepest point across each transect and preferential flows identified for the Fall 2003 and Winter 2003 surveys are documented in **Appendix K**. The actual sampling locations for the deepest point across each transect and preferential flows identified for the fall 2003 and winter 2003 surveys are shown in **Figure 5-24** through **Figure 5-27**. A total of 106 samples were collected during these surveys. Each surface water sample was a discrete "grab" type sample collected with an Alpha thief sampler as previously discussed in Section 5.1.2.1.2.

At all sampling locations, blind duplicate and equipment blank samples were collected at a frequency to represent 10 percent of the environmental samples collected, and MS/MSD samples were collected at a frequency to represent 5 percent of the environmental samples collected.

Sample Designation. Each surface water sample was designated with an alphanumeric character string set apart by hyphens. For the samples collected from the deepest point across the transect, the designation began with the ADCP transect abbreviation and number (e.g., "ADCPT1" for transect 1, etc.), followed by "SW" to indicate a surface water sample, and finally by the depth the sample was collected. For example, the surface water sample collected from transect 12 from 15 feet deep was designated "ADCPT12-SW-15' ". For the samples collected from the flow area across the transect, the designation began with the ADCP transect abbreviation and number (e.g., "ADCPT1" for transect 1, etc.), followed by "F" to indicate a flow sample, followed by a number to indicate the number of flows detected across the transect, followed by "SW" to indicate a surface water sample, and finally by the depth the sample was collected. For example, the first surface water sample collected due to flow from transect 15 at a depth of 12 feet was designated "ADCPT12F1-SW-15' ".

Blind duplicate surface water samples were designated with a fictitious number so the laboratory would not know where the sample was collected. For example, the first blind duplicate sample was designated "SW-1001". The field crew kept careful records of the designations given to the blind duplicate samples and their corresponding environmental sample so that the analytical results could be correlated with the sample locations. Each MS/MSD sample had the same designation as its associated environmental sample except that "MS" or "MSD" followed the sample designation (e.g., "ADCPT12F1-SW-15' MS" and "ADCPT12F1-SW-15' MSD"). Each equipment blank sample had the same designation as its associated sampling location except that "EB" followed the sample designation (e.g., "ADCPT12F1-SW-15' -EB").



N

Legend

ADCP Transects

Sampling Locations for the deepest point across each transect

1 0.5 0 Miles

U.S. Army Corps of Engineers

Project:

BOSQUE AND LEON RIVER WATERSHEDS STUDY

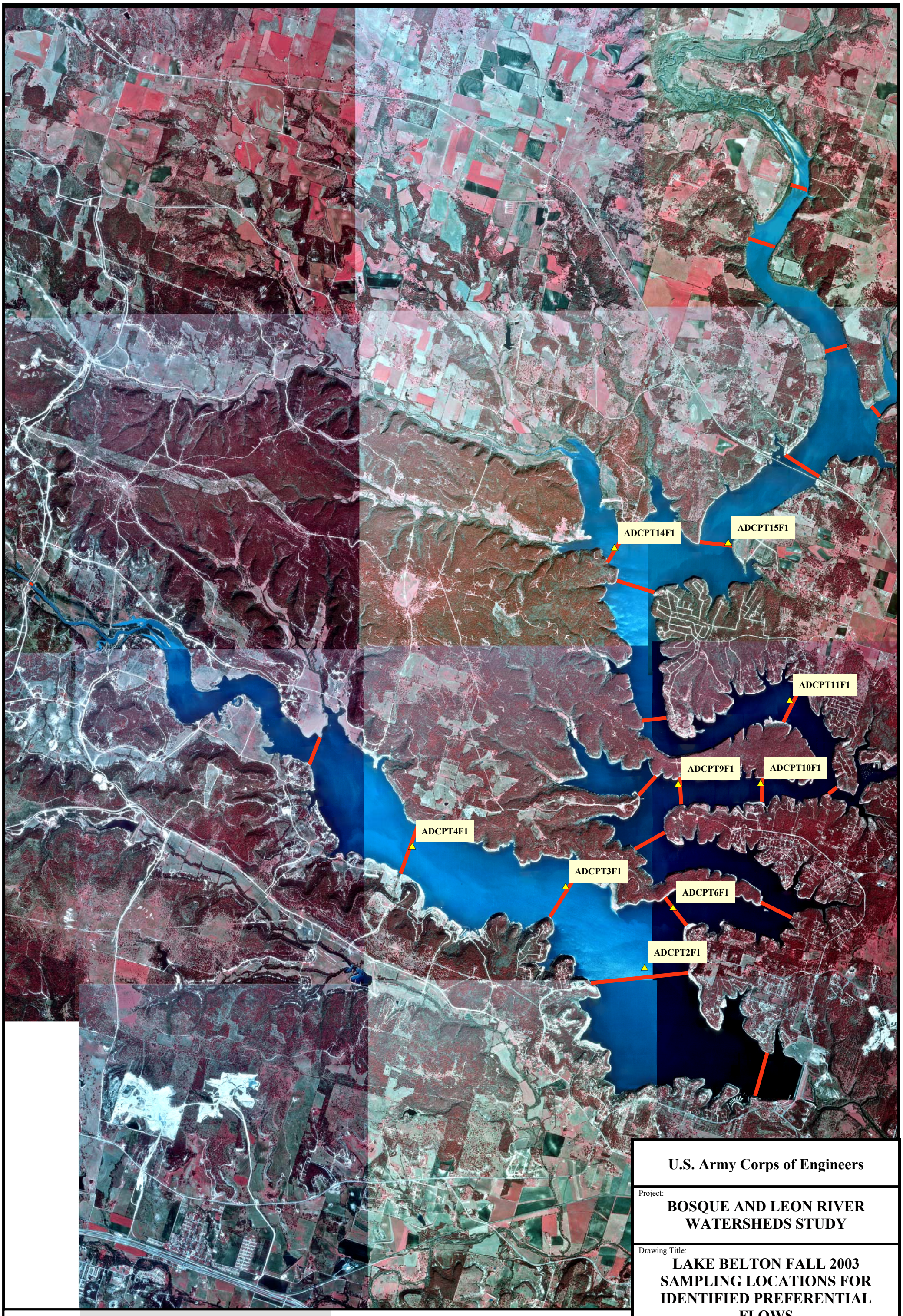
Drawing Title:

LAKE BELTON FALL 2003 SAMPLING LOCATIONS FOR DEEPEST POINT ACROSS EACH TRANSECT

Sheet 1 of 1 Sheets

SCALE: 1:67,843

FIGURE: 5-24



U.S. Army Corps of Engineers

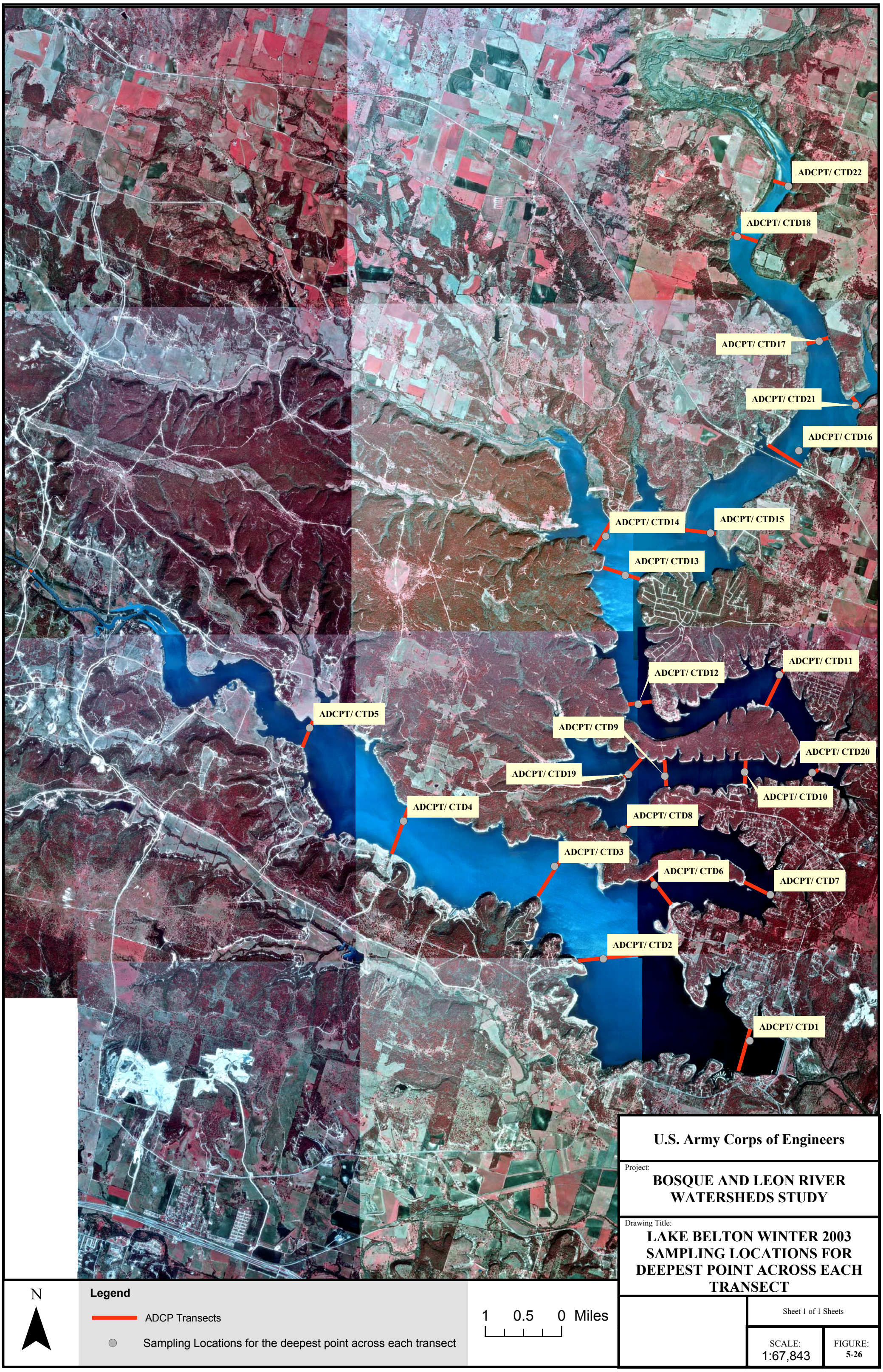
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY

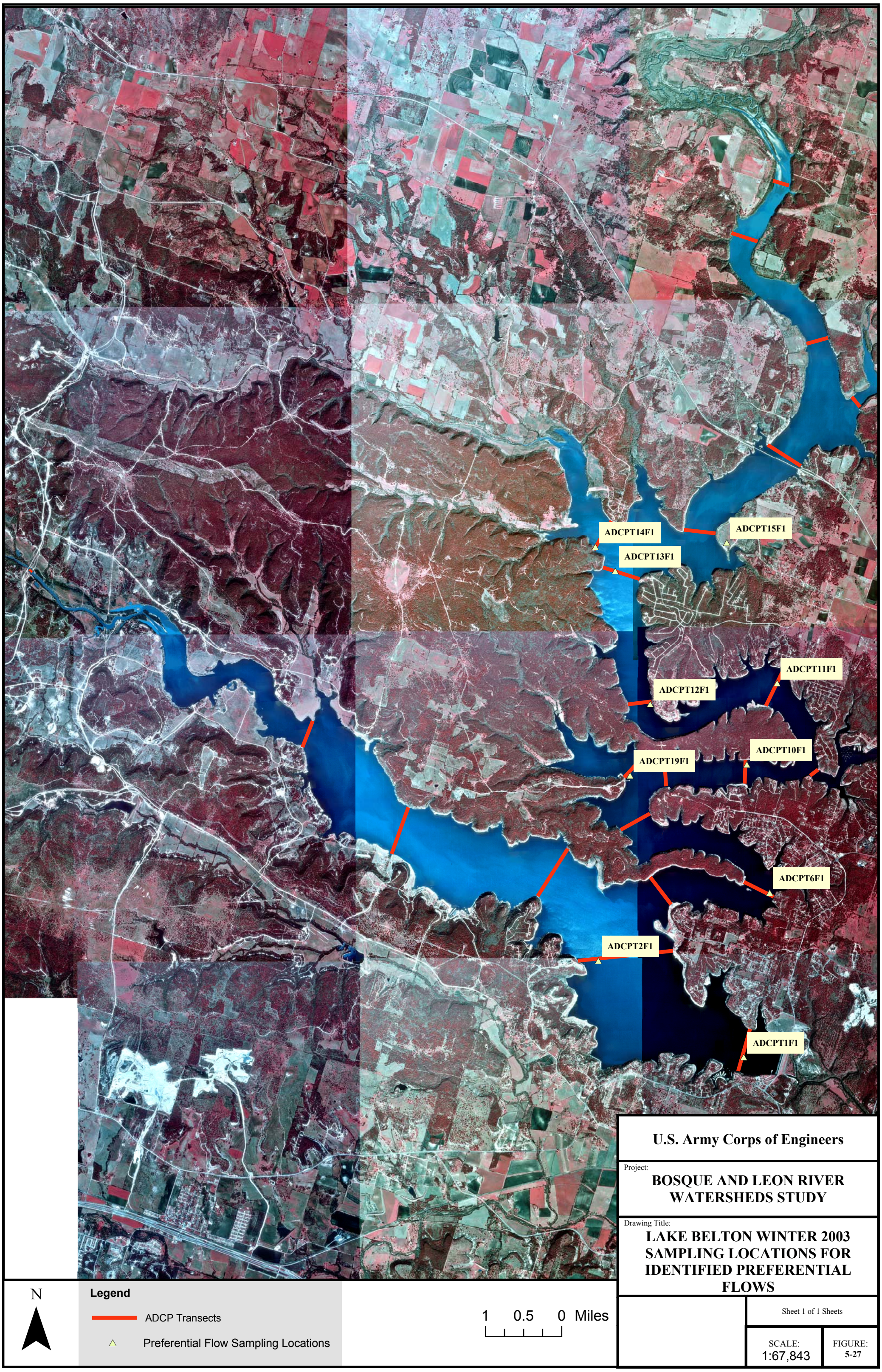
Drawing Title:
LAKE BELTON FALL 2003 SAMPLING LOCATIONS FOR IDENTIFIED PREFERENTIAL FLOWS

Sheet 1 of 1 Sheets

SCALE: 1:67,787

FIGURE: 5-25


ADCPT/ CTD22
ADCPT/ CTD18
ADCPT/ CTD17
ADCPT/ CTD21
ADCPT/ CTD16
ADCPT/ CTD14
ADCPT/ CTD15
ADCPT/ CTD13
ADCPT/ CTD11
ADCPT/ CTD12
ADCPT/ CTD5
ADCPT/ CTD9
ADCPT/ CTD20
ADCPT/ CTD19
ADCPT/ CTD10
ADCPT/ CTD4
ADCPT/ CTD8
ADCPT/ CTD3
ADCPT/ CTD6
ADCPT/ CTD7
ADCPT/ CTD2
ADCPT/ CTD1
N
Legend
ADCP Transects
Sampling Locations for the deepest point across each transect
1 0.5 0 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
LAKE BELTON WINTER 2003 SAMPLING LOCATIONS FOR DEEPEST POINT ACROSS EACH TRANSECT
Sheet 1 of 1 Sheets
SCALE:
1:67,843
FIGURE:
5-26



U.S. Army Corps of Engineers

Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY

Drawing Title:
LAKE BELTON WINTER 2003 SAMPLING LOCATIONS FOR IDENTIFIED PREFERENTIAL FLOWS

Sheet 1 of 1 Sheets

SCALE: 1:67,843

FIGURE: 5-27

Sample Analysis. All surface water samples were analyzed for perchlorate by USEPA Method 314.0 at the USACE Engineer Research and Development Center Environmental Laboratory at the Environmental Chemistry Branch in Omaha, Nebraska (See **Appendix V**). The USACE laboratory conformed to the analytical method requirements, analytical quality control requirements, instrument calibration frequency, and the laboratory quality control requirements presented in the QAPP (MWH, 2002e). The data verification report for samples analyzed by the USACE is included in **Appendix W**.

5.1.2.3.3 Data

A total of 40 water samples (including three duplicate samples) were collected from the deepest point across 23 ADCP transects and a total of 11 preferential flow samples (including two duplicate samples) were collected during the fall 2003 sampling event. A total of 43 samples (including four duplicate samples) were collected from the deepest point across the 22 ADCP transects and a total of 11 preferential flow samples (including one duplicate sample) were collected during the winter 2003 event. No perchlorate was detected in any of the ADCP samples collected during these studies. Analytical findings for the deepest point across each transect and flows for the Fall 2003 survey and the Winter 2003 survey are included in **Appendix L**.

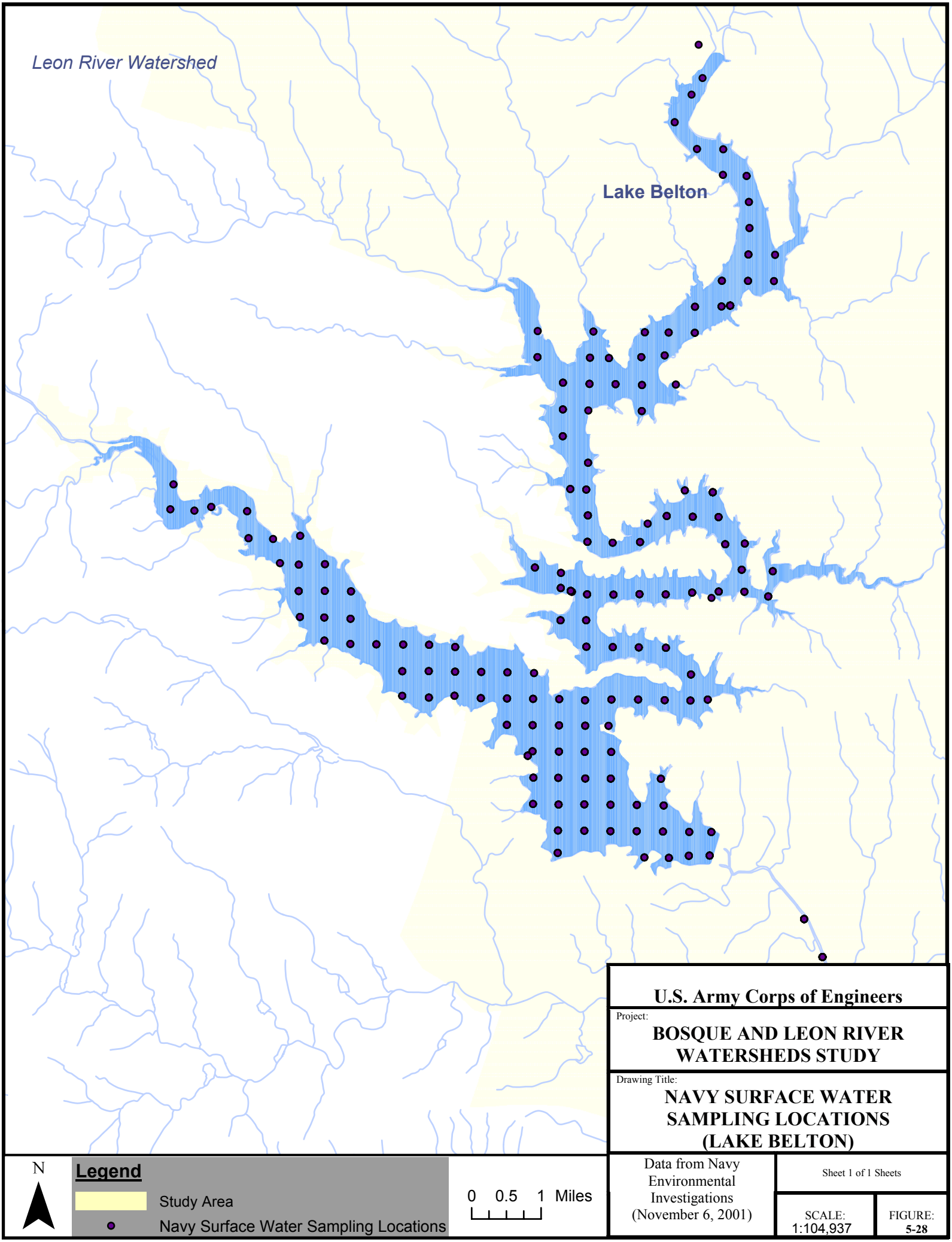
5.1.2.4 Historical Data

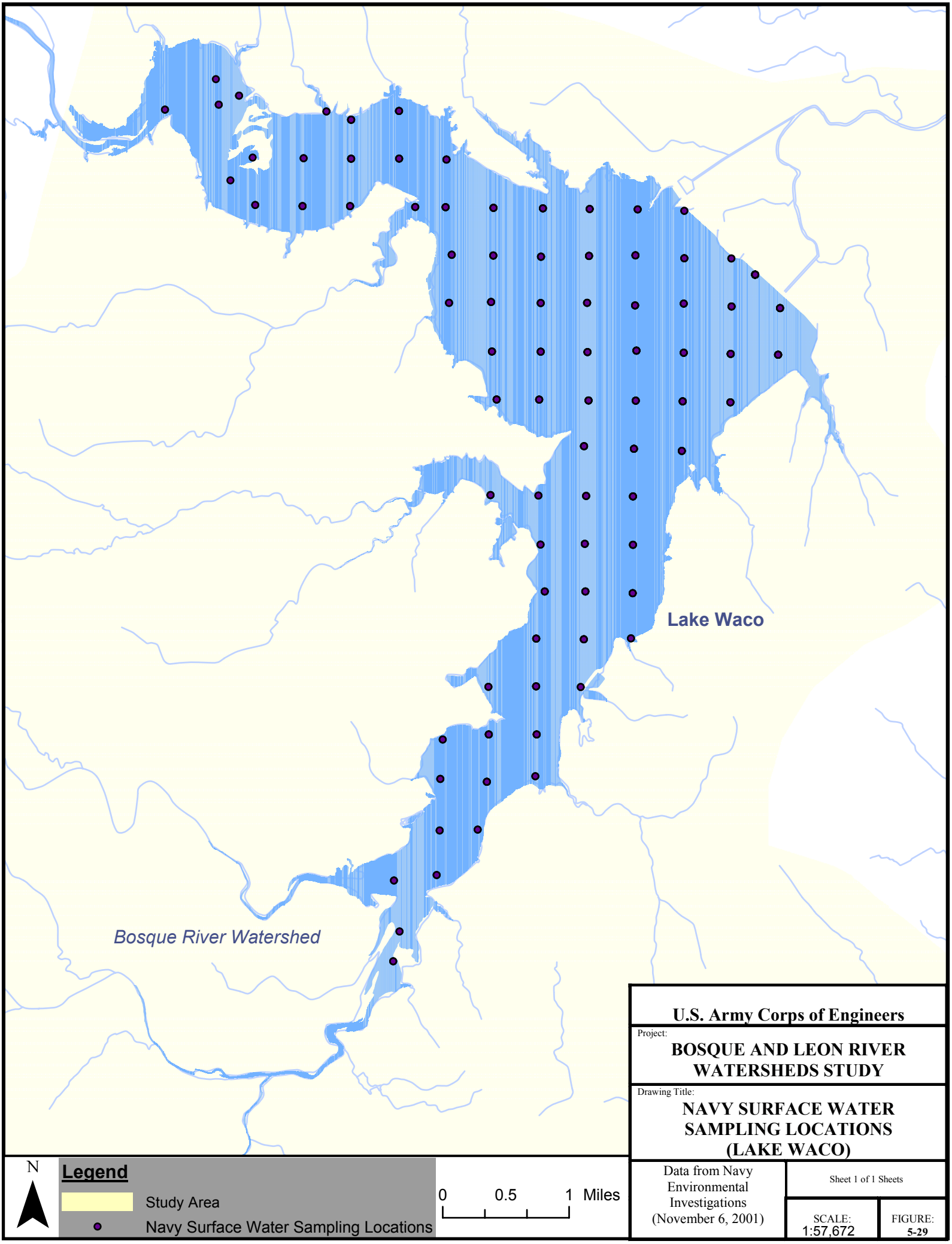
5.1.2.4.1 Introduction

The U.S. Navy conducted surface water sampling in both Lake Belton and Lake Waco between 1998 and 2001. The locations of surface water sampling for Lake Belton and Lake Waco are shown in **Figure 5-28** and **Figure 5-29**, respectively. The most extensive sampling in the lakes was conducted as part of the Phase III Groundwater Investigation at NWIRP McGregor. During this investigation, the U.S. Navy assessed water quality at Lake Belton and Lake Waco. The lake assessment approach is presented in *Lake Water Quality Assessment Work Plan* (EnSafe, 2000a). This investigation was designed to complete a thorough, yet expedited, environmental assessment of perchlorate in the lakes and produce data that could be used to assess risk to human health and the environment. EnSafe conducted two sampling events in spring 2000 and summer 2000. The Spring 2000 sampling event was conducted to assess the lakes under cool weather, cool water conditions, while the Summer 2000 sampling event was conducted to assess the lakes under warm weather, warm water conditions. The U.S. Navy also sampled all the potable water intakes in Lake Belton and Lake Waco monthly since March 1999.

5.1.2.4.2 Methodology

The U.S. Navy has completed extensive perchlorate sampling in both Lakes Belton and Waco. The overall approach, sampling techniques, and methods used for lake assessments are described in detail in the *NWIRP McGregor Final Groundwater Investigation Work Plan* (EnSafe, 1998b).


Leon River Watershed
Lake Belton
N
Legend
Study Area
Navy Surface Water Sampling Locations
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SURFACE WATER SAMPLING LOCATIONS (LAKE BELTON)
Data from Navy Environmental Investigations (November 6, 2001)
Sheet 1 of 1 Sheets
SCALE: 1:104,937
FIGURE: 5-28


Lake Waco
Bosque River Watershed
N
Legend
Study Area
Navy Surface Water Sampling Locations
0
0.5
1
Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SURFACE WATER SAMPLING LOCATIONS (LAKE WACO)
Data from Navy Environmental Investigations (November 6, 2001)
Sheet 1 of 1 Sheets
SCALE:
1:57,672
FIGURE:
5-29

Lake Belton and Lake Waco underwent two seasonal sampling events: a "cool water" event in the spring and a "warm water" event in the summer. Identical field protocols were used for each lake. To provide a sample representative of the lake surface water during each event, a 2,000-foot by 2,000-foot sampling grid was established in each lake. The sampling grids are presented as **Figure 5-28** and **Figure 5-29**.

Before collecting analytical samples, EnSafe personnel recorded the chemical and physical profile of the water column at each grid location, regardless of depth. The probe of a calibrated Horiba U-23 data logger was placed overboard and suspended with sensors just below the water surface to equilibrate. The activated datalogger was slowly hand lowered to the lake bottom, collecting a set of real-time readings during its descent. The Horiba U-23, attached to a laptop computer, measured and recorded these location specific parameters.

- pH
- Temperature
- Conductivity
- Turbidity
- Dissolved Oxygen (DO)
- Depth
- Salinity
- Total Dissolved Solids (TDS)
- Oxidation/ Reduction Potential (ORP)
- Specific Gravity
- Chloride ion (Cl^-)
- Ammonium ion (NH_4^+)
- Nitrate ion (NO_3^-)

As the Horiba measurements were being recorded and the profiles graphed, the sampling crew prepared to collect the surface water samples. Depending on water depth, up to three water samples were collected at each location. If the water depth was less than 10 feet, only one sample was collected at the bottom of the lake just above the sediment surface (hypolimnion). If the depth was between 10 and 30 feet, a surface (epilimnion) sample was also collected. If water depth was greater than 30 feet, surface, bottom, and intermediate (metalimnion) samples were collected. The primary determining factor in selecting the collection depth of the intermediate sample proved to be the temperature gradients. During both profiling events, no discernable variations in conductivity (an alternate indicator) were observed at each lake. If the water profile/graphs showed a distinct change in temperature (thermocline), or even if a minimal gradient resulted in a curved graph, an intermediate water sample was collected from immediately below it, presuming that cooler, denser water would exhibit higher perchlorate concentrations.

If a temperature gradient was absent or if the thermocline was near the bottom, the intermediate sample was collected at mathematical mid-depth. In water 30 feet or less, the intermediate sample was omitted to avoid sample redundancy.

To collect a discrete grab water sample, a messenger-activated 1.2-liter stainless steel Kemmerer bottle with a graduated line was used.

The U.S. Navy collected samples from the potable water intakes and the Wildflower Country Club irrigation intake in the study area previously discussed in Section 5.1.2.2.1. These samples were collected in accordance with the thief sampling methodologies described in Section 5.1.2.1.2 or direct sampling into appropriate containers from hosebib locations.

5.1.2.4.3 Data

Detailed observations, and findings made during both the sampling events are provided in the *Final Draft Report Lake Water Quality Assessment* (EnSafe, 2000b). A summary of these findings is presented below.

A total of 336 water samples were collected from 141 sample locations in Lake Belton during the Spring 2000 sample collection event and 331 water samples were submitted from 143 locations during the Summer 2000 event. Additionally, a total of 150 surface water samples were collected from 78 sample locations in Lake Waco during the Spring 2000 sample collection event and 142 water samples were collected from 82 sampling locations during the Summer 2000 event.

The following briefly describes the laboratory findings for both the lakes, as reported by the U.S. Navy.

- **Spring 2000 (Cool Water)** - Perchlorate was not detected in any lake water samples analyzed at or above 4 µg/L and no water sample had an estimated perchlorate concentration less than 4 µg/L, the pratical quantitation limit (PQL).
- **Summer 2000 (Warm Water)** – Perchlorate was detected in a single water sample from Lake Waco during the summer sampling event. Intermediate water sample WACW204102 from the center of the lake, 23 feet below the surface had a perchlorate detection of 17.4 µg/L reported by the laboratory. The surface water and bottom interval samples from this location were below detectable levels for perchlorate. The U.S. Navy data validator reviewed the laboratory's findings and flagged the 17.4 µg/L result with a "J" qualifier, meaning it is an estimated value. A memorandum discussing the validation steps for this single perchlorate detection and rationale for no further action is provided in detail in Appendix I of the *Final Draft Report Lake Water Quality Assessment* (EnSafe, 2000b). The location of this sample is shown in **Figure 5-30**. The U.S. Navy decided that that they would continue to report the detection as 17.4 µg/L "J". However, based on the analytical measures performed by the laboratory and a comparison to data collected from the

sample point and surrounding points, the U.S. Navy concluded that the isolated detection of perchlorate in Lake Waco should be considered an unreproducable anomaly and not representative of contamination in Lake Waco.

Of all the 959 lake water samples collected during both sampling events, the U.S. Navy reported only one questionable detection, which represents 0.1% of the samples. The analytical data for this investigation are discussed in detail in the *Final Draft Report Lake Water Quality Assessment* (EnSafe, 2000b).

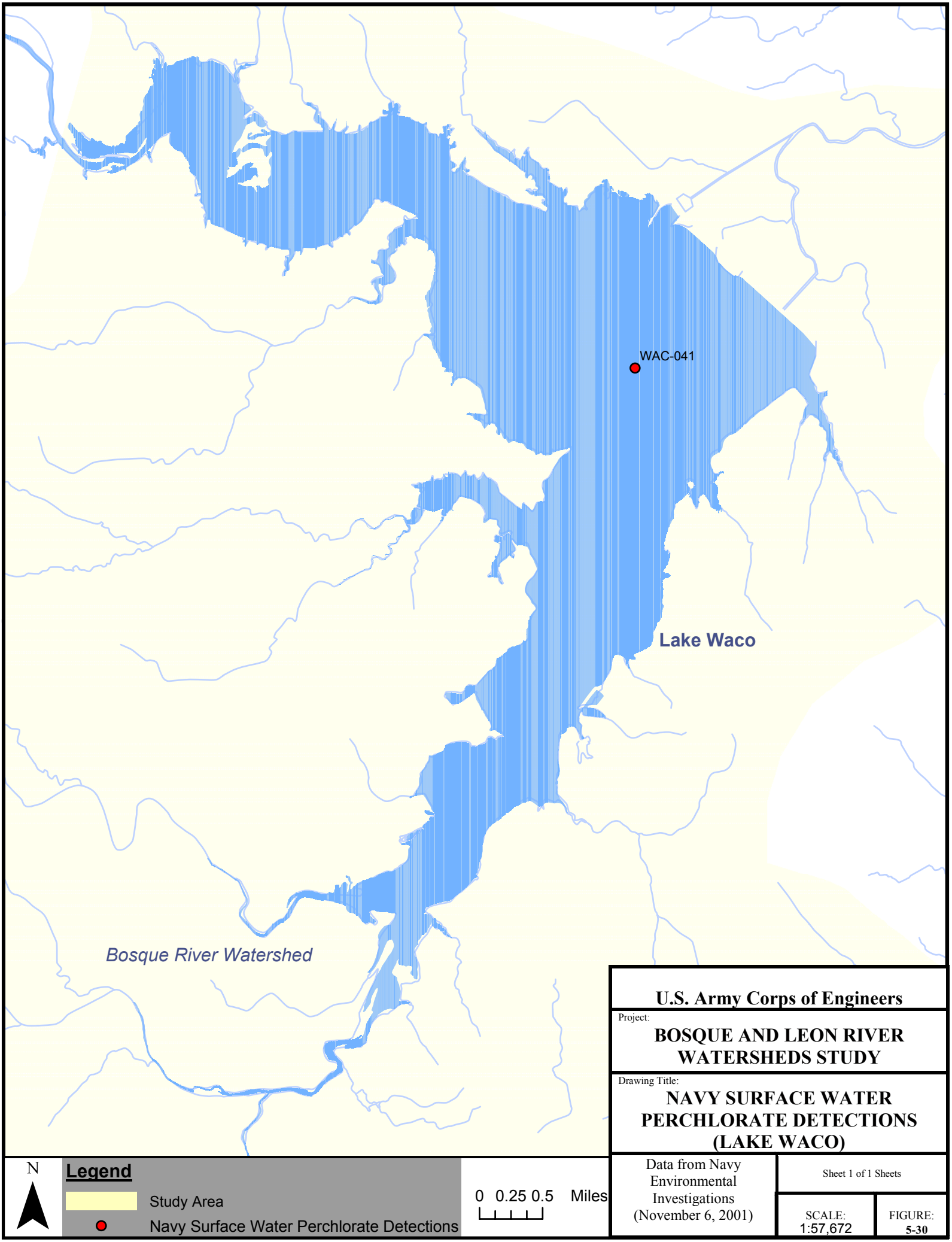
However, based on a review of previous EnSafe reports, MWH confirmed that there has been one other perchlorate detection that has occurred in Lake Belton as part of U.S. Navy studies. This sample was collected from the southern portion of Lake Belton (BEL-071, 7 µg/L).

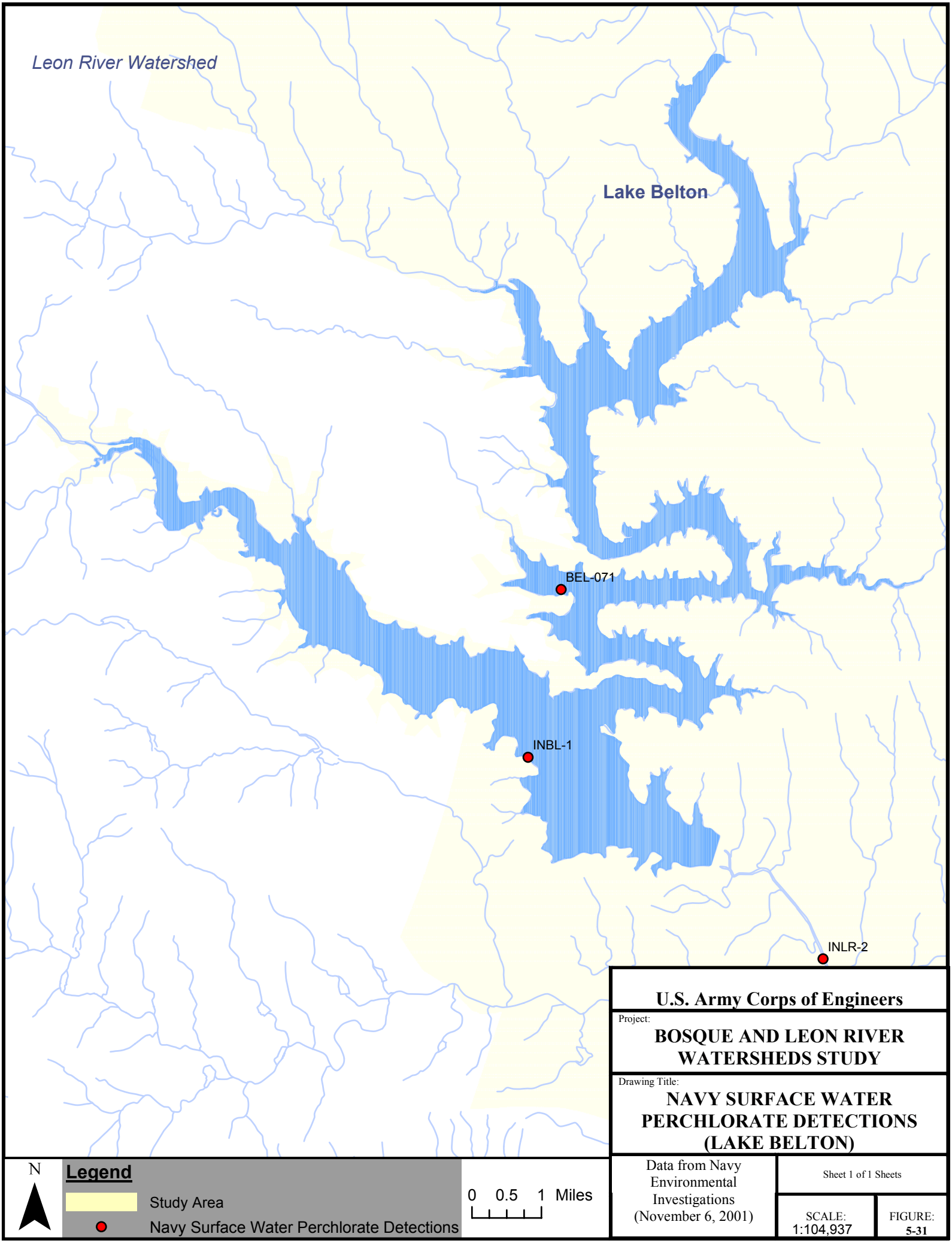
The U.S. Navy collected a total of around 243 intake samples at Lake Intakes since February 1999 through December 2002. Most of the monthly samples collected by the U.S. Navy from the five water intakes have been below detection levels for perchlorate. The only three detections that have occurred in the intakes sampled by the U.S. Navy were at the Bell County Raw-Water Intake (INBL-1, 4.1 µg/l in October 1999) and the Wildflower Country Club irrigation intake (INLR-2, 0.67 µg/l in March 1999 and 6 µg/l in July 2000).

The locations of all samples that had perchlorate detections in Lake Belton are shown in **Figure 5-31**.

5.1.2.5 Discussion

Based on the historical sampling data reviewed and discussed above, previous detections in the lakes are limited to one sample in Lake Waco (17.4 µg/L) that was considered to be an anomaly by the U.S. Navy, two samples in Lake Belton (4.1 µg/L and 7 µg/L) and two samples downstream of the Lake Belton dam (0.67 µg/L and 6 µg/L). These are the only five detections out of approximately 1,202 samples collected during these studies. If perchlorate were reaching the lakes, detections would most likely occur in the delta areas of these reservoirs as these areas receive direct discharge from the Bosque and Leon River watersheds and collect the greatest sediment deposition in each lake. The project team sampled these delta areas during this study and no perchlorate was detected in the delta area samples collected. In addition, no perchlorate was detected during ADCP transect sampling within Lake Belton or any of the water or irrigation intakes sampled within both the lakes. Based on the data collected by the U.S. Navy and the USACE project team, perchlorate detections have been limited to two random hits in Lake Belton, two hits downstream of the Lake Belton dam and one questionable detection in Lake Waco. Based on all of the non-detect results collected as part of the delta area, ADCP transect, and intake studies performed by the project team and the historical studies discussed, significant perchlorate concentrations are not consistently being detected within either of the lakes.


WAC-041
Lake Waco
Bosque River Watershed
N
Legend
Study Area
Navy Surface Water Perchlorate Detections
0 0.25 0.5 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SURFACE WATER PERCHLORATE DETECTIONS (LAKE WACO)
Data from Navy Environmental Investigations (November 6, 2001)
Sheet 1 of 1 Sheets
SCALE:
1:57,672
FIGURE:
5-30


Leon River Watershed
Lake Belton
BEL-071
INBL-1
INLR-2
N
Legend
Study Area
Navy Surface Water Perchlorate Detections
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SURFACE WATER PERCHLORATE DETECTIONS (LAKE BELTON)
Data from Navy Environmental Investigations (November 6, 2001)
Sheet 1 of 1 Sheets
SCALE: 1:104,937
FIGURE: 5-31

5.1.3 Groundwater/Surface Water Interactions

5.1.3.1 Introduction

To assist in determining how perchlorate may move between groundwater and surface water, a groundwater/surface water interaction study was performed during this study. This portion of the Study was conducted as part of the Longitudinal Stream Sampling Study. All the methodologies and protocol followed are detailed in the *Final Longitudinal Stream Sampling Study Field Sampling Plan* (MWH, 2002b). Any deviations from the Field Sampling Plan are discussed further below.

5.1.3.2 Methodology

The methodology regarding the installation and setup of the automated sampling stations was previously discussed in detail in Section 5.1.1.2.2. Each station was equipped with an ISCO 4230 Flow Meter (equipped with a bubbler and a rain gauge) to monitor stream level and rainfall on a 15 minute basis. This study also included the installation of shallow groundwater monitoring wells near the surface water monitoring stations and installation of a water level pressure transducer into each well to monitor groundwater levels. Groundwater levels were also collected every fifteen minutes. Monitoring wells were constructed at eleven of the 15 automated sampling station locations, as listed in **Table 5-5**.

Table 5-5
Groundwater Monitoring Well Locations

Monitoring Station ID	Groundwater Monitoring Well Installed (Stations with a nearby well marked with an X)	Watershed
TRM1	X (shared well with station SC1)	Lake Belton
OC1	X	Lake Belton
SC1	X (shared well with station TRM1)	Lake Belton
SC3	X	Lake Belton
SC5	X	Lake Belton
LR1	X	Lake Belton
CHC1	No well installed	Lake Belton
SBR1	X	Lake Waco
SBR2	X	Lake Waco
SBR4	X	Lake Waco
HC1	X	Lake Waco
HC2	X	Lake Waco
SBR3	No well installed due to accessibility issues	Lake Waco
SBR5	X	Lake Waco
MBR1	No well installed due to accessibility issues	Lake Waco

The monitoring wells were located such that groundwater elevation measurements collected (in the shallow water-table aquifer) could be compared with the measurements collected at the nearby surface water monitoring stations. The resulting data were used to assess groundwater and surface water interactions and determine how groundwater levels were impacted by seasonal rainfall and storm events. Information on monitoring well construction and well completion data is included in **Appendix M**.

All completed monitoring wells were equipped with In-Situ, Inc. miniTROLL digital pressure transducers. The probes were ordered on a well-specific basis from In-Situ Inc. and cable lengths were pre-cut to fit the individual wells. The probes were installed into the wells to a depth of approximately 1-foot above bottom. This setting depth allows the probe to collect water readings even if water levels fall to near the bottom of the well, and allows the probe to be kept off of the well bottom where the pressure sensor may become surrounded by sediment. Probes do not need to be removed from the well to access data, as the support cable can be accessed at the surface with an interface cable. The pressure transducers were programmed to record the water level in the well every 15 minutes. This is the same data collection period as the Sampling Stations.

The data logger converts the pressure value sent by the transducer into feet of water above the transducer and records the values in its memory. The data were then downloaded from the logger to a PC computer using an RS-232 port. Each transducer had specific parameters that were input to the data logger to make the appropriate conversions from pressure units to feet of water. The field sampling team collected these data each time they mobilized to the sampling stations during scheduled sampling rounds.

5.1.3.3 Data

MWH performed a detailed, manual evaluation of the extensive data sets generated by the automated stream level and groundwater level equipment. These data evaluations were required prior to processing and analysis of the data to ensure the integrity of the data and conformance with the data quality objectives for the study.

Time Corrections

The time change between Standard Time (October 27, 2002) and Daylight Saving Time (April 6, 2003) for the sampling stations required data correction. Some stations were not reprogrammed immediately to reflect the time changes, which required the raw time data to be changed by an hour (back or forward depending on which time change) until the equipment was adjusted to record actual time. These corrections were documented with comments in the data spreadsheets.

Rainfall Data Corrections

Due to intermittent occurrences of equipment errors and dead batteries associated with the ISCO rainfall monitoring equipment, retrieved data had to be corrected to more closely reflect actual rainfall occurrences. To accomplish this task, accurate rainfall data from the closest possible sampling station were used to replace erred data. These occurrences were documented in the data spreadsheets and in the rainfall graphs that were generated and are as follows:

- For monitoring station CHC1, rainfall data from monitoring station LR1 were used from November 5, 2002 to March 24, 2003 due to equipment error.
- For monitoring station SC1, rainfall data from monitoring station SC3/OC1 rainfall data were used from August 18, 2003 to October 20, 2003 due to equipment error.
- For monitoring station TRM1, rainfall data from monitoring station SC3/OC1 rainfall data were used from August 18, 2003 to October 20, 2003 due to equipment error.

Monitoring station SBR5 was located under an overpass where rainfall could not be recorded. Consequently, rainfall data from the closest monitoring station, MBR1, were used to populate the rainfall data for monitoring station SBR5. The rainfall data were added to the monitoring station SBR5 data set because it was important to see trends in surface and ground water levels in conjunction with the rainfall events.

GW/SW Data Corrections

Due to intermittent occurrences of equipment errors, dead batteries, and high sediment levels accumulating on the strainer, the surface water levels and ground water levels retrieved were corrected to more closely reflect manual quality assurance and control levels taken at approximately two week intervals. Surface water levels at two monitoring stations, SBR3 and LR1, particularly toward the end of the sampling period, showed signs of high sedimentation that blocked the strainer and prevented reading surface water levels and retrieving storm samples. Some data from these two stations were discarded from the data set used for analysis, due to uncorrectable inaccuracies.

Based on the detailed review and evaluation of the extensive data generated by the surface water and groundwater level monitoring equipment, the vast majority of the collected data are of appropriate quality and were determined to be suitable for use in analysis of surface water and groundwater interactions. These data are all included in **Appendix N** and discussed below.

5.1.3.4 Discussion

One of the purposes of simultaneous monitoring of groundwater levels, stream levels, and rainfall at various locations throughout the study area was to obtain a more systematic understanding of watershed hydrology and the interrelationship of these three key components. As such, similar to the perchlorate concentration data from the monitoring stations, these data will be discussed in terms of stream segments, moving from upstream to downstream, as listed in **Table 5-6**:

Table 5-6
Longitudinal Monitoring Station Locations, by Stream Segment

STREAM SEGMENT	MONITORING STATIONS
NWIRP to Lake Belton, Leon River	
Station Creek above Tributary M	SC1
Station Creek between Tributary M and Onion Creek	SC3, TRM1
Station Creek below Onion Creek	SC5, OC1
Leon River below Station Creek	LR1
Fort Hood to Lake Belton	
Cowhouse Creek	CHC1
NWIRP to Lake Waco	
Harris Creek	HC1, HC2, SBR3
South Bosque River upstream of Harris Creek	SBR1, SBR2, SBR4
South Bosque River between Harris Creek and Middle Bosque River	SBR5
Middle Bosque River	MBR1

Plots of groundwater level, surface water level, and daily rainfall data from all stations are included in **Appendix N**.

5.1.3.4.1 NWIRP to Lake Belton

Station Creek Above Tributary M

The segment of Station Creek upstream of its confluence with Tributary M is characterized by monitoring station SC1. The stream level, groundwater level, and rainfall data are graphically depicted on the monitoring station SC1 plot included in **Appendix N**. The monitoring period time line spans the x-axis. Stream level and groundwater level are depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at this location varied by approximately 7 feet during the monitoring period, ranging from dry (approximately 753.5 fmsl) to 760.5 fmsl. Rainfall amounts ranged from 0 to approximately 5.5 inches per day. Ten rainfall events exceeded 1.0 inch per day. Eight of these ten events coincide with stream level increases of at least two feet. These stream level increases are very short in duration, with the majority of the runoff peak from the rainfall event passing within 30 hours of the cessation of the event.

One of the other two events in excess of one inch per day coincides with a stream level increase of approximately 0.8 foot. The remaining rainfall event greater than one inch per day did not coincide with a stream level increase; rather, the stream remained dry during this event. A comparison of these rainfall data to those measured at nearby monitoring stations suggests that this rainfall event was localized. As a result, it is thought that rainfall from this event measured at monitoring station SC1 did not extend over a large enough area to generate significant runoff at this station. All significant stream level spikes observed at monitoring station SC1 appear to coincide with rainfall events.

Groundwater levels at monitoring station SC1 varied by approximately 3.5 feet during the monitoring period, ranging from approximately 756 to 759.5 fmsl. Groundwater level increases were generally sudden events, similar to stream level spikes. Conversely, groundwater level decreases occurred over a period of days or weeks, with the rate of drop decreasing with time. This is considered to be a classic response pattern in groundwater systems. All recorded groundwater level increases at this monitoring station coincided with recorded rainfall events. However, not all rainfall events resulted in groundwater level increases. As with stream level, this is likely a result of the very localized nature of some of the rainfall events.

Throughout the monitoring period, groundwater levels in the monitoring well were always higher than stream levels, suggesting that groundwater discharges to the stream. Therefore, this segment of the stream is characterized as a gaining stream.

Station Creek Between Tributary M and Onion Creek

The segment of Station Creek between tributary M and Onion Creek is characterized by monitoring stations TRM1 and SC3. The stream level, groundwater level, and rainfall data are graphically depicted on the plots for monitoring stations TRM1 and SC3 included in **Appendix N**. The monitoring period time line spans the x-axis. Stream level and groundwater level are depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at monitoring station SC3 varied by approximately 4.5 feet during the monitoring period, ranging from dry (approximately 719.5 fmsl) to 724 fmsl. Rainfall ranged from 0 to approximately 1.4 inches per day. Eight rainfall events exceeded 1 inch per day. All of these six events coincide with stream level increases ranging from approximately 1 to 3 feet. As with the upstream segment of Station Creek, these stream level increases are very short in duration.

Stream levels at monitoring station TRM1 varied by approximately 5 feet during the monitoring period, ranging from dry (approximately 755.5 fmsl) to 760.5 fmsl. Rainfall ranged from 0 to 5.5 inches per day. Nine rainfall events exceeded 1 inch per day. Eight of these nine events coincide with stream level increases ranging from approximately 0.5 to 5 feet. As with the monitoring station SC3, these stream level increases are very short in duration.

The remaining rainfall event in excess of 1 inch per day did not coincide with a large stream level increase. The response to this event is not clearly discernable from other events that influence stream levels at this location.

Numerous stream level spikes that do not coincide with rainfall events were recorded at monitoring station TRM1. These stream level increases are believed to be a result of discharges of water in association with the U.S. Navy's remediation efforts at NWIRP. These events are particularly discernable on the plot for monitoring station TRM1 (**Appendix N**) during the period of February through May 2002. While more muted, these same patterns are discernable in stream levels at the downstream monitoring station SC3.

Groundwater levels at monitoring station TRM1 varied by approximately 3.5 feet during the monitoring period, ranging from approximately 756 to 759.5 fmsl. This monitoring well is shared between monitoring station SC1 and TRM1. Groundwater level increases were generally sudden events, similar to stream level spikes. Conversely, groundwater level decreases occurred over a period of days or weeks, with the rate of drop decreasing with time. This is considered to be a classic response pattern in groundwater systems. All recorded groundwater level increases at this monitoring station coincided with recorded rainfall events. However, not all rainfall events resulted in groundwater level increases. As with stream level, this is likely a result of the very localized nature of some of the rainfall events.

Groundwater levels at monitoring station SC3 varied by approximately 8 feet, ranging from approximately 717 to 725 fmsl. While the larger increases in groundwater levels were sudden and coincident with rainfall, a pattern of smaller increases, on the order of a few tenths of a foot, was clearly observable in this monitoring well. In addition to a difference in magnitude, these fluctuations occur over a more extended period of time. The resultant shape of the hydrograph from these events is a hump rather than a spike, as seen during the month of April 2002 on the plot for monitoring station SC3 (**Appendix N**). These groundwater level humps at monitoring station SC3 coincide with the non-rainfall related stream level increases recorded at monitoring station TRM1.

At monitoring station TRM1, groundwater levels in the monitoring well were higher than stream levels, except during several of the peaks associated with the water level increases attributed to NWIRP discharges. During these discharge events, Tributary M appears to transition from a gaining stream to a losing stream, with surface water flowing into the groundwater system during these discharge events. The effect of this groundwater recharge is reflected in increases in groundwater levels at the downstream monitoring station SC3. Relative to stream level, groundwater levels at monitoring station SC3 vary from above to below stream, depending on the frequency and duration of rainfall events. Therefore, this segment of the stream is characterized as transitional between a gaining stream and losing stream, depending on both stream flow and rainfall.

Station Creek Below Onion Creek

The segment of Station Creek below Onion Creek is characterized by monitoring stations OC1 and SC5. The stream level, groundwater level, and rainfall data are graphically depicted on the plots for monitoring stations OC1 and SC5 included in **Appendix N**. The monitoring period time line spans the x-axis. Stream level and groundwater level are depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at monitoring station SC5 varied by nearly 16 feet during the monitoring period, ranging from dry (602.5 fmsl) to just over 618 fmsl. Rainfall ranged from 0 to nearly 3 inches per day. Six rainfall events exceeded 1 inch per day. Five of these six events coincide with stream level increases of at least two feet. These stream level increases are very short in duration, with the majority of the runoff peak from the rainfall event passing within 72 hours of the cessation of the event. The remaining rainfall event in excess of 1 inch per day coincided with a much smaller stream level increase.

Four stream spikes at monitoring station SC5 do not coincide with large rainfall events recorded at this same monitoring station. However, rainfall data from upstream monitoring stations suggest that this stream water level peak was, in fact, a result of rainfall runoff. This rainfall event appears to have been localized to the extent that only minor rainfall was recorded at monitoring station SC5 despite the measurement of the stream level increase.

Stream levels at monitoring station OC1 varied by approximately 4.5 feet during the monitoring period, ranging from dry (707.5 fmsl) to 712 fmsl. Rainfall ranged from 0 to approximately 5.5 inches per day. Twelve rainfall events exceeded 1 inch per hour. Nine of these twelve events coincide with stream level increases of at least one foot. These stream level increases are very short in duration, with the majority of the runoff peak from the rainfall event passing within 48 hours of the cessation of the event. It is presumed that the remaining three rainfall events in excess of 1 inch per day were either not of sufficient duration or did not extend over a large enough area to generate sufficient runoff to measurably increase water levels at this location.

Groundwater levels at monitoring station SC5 varied by over 11 feet during the monitoring period, ranging from approximately 602.5 to 614 fmsl. Groundwater level increases were generally sudden events, similar to stream level spikes. Conversely, groundwater level decreases occurred over a period of days or weeks, with the rate of drop decreasing with time. This is considered to be a classic response pattern in groundwater systems. All groundwater level increases at this monitoring station in excess of a few tenths of a foot coincided with recorded rainfall events. However, as observed at other monitoring stations, not all rainfall events resulted in groundwater level increases.

With the exception of late summer 2002, groundwater levels in the monitoring well were always higher than stream levels, suggesting that groundwater discharges to the stream. However, during the late summer, groundwater levels dropped below stream levels,

indicating this segment of the stream to be transitional with respect to gaining and losing conditions, like the preceding stream segment.

Groundwater levels at monitoring station OC1 varied by approximately 8 feet during the monitoring period, ranging from approximately 702 to 710 fmsl. Groundwater level increases on the order of 1 foot were coincident with rainfall events and generally sudden, as observed at other locations. However, an extensive number of hump-shaped water level fluctuations, on the order of a few tenths of a foot, were recorded. The peaks and troughs of these fluctuations are coincident with those recorded at monitoring station SC3 and attributed to discharges from NWIRP. However, no discharges from NWIRP are known to occur in Onion Creek. Groundwater levels at monitoring station OC1 are consistently lower than the stream levels, indicating that this segment of Onion Creek is a losing stream.

Leon River Below Station Creek

The segment of the Leon River below Station Creek is characterized by monitoring station LR1. The stream level, groundwater level, and rainfall data are graphically depicted on the plot for monitoring station LR1 included in **Appendix N**. The monitoring period time line spans the x-axis. Stream level and groundwater level are depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at this location varied by approximately 18 feet during the monitoring period, ranging from 594 to 611 fmsl. Rainfall amounts ranged from 0 to over 2.4 inches per day. Nine rainfall events exceeded 1 inch per day. Five of these nine events appear coincident with stream level increases from approximately two to twelve feet. These stream level increases are somewhat longer in duration than those observed farther upstream in the watershed, with the majority of the runoff peak from the rainfall event passing within 10 days of the cessation of the event.

The remaining rainfall events in excess of 1 inch per day did not coincide with large stream level increases; rather, the observed stream level increases were on the order of a few tenths of a foot to one foot. It is presumed that this rainfall event was either not of sufficient duration or did not extend over a large enough area to generate sufficient runoff to measurable increase water levels at this location.

Contrary to the high coincidence of rainfall with stream level increases, very few stream level increases were coincident with rainfall. In fact, particularly during the summer months of 2003, daily variations in stream level at monitoring station LR1 are typical. This indicates that the stream flow characteristics at this location are dominated by management actions, such as releases for irrigation, on the Leon River upstream of Station Creek. Given the importance of the Leon River to the region's water supply, this result is expected.

Groundwater levels at monitoring station LR1 varied by nearly ten feet during the monitoring period, ranging from approximately 592 to over 601 fmsl. Groundwater level

increases were generally sudden events, similar to stream level spikes. Conversely, groundwater level decreases occurred over a period of weeks, with the rate of drop decreasing with time. This is considered to be a classic response pattern in groundwater systems. All recorded groundwater level increases at this monitoring station coincided with recorded rainfall events. However, not all rainfall events resulted in groundwater level increases. As with stream level, this is likely a result of the very localized nature of some of the rainfall events.

Throughout the monitoring period, groundwater levels in the monitoring well were always lower than stream levels, suggesting that this segment of the Leon River is a losing stream. However, there does not appear to be an appreciable response of groundwater level to the stream level fluctuations that dominate the Leon River during the summer months. As a result, the connection between the stream and groundwater systems at this location appears to be weak.

5.1.3.4.2 Cowhouse Creek

The entire drainage area of Cowhouse Creek tributary to Lake Belton is characterized by monitoring station CHC1. The stream level and rainfall data are graphically depicted on the plot for monitoring station CHC1 included in **Appendix N**. No groundwater level data were obtained. The monitoring period time line spans the x-axis. Stream level is depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at this location varied by approximately 6 feet during the monitoring period, ranging from approximately 574.5 to 580.5 fmsl. Rainfall ranged from 0 to 2.43 inches per day. Fourteen rainfall events were 1 inch per day or greater. Six of these fourteen events coincide with stream level increases of approximately 1 foot or more. These stream level increases are relatively long in duration, with the majority of the runoff peak from the rainfall event passing within 2 weeks of the cessation of the event. The remaining eight rainfall events in excess of 1 inch per day did not coincide with large stream level increases; rather, the observed stream level increases were either smaller or not discernable from other stream level variations.

5.1.3.4.3 NWIRP to Lake Waco

Harris Creek

The segment of Harris Creek upstream of its confluence with the South Bosque River is characterized by monitoring stations HC1, HC2, and SBR3. The stream level, groundwater level, and rainfall data are graphically depicted on the plots for monitoring stations HC1, HC2, and SBR3 included in **Appendix N**. The monitoring period time line spans the x-axis. Stream level and groundwater level are depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at monitoring station HC1 varied by approximately 4 feet during the monitoring period, ranging from 664.5 to 668.5 fmsl. Rainfall ranged from 0 to 1.75 inches per day. Five rainfall events exceeded 1 inch per day. All five of these events coincide with stream level increases ranging from approximately 1 to 3 feet. As with the

streams from NWIRP to Lake Belton, these stream level increases are very short in duration.

Stream levels at monitoring station HC2 varied by approximately 12 feet during the monitoring period, ranging from 593 fmsl to approximately 605 fmsl. Rainfall ranged from 0 to slightly over 3.5 inches per day. Eleven rainfall events exceeded 1 inch per day. All but three of these events coincide with stream level increases ranging from approximately 1.5 to 11 feet. As with other monitoring stations, these stream level increases are very short in duration. Three rainfall events in excess of 1 inch per day resulted in smaller stream level increases of only a few tenths of a foot. This is likely a result of the localized nature and/or short duration of rainfall events in the study area.

Stream levels at monitoring station SBR3 varied by approximately 8 feet during the monitoring period, ranging from approximately 492 to 500 fmsl. Rainfall ranged from 0 to 1.9 inches per day. Ten rainfall events exceeded 1 inch per day. Due to high sedimentation, the stream level data set for this monitoring station is incomplete. Where data are available, all rainfall events in excess of 1 inch per day coincide with stream level increases of at least 0.5 feet. As with other monitoring stations, these stream level increases are very short in duration.

Groundwater levels at monitoring station HC1 varied by over 5 feet during the monitoring period, ranging from approximately 663 to 668 fmsl. Groundwater level increases in excess of a few tenths of a foot were generally sudden events, similar to stream level spikes. Conversely, groundwater level decreases occurred over a period of days or weeks, with the rate of drop decreasing with time. This is considered to be a classic response pattern in groundwater systems. Six groundwater level increase events in excess of 1 foot were recorded at this monitoring station. Of these, only two clearly coincided with a recorded rainfall event in excess of 1 inch per day. Rainfall records from the nearby monitoring station HC2 indicate rainfall events that were coincident with the other four groundwater level rises. This indicates the localized nature of some of the rainfall events and is consistent with findings from monitoring stations on the NWIRP to Lake Belton tributary system.

Beginning in mid to late June 2003, groundwater levels at monitoring station HC1 exhibit a daily pattern of peaks and troughs with a total daily variation of a few tenths of a foot. The peaks typically occur near 10 AM and the troughs near 5 PM. This water level variation may be attributable to operation of a nearby well for irrigation purposes. Plant transpiration is not a likely cause given the timing of the peaks and troughs.

Groundwater levels at monitoring station HC2 varied from 570 to 602 fmsl. However, the pattern of groundwater level variation in this monitoring well is very different from all other monitoring stations, gradually rising until early April 2003 and then gradually decreasing throughout the remainder of the monitoring period. The only exception to this is a sudden increase of approximately 5 feet that appears to coincide with a rainfall event on October 9, 2003. These data suggest that the groundwater system at this location is largely disconnected from the surface hydrology dynamics. A review of the well log for

this monitoring well indicates that earth materials at this location were very dense, unfractured, and that water was not encountered during borehole drilling.

Groundwater levels at monitoring station SBR3 varied from approximately 494 to 498 fmsl. The pattern of groundwater level variation was typical of that observed at other monitoring stations. Strong coincidence of groundwater level increases to rainfall events was recorded.

Groundwater levels at monitoring station HC1 are variable relative to stream level, suggesting that the stream is transitory between gaining and losing conditions. At monitoring station SBR3, groundwater levels are consistently higher than stream levels, suggesting gaining stream conditions at this location.

South Bosque River Upstream of Harris Creek

The segment of the South Bosque River upstream of its confluence with Harris Creek is characterized by monitoring stations SBR1, SBR2, and SBR4. The stream level, groundwater level, and rainfall data are graphically depicted on the plots for monitoring stations SBR1, SBR2, and SBR4 included in **Appendix N**. The monitoring period time line spans the x-axis. Stream level and groundwater level are depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at monitoring station SBR1 varied by approximately 3.5 feet during the monitoring period, ranging from 607 to 610.5 fmsl. Rainfall ranged from 0 to 1.88 inches per day. Nine rainfall events exceeded 1 inch per day. Only four of these nine events coincide with stream level increases of approximately 1 foot or more. Based on review of rainfall data from nearby monitoring stations, the other five rainfall events of this magnitude were very localized and/or of short duration, thus significant surface runoff did not result.

Stream levels at monitoring station SBR2 varied by approximately 12 feet during the monitoring period, ranging from approximately 530 fmsl to 542 fmsl. Rainfall ranged from 0 to just over 1.4 inches per 15 day. The stream level data are characterized by a preponderance of one- to three-foot rapid variations in water levels during the period from December 2002 through April 2003. These variations are unique in both frequency and magnitude and are not coincident with rainfall or other known surface hydrologic events.

Stream levels at monitoring station SBR4 varied by approximately 12 feet during the monitoring period, ranging from approximately 501 to 513 fmsl. Rainfall ranged from 0 to just over 1.5 inches per day. Ten rainfall events exceeded one inch per day. Each of these rainfall events coincides with stream level increases of at approximately 0.5 feet or more. As with other monitoring stations, these stream level increases are very short in duration. The unique stream level fluctuations recorded at the upstream monitoring station SBR2 are not observable at monitoring station SBR4.

Groundwater levels at monitoring station SBR1 varied by nearly 5 feet during the monitoring period, ranging from approximately 608 to 613 fmsl. Groundwater level increases in excess of a few tenths of a foot were generally sudden events, similar to stream level spikes. Conversely, groundwater level decreases occurred over a period of days or weeks, with the rate of drop decreasing with time. This is considered to be a classic response pattern in groundwater systems. Six groundwater level increase events in excess of 0.5 feet were recorded at this monitoring station. Each of these coincided with a recorded rainfall event.

Groundwater levels at monitoring station SBR2 varied from 529 to 532 fmsl. However, the pattern of groundwater level variation in this monitoring well is dissimilar from the monitoring wells at upstream locations. The pattern of water level exhibited on the plot for monitoring station SBR2 (**Appendix N**) is hump-shaped rather than spiky, and looks very much like that observed at monitoring stations SC3 and OC1. At these other two locations, the hump-shaped pattern was attributed to groundwater recharge from surface water discharge events at NWIRP. Similarly, the pattern of groundwater level change at monitoring station SBR2 is suggestive of groundwater recharge from the apparent surface water discharges recorded at this monitoring station.

No groundwater monitoring wells were installed at monitoring station SBR-4.

Groundwater levels at monitoring station SBR1 are consistently higher than stream levels, suggesting that this location is in a gaining stream condition. Groundwater levels at monitoring station SBR2 are typically lower or only slightly higher than stream water levels, suggesting this location to typically function as a losing stream.

South Bosque River Between Harris Creek and Middle Bosque River

The segment of the South Bosque River downstream of the confluence with Harris Creek and upstream of the confluence with the Middle Bosque River is characterized by monitoring station SBR5. The stream level, groundwater level, and rainfall data are graphically depicted on the plot for monitoring station SBR5 included in **Appendix N**. The monitoring period time line spans the x-axis. Stream level and groundwater level are depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at monitoring station SBR5 varied by approximately 10 feet during the monitoring period, ranging from 453 to 463 fmsl. Rainfall ranged from 0 to approximately 1.7 inches per day. Eight rainfall events exceeded 1 inch per day. Each of these events coincides with stream level increases of approximately 0.5 feet or more. As with other monitoring stations, these stream level increases are very short in duration.

Groundwater levels at monitoring station SBR5 varied by nearly 10 feet during the monitoring period, ranging from approximately 453 to 463 fmsl. Groundwater level increases in excess of a few tenths of a foot were generally sudden events, similar to stream level spikes. Smaller groundwater level variations of a few tenths of a foot during the January through April 2003 period were characteristically hump-shaped, similar to

those observed at the upstream monitoring station SBR2. In fact, these variations appear to coincident with or perhaps slightly lagging those at monitoring station SBR2.

Groundwater levels at monitoring station SBR5 are typically higher than stream levels, suggesting that this location is in a gaining stream condition.

Middle Bosque River downstream of the South Bosque River
The Middle Bosque River downstream of the confluence with the South Bosque River and upstream of Lake Waco is characterized by monitoring station MBR1. The stream level and rainfall data are graphically depicted on the plot for monitoring station MBR1 included in **Appendix N**. No groundwater level data were obtained. The monitoring period time line spans the x-axis. Stream level is depicted on the left y-axis in feet mean sea level (fmsl). Rainfall is depicted on the right y-axis in inches of rainfall per day.

Stream levels at this location varied by approximately 5 feet during the monitoring period, ranging from 445 to 450 fmsl. Rainfall ranged from 0 to over 1.7 inches per day. Six rainfall events exceeded 1 inch per day. There is not a clear response to 1-hour rainfall at this station, possibly because this location is far downstream in the watershed and is reported to be in the backwater of Lake Waco.

5.1.4 Flow

Streamflows at most of the 15 longitudinal sampling stations were estimated throughout the duration of the sampling to determine how any potential perchlorate detections might impact Lakes Belton and Waco. In addition to flows at each of the stations, acoustic doppler technology was used to characterize potential preferential flow paths through Lake Belton. A detailed discussion of the ADCP flow study is provided in Section 5.1.4.2.

5.1.4.1 Streams

5.1.4.1.1 Introduction

The amount of flow reaching Lakes Belton and Waco from the NWIRP property compared to flows reaching these lakes from other watershed areas is critical both in estimating the potential impact of perchlorate concentrations detected in the streams and in assessing the potential for water supply contamination from the NWIRP source. Flow data from two flow surveys were used in conjunction with the surface water level data described in Section 5.1.3.3 to estimate flows. Where available, flow data from the U.S. Navy Environmental Investigations, the USGS, and ADCP studies performed in the Leon River just upstream of Lake Belton were used to corroborate the estimated flows.

5.1.4.1.2 Methodology

TIAER surveyed the stream channel cross-section and centerline longitudinal profile at each of the stream monitoring locations. They also attempted to measure the flow at each station in March, 2003. BRA conducted a second flow measurement study at each station in October, 2003. Flows over the duration of the one-year monitoring period were then estimated using water level data, channel geometry data and Manning's flow equation.

This section documents the methodologies used in measuring and calculating stream flows. All associated data, including stream channel survey data, are presented in Section 5.1.4.1.3.

5.1.4.1.2.1 Stream Channel Survey

TIAER surveyed the stream channel at each monitoring station location to determine the appropriate stream cross-section and slope characteristics needed to estimate actual stream flows. The survey included:

- Establishing a benchmark.
- Measuring elevations across the floor of the stream channel (perpendicular to flow) to establish the stream channel cross-section.
- Measuring elevations along the floor of the stream channel (parallel to flow) to determine the slope.
- Measuring the elevation of the bubbler, top of stream, and bottom of stream at the bubbler location.

The results from this survey are included in **Appendix O**.

5.1.4.1.2.2 Measured Stream Flow

Flow at or near most of the stations was measured twice during this study, once by TIAER in March 2003 and once by BRA in October 2003. Flow could not be measured at stations SBR5, MBR1, or CHC1. Sites MBR1 and CHC1 were reported to be in the backwater of Lakes Waco and Belton, respectively. Flow at SBR5 may also be affected by backwater from Lake Waco; flow at this site was very slow and could not be measured during the flow survey.

Per standard methodology, velocity was measured at 60% of the flow depth. Both velocity and depth of flow were measured at several points across the stream cross-section. The measured velocity, depth, and width of each sub-section were used to compute the volumetric flow across each sub-section, which was then summed to determine the total flow at that point in the stream.

The depth of flow recorded by the water level meter during the first flow survey was noted in order to relate the water level data to the measured flows. The second flow survey followed a large storm that washed away, broke, or submerged the water level meters at all of the stations except CHC1 and OC1 . Therefore, depths of flow reported at water level meters during this second survey are approximate and must be used with caution.

5.1.4.1.2.3 Flow Calculations

Ideally, the flow surveys could have been used to determine a level-discharge relationship to provide flow data from the level data collected over the course of this study. However, a valid level-flow relationship could not be determined based on the available flow data from the two surveys previously discussed. Several of the water levels reported during the second flow survey were very close to levels reported during the first

survey with respect to the range of levels seen over the course of the study. Additionally, levels reported during the second survey can only be considered approximate, as most of the water level meters had been washed away. Therefore, the project team chose to estimate flows at each station using Manning's equation, which predicts flow under uniform flow conditions, where Q = flow in cfs, A = cross-sectional area of flow, R = hydraulic radius, S = slope, and n = Manning roughness coefficient:

$$Q = \frac{1.49}{n} A R^{2/3} S^{1/2}$$

Although flow in these streams is not uniform, the Manning equation can still result in reasonable estimates of stream flow if n values are carefully chosen and results are compared to actual flow data.

Channel Geometry

The channel geometry parameters necessary for use of the Manning equation were calculated using CGAP 3.5, a publicly available software program developed by the USGS. The area of flow, wetted perimeter, and hydraulic radius were calculated for each stream cross-section over the range of water levels observed at each station at intervals ranging from 0.03 foot to 0.10 foot. Channel geometries at water levels between those calculated by the USGS program were then interpolated. At several stations, the highest measured water level was above the extent of the surveyed cross-section. In these cases, the program extrapolated the cross section to the necessary level.

Channel slope at each station was determined by a longitudinal stream centerline survey over 400 feet of the channel length near the station. In a few cases, the survey crew reported a slight negative slope. While a slight negative slope can be valid in some sections of a natural channel, use of the Manning equation requires a positive slope. In these cases, a slight positive slope was estimated by looking at points along the longitudinal survey other than the end points.

Photos of well-studied river channels with known Manning's n values, as well as tabulated values, were used to make initial estimates of the channel n value to use at each site. These initial estimates were refined by comparing the resulting depth-flow relationship to both flow surveys.

Several of the stream channel cross-sections show a defined main channel with distinct floodplains. Such compound channels often have very different roughness coefficients (n values) in the main channel than on the floodplains. Two n values were estimated at stations with this type of cross section, and a compound roughness coefficient was used when the depth of flow exceeded the flood plain level. This compound roughness coefficient was calculated by scaling the two n values based on the wetted perimeter associated with each n value, according to the following equation, where n_c is the composite roughness coefficient and P is the wetted perimeter:

$$n_c = \left(\frac{\sum P_i (n_i)^{3/2}}{\sum P_i} \right)^{2/3}$$

Verification

Calculated flows in the Leon River were compared not only to the two flow surveys performed at the site, but also to flows measured during two ADCP studies downstream of station LR1 on the Leon River. USGS data at an upstream station near Gatesville provided additional verification that the flows calculated for the Leon River were reasonable. Flows at stations SC1, TRM1, and HC1 were compared to flow data previously collected by the U.S. Navy at nearby locations to ensure that calculated flows were within a reasonable range.

5.1.4.1.3 Data

Data collected during the stream channel survey, both flow surveys, and the resulting calculated flows over the duration of monitoring are presented in this section. These data are organized by watershed, with the Leon River watershed data presented first and the Bosque River watershed data presented second. Although flows were not calculated at Cowhouse Creek, stream channel survey data at monitoring station CHC1 are presented following the Bosque River watershed data.

5.1.4.1.3.1 Leon River Watershed

The NWIRP to Lake Belton portion of the Leon River Watershed studied during this investigation includes Tributary M, Onion Creek, Station Creek, and the Leon River downstream of Station Creek.

Leon River Watershed Measured Data

Appendix O includes photographs of each stream monitoring location, plots of the surveyed cross-section, plots of the surveyed longitudinal centerline profile, and three-dimensional cross-section representations of the stream channel.

Table 5-7 summarizes the results of the two flow surveys at each station in the Leon River Watershed, including date, time, depth of stream at the bubbler location, and measured flow. As discussed previously, the second flow survey was completed following flooding that washed away all sampling equipment. Therefore, depths at the bubbler reported during the second flow survey may or may not be comparable to depths reported during the first flow survey. The flow measurements are less sensitive to the exact measurement locations, and therefore can be considered accurate for both surveys.

Table 5-7
NWIRP to Lake Belton Flow Survey Results

Monitoring Station ID	Flow Survey 1			Flow Survey 2		
	Date/Time	Depth at Bubbler (ft)	Flow (cfs)	Date/Time	Depth at Bubbler (ft)	Flow (cfs)
TRM1	3/4/03 18:55	0.90	2.84	10/16/03 10:05	1.20	0.52
OC1	3/5/03 10:45	0.40	4.46	10/16/03 10:30	0.80	0.51
SC1	3/4/03 18:25	0.83	10.42	10/16/03 9:45	1.30	0.84
SC3	3/5/03 09:30	1.73	16.38	10/16/03 9:15	1.15	3.56
SC5	3/5/03 12:45	0.65	38.03	10/16/03 11:10	1.20	5.91
LR1	3/25/03 11:30	3.00	189.34	11/6/03 9:15	2.70	102.33

Leon River Watershed Flow Calculations.
As discussed in the methodology section, flows were estimated over the duration of monitoring based on measured water level, channel geometry determined during surveying, and the Manning equation for uniform flow. Use of the Manning equation requires that a roughness coefficient, Manning's n, be estimated. **Table 5-8** summarizes the Manning's n values used at each station, including comments on why these values were chosen.

Table 5-8
NWIRP to Lake Belton Manning's n Values

Station ID	Manning's n		Comments
	Main Channel	Flood Plain	
TRM1	0.30	0.03	Small, meandering channel. Grassy floodplain.
OC1	0.11	0.25	Thickly vegetated floodplain.
SC1	0.11	0.035	Weedy, meandering main channel. Grassy floodplain.
SC3	0.50	NA	Very high value used for main channel to correct for unusual flow conditions at this station. Stagnant water at the bubbler location has frequently been observed, which would cause on overestimation of flow. Additionally, this site is upstream of a debris screen, which could cause depths greater than the normal uniform flow depth.
SC5	0.06	0.035	Relatively straight channel section.
LR1	0.09	0.03	Relatively straight channel section.

With the exception of station SC3, the n values used typically decrease as channel size increases. This trend is as expected, since roughness, changes in cross-section, or channel meanders will have a much more significant effect on very small channels than on larger ones. With the exception of station OC1, which has a thickly vegetated floodplain (see the photo in **Appendix O**), this watershed is characterized by grassy floodplains that have fairly low roughness coefficients.

Plots of the resulting calculated flows over the monitoring duration are included in **Appendix P**. The two manual flow measurements taken at each monitoring station are also shown on these plots. **Figure 5-32** shows flows from each monitoring station from upstream on Station Creek and Tributary M down to monitoring station SC5 plotted on the same graph for comparison.

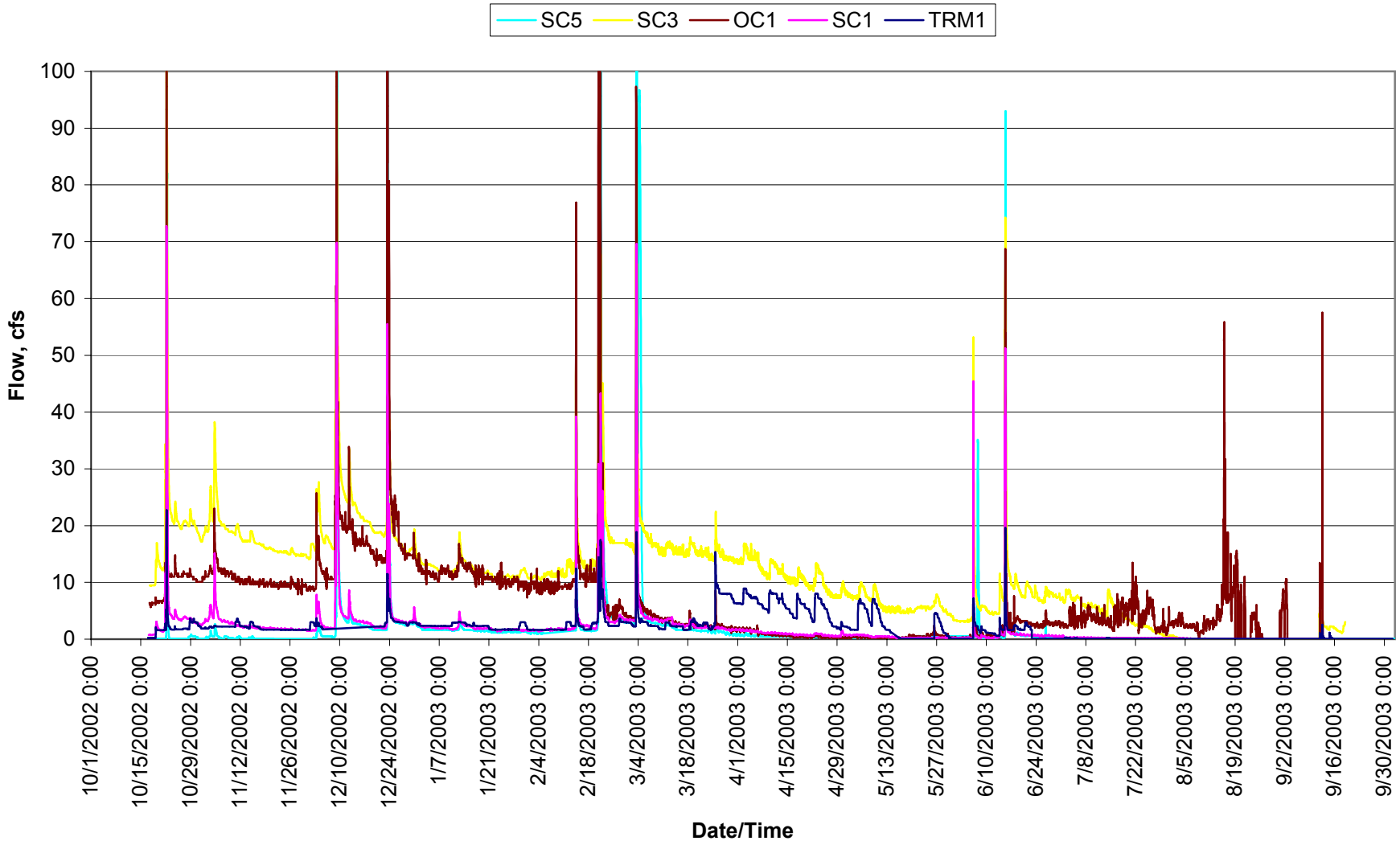
Verification of Calculated Flows

Calculated flows in the Leon River Watershed were compared against flow measurements and estimates from other sources to verify that flows were within a reasonable range. These other flow data included: manual flow measurements performed at each stream during this study; two flow measurements performed during the ADCP study at the Leon River, 6 miles downstream from monitoring station LR1; current USGS flow data from the Leon River near Gatesville, approximately 35 miles upstream from monitoring station LR1; and previous U.S. Navy flow data at sites just upstream of monitoring stations SC1 and TRM1.

Manual Flow Measurements. Two manual flow surveys were completed during this study at each monitoring station not affected by backwater from the lakes. These manual flow measurements were plotted on the flow data graphs included in **Appendix P**. However, because the second flow survey was completed after several of the water level meters had been washed away by a high rainfall event, flow measured during this second survey could not be compared directly to a calculated flow at several stations. Additionally, at stations where level data were available during the second flow survey, measured flows could not be correlated for both flow surveys with flows estimated using Manning's flow equation. Therefore, the depth-discharge curve for each station based on the calculated flows was plotted and compared to the flows and depths reported during the two flow surveys. As seen on these graphs, also included in **Appendix P**, the calculated depth-flow curve generally falls between the two manual flow survey points.

Leon River ADCP Measurements. Two additional flow measurements using ADCP technology were performed at the Leon River during the ADCP portion of this study. These measurements were made approximately 6 miles downstream from monitoring station LR1. These additional two points are plotted with the flow data for monitoring station LR1 and on the depth-discharge curve described above, both included in **Appendix P**. The depth used in plotting these points was that measured at the bubbler at LR1 at the time the ADCP measurement was taken.

Figure 5-32
Comparison of Flows at Monitoring Stations Upstream of the Leon River



Leon River USGS Data. A USGS station exists on the Leon River near Gatesville, approximately 35 miles upstream of monitoring station LR1. Flows reported at this USGS station during the manual flow surveys and ADCP measurements were approximately half of the measured flow at monitoring station LR1. Flows at monitoring station LR1 and the USGS station were compared throughout the study to ensure that calculated flows at much higher stages than those at which the flow measurements were taken were reasonable.

U.S. Navy Data. The U.S. Navy collected data from several streams at or near the NWIRP site between March and May 2000. One location was approximately 0.4 mile upstream of monitoring station SC1, and one was approximately 0.6 mile upstream of monitoring station TRM1. Although the U.S. Navy data were reported for a different period than flows calculated for this study, these data could still be used to verify the general range of flow calculated during this study. **Table 5-9** summarizes the U.S. Navy flows reported near these two stations compared to flows calculated during this study.

Table 5-9
Comparison of Monitoring Station TRM1 and SC1 Flows to U.S. Navy Data

Station ID	Dates	Typical Range in Dry Weather Flow (cfs)	Typical Range in Peak Storm Water Flow* (cfs)
TRM1	October 2002 through October 2003	0 – 2	8 – 15 during typical storms, with a peak flow of approximately 75 cfs during October 2003 storm.
0.6 mile Upstream of TRM1 (U.S. Navy Data)	March 2000 through May 2000	0 – 0.2	Almost no response, with most spikes < 10 cfs. One spike of 115 cfs
SC1	October 2002 through October 2003	0 – 3	10 – 70 during typical storms, with a peak flow of approximately 700 cfs during October 2003 storm.
0.4 mile Upstream of SC1 (U.S. Navy Data)	March 2000 through May 2000	0 – 1.5	20 - 270

Discussion of Leon River Watershed Monitoring Stations

The pattern of the calculated flow at each of these stations mimics that of the recorded water levels: low values during dry periods and rapid, sharp spikes following significant rainfall events. This pattern was described in detail in Section 5.1.3 for each stations; therefore, a detailed description of the response to rainfall events is not repeated here. **Table 5-10** summarizes the typical dry weather and storm flows estimated for each site.

Table 5-10
Leon River Watershed, Typical Flows

Station ID	Typical Range in Dry Weather Flow (cfs)	Typical Range in Peak Storm Water Flow* (cfs)	Comments
TRM1	0 – 2	8 – 15	Peak flow of approximately 75 cfs during October 2003 storm.
OC1	0.2 – 10	60 – 130	Peak flow of approximately 400 cfs during October 2003 storm.
SC1	0 – 3	10 – 70	Peak flow of approximately 700 cfs during October 2003 storm.
SC3	0 – 15	30 – 100	Level data during October 2003 storm not available.
SC5	0 – 2	40 – 230	Peak flow of nearly 6,000 cfs during October 2003 storm.
LR1	100 – 300	500 – 2,000	Level data during October 2003 storm not available. Flow at an upstream USGS station on the Leon River near Gatesville peaked at about 6,000 cfs during October 2003 storm. Flow at monitoring station LR1 is typically about twice that at the USGS station.

* Peak Storm Flow does not include the large storm in October, 2003 as this storm caused a much larger response than any previous storm during monitoring. This storm washed away many of the meters, so data for this storm are not available at all stations. See the comments for each station for any information about peak flow during this storm.

Overall, flows in the streams discharging to the Leon River are characterized by very low or no flow during dry periods with sharp spikes in flow during storm events. Spikes in response to rainfall typically last only one to two days at monitoring stations upstream of monitoring station LR1. Due to the much larger drainage area contributing to monitoring station LR1, elevated flow at this station due to storm events typically lasts much longer, generally exceeding one week. Flow at monitoring station SC5 is typically lower than at upstream stations under dry weather conditions. Monitoring station SC5 is frequently dry; however, flow spikes are higher here than at upstream stations during storm events. This observation based on estimated flows is corroborated by field observations. The highest dry weather flow upstream of monitoring station LR1 typically occurs at monitoring station SC3. Based on the groundwater and surface water level data presented in Section 5.1.3, the flow at station SC3 may be higher than the combined flow from upstream stations because stream reaches upstream of this monitoring station are typically gaining streams (See Chapter 6).

These observations are significant because they indicate that stream flow is lost to evapotranspiration or to groundwater between monitoring stations SC3 and SC5 under typical (non-storm) conditions. Therefore, very little, if any, surface drainage from the NWIRP area reaches the Leon River via surface flow during normal dry weather

conditions. Dry weather flow reaching the Leon River from Station Creek is estimated to be typically less than 2% of the total flow in the Leon River at monitoring station LR1. During certain storm events recorded during this study, this percentage is conservatively estimated to be as high as 25%. Additional discussion on this flow as a component of inflow into Lake Belton is included in Chapter 6.

5.1.4.1.3.2 Bosque River Watershed

The portion of the Bosque River Watershed between NWIRP and Lake Waco studied during this investigation includes Harris Creek, the South Bosque River, and the Middle Bosque River downstream of the South Bosque River.

Bosque River Watershed Measured Data

Appendix O includes photographs of each stream monitoring location, plots of the surveyed cross-section, plots of the surveyed longitudinal centerline profile, and three-dimensional cross-section representations of the stream channel.

Two flow surveys were completed at six of the eight monitoring stations in the Bosque River watershed. Flows could not be measured at two of the monitoring stations: SBR5 and MBR1. As previously discussed, MBR1 was reported to be in the backwater of Lake Waco. Flow at SBR5 was likewise very slow. This station may also be affected by backwater from Lake Waco. **Table 5-11** summarizes the results of these flow surveys, including date and time, depth of stream at the bubbler location, and measured flow. As discussed previously in the methodology section, the second flow survey was completed following flooding that washed away all sampling equipment. Therefore, depths at the bubbler reported during the second flow survey may or may not comparable to depths reported during the first flow survey. The flow measurements are less sensitive to the exact measurement locations and therefore should be accurate for both surveys.

Table 5-11
NWIRP to Lake Waco Flow Survey Results

Station ID	Flow Survey 1			Flow Survey 2		
	Date/Time	Depth at Bubbler (ft)	Flow (cfs)	Date/Time	Depth at Bubbler (ft)	Flow (cfs)
HC1	3/4/03 12:40	0.85	47.13	10/16/03 12:35	0.75	12.20
HC2	3/4/03 14:50	1.50	55.18	10/16/03 8:30	1.05	13.31
SBR3	3/6/03 9:15	1.50	57.58	10/16/03 9:10	2.50	17.46
SBR1	3/4/03 17:00	0.80	56.36	10/16/03 13:05	1.70	7.62
SBR2	3/5/03 15:20	1.80	67.18	10/16/03 13:40	0.30	14.13
SBR4	3/5/03 17:45	1.90	79.82	10/16/03 14:10	1.50	10.53

Bosque River Watershed Flow Calculations

As discussed in the methodology section, flows were estimated over the duration of monitoring based on measured water level, channel geometry determined during surveying, and the Manning equation for uniform flow. Use of the Manning equation requires that certain channel properties be known or estimated. **Table 5-12** summarizes the slope and Manning's n values used at each station, including comments on how these values were estimated. Flows were not estimated at monitoring stations SBR5 or MBR1. Flows at these stations could not be measured during the flow surveys because flow was very slow and possibly affected by the backwater of Lake Waco. Use of Manning's equation at these stations would have led to highly erroneous results and there are no alternative data for comparison.

Table 5-12
NWIRP to Lake Waco Manning's n Values

Monitoring Station ID	Manning's n		Comments
	Main Channel	Flood Plain (winter/summer)	
HC1	0.11	0.16 / 0.20	Thick weeds on flood plain with some trees.
HC2	0.08	0.03	Relatively straight channel. Grassy floodplain.
SBR3	0.12	0.16 / 0.20	Thick weeds with trees on upper part of channel and on floodplain.
SBR1	0.11	0.04	Meandering channel with weeds. Grassy floodplain.
SBR2	0.10	0.16 / 0.20	Thick weeds with trees on floodplain.
SBR4	0.10	0.13 / 0.17	Trees and brush on floodplain.

Plots of the resulting calculated flows over the monitoring period duration are included in **Appendix P**. The two manual flow measurements taken at each site are also shown on these plots. **Figure 5-33** shows flows from monitoring station HC1 downstream to monitoring station SBR3 and **Figure 5-34** shows flows from monitoring station SBR1 downstream to monitoring station SBR4 for comparison between monitoring stations.

Verification of Calculated Flows.

Fewer opportunities for flow comparisons exist in the Bosque River Watershed than in the Leon River Watershed. However, calculated flows in the Bosque River Watershed could be compared against a few flow measurements and estimates from other sources to verify that flows were within a reasonable range. These other flow data included: manual flow measurements performed at each stream during this study; USGS data at nearby USGS monitoring stations; previous U.S. Navy flow data at sites upstream of station HC1; and visual observations by the project team in comparison to Leon River watershed stations.

Figure 5-33
Comparison of Flows at Monitoring Stations HC1, HC2, and SBR3

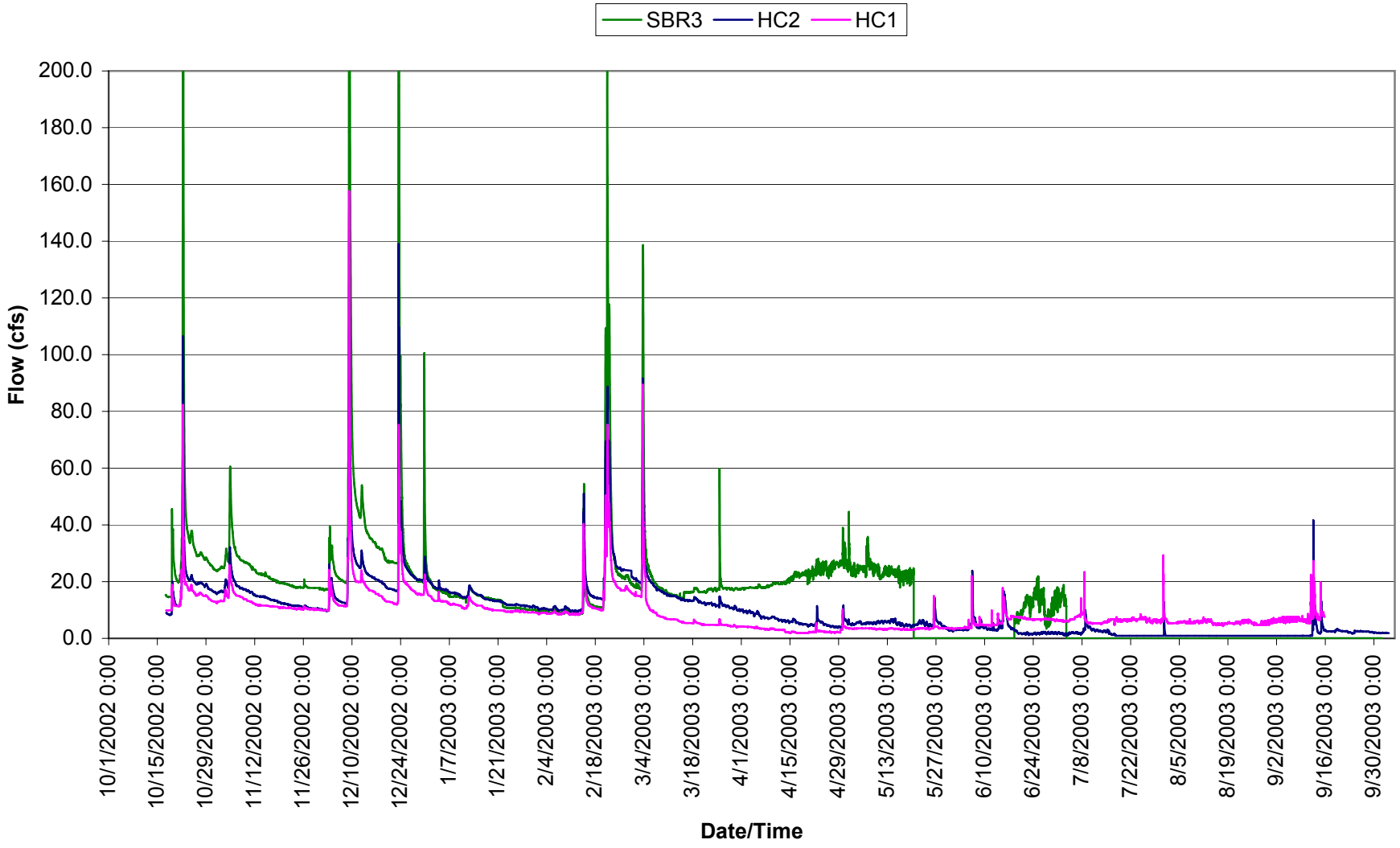
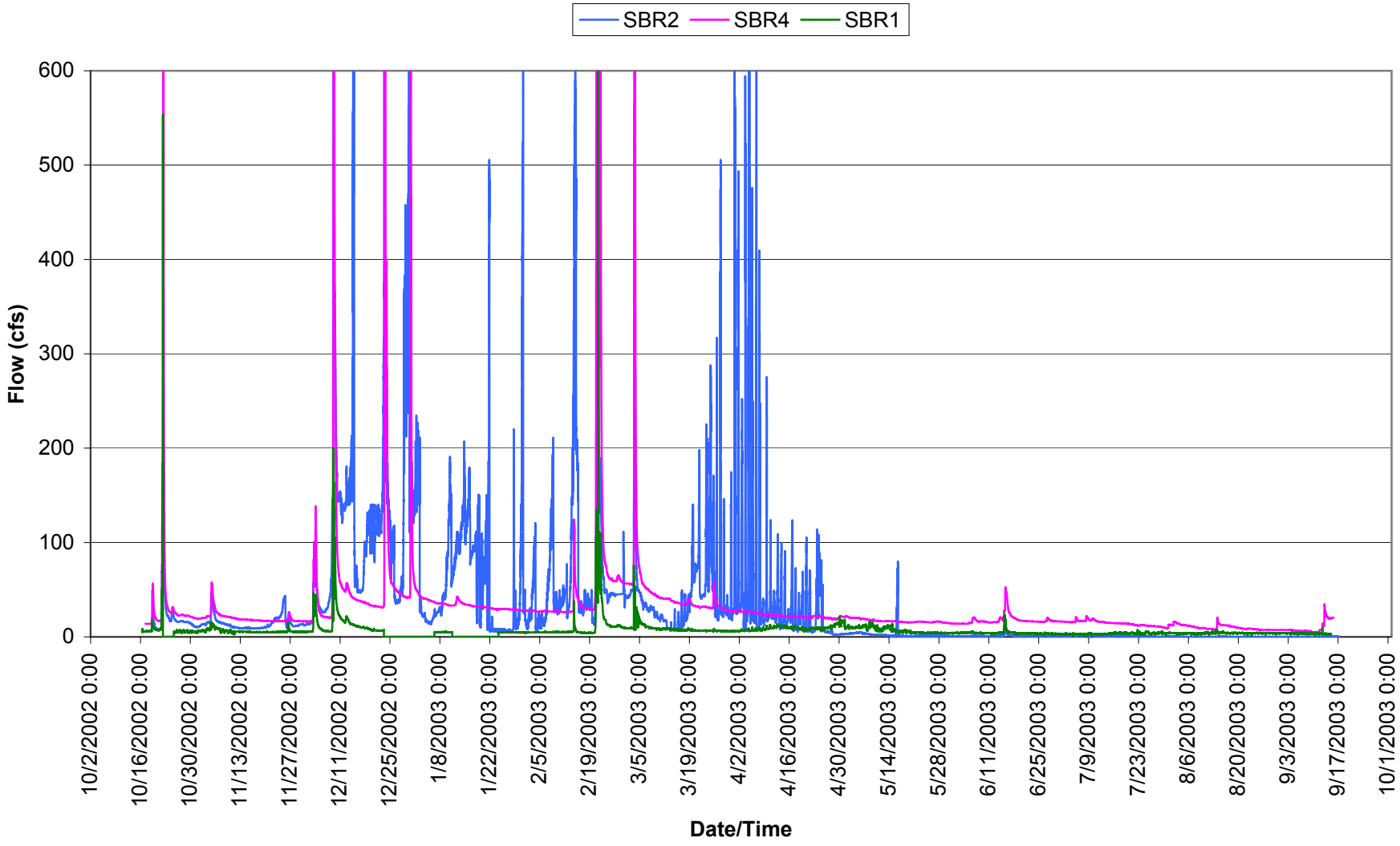


Figure 5-34
Comparison of Flows at Monitoring Stations SBR1, SBR2, and SBR4



Manual Flow Measurements. As discussed previously in this section, two manual flow surveys were completed during this study at each station not affected by backwater from the lakes. These manual flow measurements were plotted on the flow data graphs included in **Appendix P**. However, because the second flow survey was completed when several of the level meters were no longer in place, flow measured during this second survey could not be compared directly to a calculated flow at several stations. Additionally, at stations where level data were available during the second flow survey, flows calculated with Manning's flow equation could not match both the first and second flow survey measurements. Therefore, the stage-discharge curve for each station based on the calculated flows was plotted and compared to the flows and depths reported during the two flow surveys. As seen on these graphs, included in **Appendix P**, the calculated stage-discharge curve generally falls between the two manual flow survey points.

USGS Data. Two USGS stream monitoring stations exist upstream of monitoring stations for this study in the Bosque River Watershed. Unfortunately, flow data at these locations are not available during the period this study was conducted. However, these data could still be used to perform a limited amount of verification of calculated flows.

One of the USGS stations was located on the South Bosque River near McGregor, approximately 1.7 miles upstream of monitoring station SBR1. The only data available for this station were annual peak flows between 1967 and 1974. Reported peak flows ranged from approximately 200 cfs to approximately 4,200 cfs. Peak flow calculated at monitoring station SBR1 during this study was approximately 800 cfs, within the range of the USGS data.

The USGS also has a stream monitoring station on the Middle Bosque River approximately 8 miles upstream of station MBR1. Peak flows are available at this station from year 1959 through year 2001. Reported peaks at this station range from approximately 1,000 cfs to approximately 34,000 cfs, with most peaks around 10,000 cfs. While flow could not be calculated at station MBR1 or SBR5, this USGS station can at least provide some guidance in evaluating flows at SBR4 and SBR3. These two stations are located on the main tributaries discharging to SBR5, which is the largest tributary discharging to the Middle Bosque River downstream of the USGS station. Peak flows calculated at monitoring stations SBR3 and SBR4 are approximately 500 cfs and 3,300 cfs, respectively. These peak flows seem reasonable considering the peak flows reported at the USGS station on the Middle Bosque River.

U.S. Navy Data. The U.S. Navy collected data from several streams at or near the NWIRP site between March and May in year 2000. Two U.S. Navy monitoring stations are located approximately 3 miles upstream of monitoring station HC1 on tributaries to Harris Creek. The maximum peak in flow at these U.S. Navy stations that appeared to be due to rainfall totaled approximately 100 cfs. Therefore, the calculated peak flow of approximately 160 cfs at monitoring station HC1 seems reasonable.

Field Observations. The project field crew reported that flow in the streams monitored in the Bosque River Watershed typically (non-storm conditions) appeared to have more

flow than observed in Station Creek and Tributary M in the Leon River Watershed. Additionally, unlike monitoring stations on Station Creek and Tributary M, stations in the Bosque River Watershed did not go dry at any time during this study. The observations support the flow calculations in that calculated dry weather flows in the Bosque River Watershed were significantly higher than in streams discharging to the Leon River.

Discussion of Bosque River Watershed Stations

The pattern of the calculated flow at each of these monitoring stations mimics that of the recorded water levels, with fairly low values during dry periods and rapid, sharp spikes following significant rainfall events. Since the pattern at each monitoring station was described in detail in Section 5.1.3, a detailed description of the response to rainfall events is not repeated here. **Table 5-13** summarizes the typical dry weather and storm flows estimated for each site.

Table 5-13
NWIRP to Lake Waco, Typical Flows

Monitoring Station ID	Typical Range in Dry Weather Flow (cfs)	Typical Range in Peak Storm Water Flow* (cfs)	Comments
HC1	2 – 15	60 – 160	Level data not available during October 2003 storm.
HC2	2 – 15	50 – 150	Peak flow of nearly 1,200 cfs during October 2003 storm.
SBR3	9 – 25	100 – 480	Level data not available during October 2003 storm.
SBR1	4 – 8	40 – 800	Level data during October 2003 storm not available.
SBR2	1 – 15	400 – 1,600	Dry weather flows highly variable due to apparent daily discharge from some source. Flows here are approximate natural flows. Flows due to apparent discharge reach much higher levels (hundreds of cfs). Likewise, it is difficult to discern the difference between peak flow due to rainfall and daily discharges. Level data during October 2003 storm not available.
SBR4	5 – 30	150 – 3,000	Level data during October 2003 storm not available.

* Peak Storm Flow does not include the large storm in October, 2003 as this storm caused a much larger response than any previous storm during the monitoring period. This storm washed away many of the meters, so data for this storm are not available at all stations. See the comments for each station for any information about peak flow during this storm.

As seen on **Figure 5-33**, flows at monitoring stations HC1 and HC2 were similar during non-storm conditions, with flow at the upstream station, HC1, slightly lower during the winter months. By June, however, flow at monitoring station HC1 was somewhat higher than flow calculated at monitoring station HC2, indicating that the stream reach between monitoring stations HC1 and HC2 lost water during the summer season to evapotranspiration or to groundwater. Flow farther downstream at station SBR3 was typically about the same or somewhat higher than at station HC2, but peak flows during storm events were typically two to three times greater at monitoring station SBR3 than those estimated at upstream stations.

As seen on **Figure 5-34**, average flow in the South Bosque River, characterized by monitoring stations SBR1, SBR2, and SBR4, typically increased from upstream to downstream. As in Harris Creek, however, this trend appears to change during the summer months, with calculated flow at upstream monitoring station SBR1 exceeding flow at monitoring station SBR2. Again, this change indicates that this stream reach loses more water to evapotranspiration or to groundwater than it gains from tributaries during the summer.

Monitoring stations along the South Bosque River also showed some daily fluctuations in flow not observed at other stations. Fluctuations at monitoring station SBR1 are small, approximately one to two cfs, with a daily peak in flow around 4:00 PM. This magnitude of flow change is on the order of what might be released from a small sanitary WWTP and could be due to discharges from an upstream plant. Monitoring station SBR2, approximately 5 miles downstream from SBR1, showed very large daily fluctuations in flow, on the order of hundreds of cfs, during part of the monitoring period. The validity or potential cause of these fluctuations is unknown. These fluctuations are not apparent downstream at monitoring station SBR4.

Although flows are unknown at monitoring stations SBR5 and MBR1, a general estimate of the amount of flow reaching these stations from Harris Creek and the South Bosque River can still be made. Flow at monitoring station SBR5 is expected to come primarily from these upstream monitoring stations on the South Bosque River and Harris Creek, as tributaries flowing into the South Bosque River between SBR4 and SBR5 appear to be very small. The amount of flow reaching the lower South Bosque River (SBR5) from the Harris Creek route (HC1, HC2, SBR3) is likely similar to that from the South Bosque route (SBR1, SBR2, SBR4) during dry weather. However, peak storm flows coming from the South Bosque River could be up to four times greater than those from Harris Creek. Sufficient data are not available to estimate the percentage contribution of flow from the South Bosque River to the Middle Bosque River, although it is thought to be somewhat higher than the contribution of Station Creek to the Leon River as discussed in the previous section. Further information on estimated flows compared to the total flow entering Lake Waco is included in Chapter 6.

5.1.4.1.3.3 Cowhouse Creek

As discussed previously, flows were not calculated at Cowhouse Creek because this monitoring station was reported to be in the backwater of Lake Belton. However, a

photograph of the stream at station CHC1, a plot of the cross-section, a plot of the centerline profile, and a three-dimensional representation of the cross-section are included in **Appendix O**.

5.1.4.2 Lakes (ADCP)

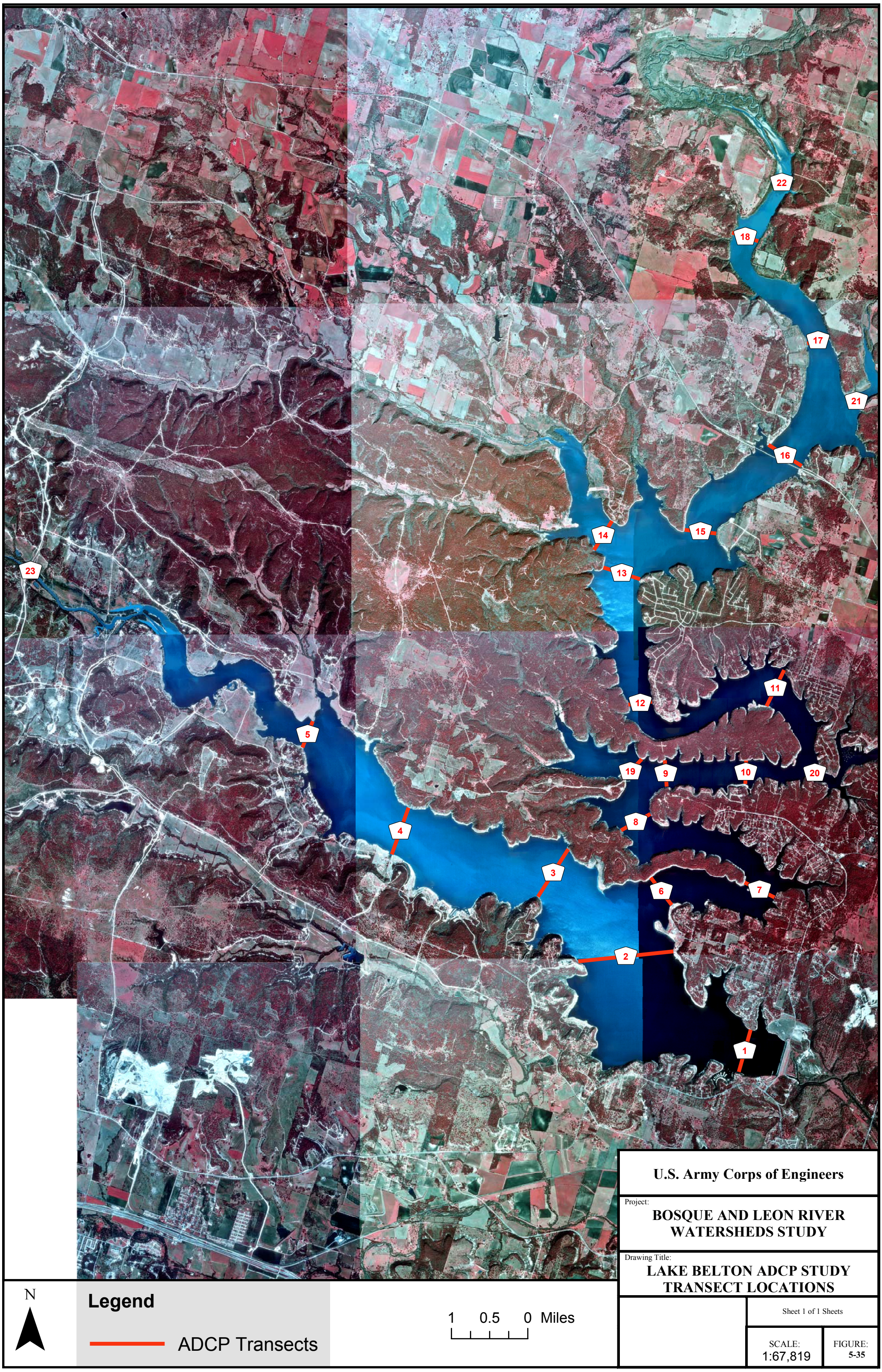
The Acoustic Doppler Current Profiler (ADCP) was first shown to be a promising tool for determining river current velocity in 1982 (USGS, 2001). ADCP equipment is now used to measure current velocity in oceans, lakes, rivers, and estuaries. ADCP is used over a wide range of depths and varying field conditions making it a powerful tool for the measurement of water current velocity in settings where conventional discharge measurements could not previously be performed.

The ADCP equipment measures water velocity using a principle of physics known as the doppler shift, where the change in frequency of a source of sound is related to both the velocity of the source and the observer. ADCP functions by emitting sound waves from near the water surface to the bottom of the water body. The ADCP equipment bounces an ultrasonic sound pulse off small particles of sediment and other material being carried by the current in the water column (collectively referred to as backscatters) and records the return echo from the acoustic backscatters. Upon receiving the return echo, the ADCP's onboard signal processing unit calculates the Doppler shift and thus determines the velocity of the backscatters, which is equal to the velocity of the water.

For this study, an ADCP was attached to a boat and used to determine current velocity at 21 transects, as shown on **Figure 5-35**, by motoring across Lake Belton. A total of four ADCP measurements were obtained at all transects, one during each season of the year, in order to better understand how lake currents change throughout the year and to ascertain what effect changing flow patterns may have on perchlorate fate and transport.

The overall rationale for the selected ADCP transects was to survey specific locations within the lake that could be helpful in identifying flow patterns within the lake, especially potential deep-water currents that were hypothesized to exist along the old river channel or thalweg of the lake. These deep-water currents, if encountered, could provide a preferential flow path for perchlorate that may be entering the lake.

The transect locations were selected based on a review of historical topographic maps, to provide an evenly spaced distribution along the length of the thalweg. Transects were also located at the mouths of major streams entering the lake. Three transects were located near the Bell County WCID No. 1, Blue Bonnet, and City of Gatesville water intake structures to determine if any preferential flows within the lake are located near these structures. The ADCP survey was limited to Lake Belton based on the assumption that Lake Waco is a more well-mixed, homogeneous environment, both because of its shallow configuration and the presence of a mechanical aeration system.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LAKE BELTON ADCP STUDY TRANSECT LOCATIONS
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5-35

In addition to the 21 original transects, an additional transect across the Leon River was conducted during the summer, fall, and winter surveys to assist in estimating flows in this stream and to validate calculated sampling station flow meter readings. Also, an additional transect across Cowhouse Creek was conducted during the fall survey to obtain better information about the discharge flows into the lake from this creek. This portion of the Study was conducted as part of the Acoustic Doppler Current Profiler Survey. All the methodologies and protocols followed during these studies are detailed in the *Final Lake Belton Acoustic Doppler Current Profiler Field Sampling Plan* (MWH, 2003). Any deviations from the Field Sampling Plan are discussed further below.

5.1.4.2.1 Methodology

Pre-Survey ADCP Activities. Prior to deployment of the ADCP, the instrument's compass was calibrated on land according to manufacturer's guidelines. Following compass calibration, the ADCP was attached to the side of the boat using a special mount and set at a depth of at least three inches below the water surface based on the manufacturer's guidelines. The depth to which the ADCP was installed below the water surface was recorded in the field notes. The ADCP was programmed, operated, and maintained according to the manufacturer's guidelines by an experienced professional.

ADCP Survey Activities. An ADCP pre-run was performed at the beginning of each field day to verify that all equipment was functioning properly. An experienced boat operator controlled the boat across the transect in order to ensure representative and accurate ADCP data. Each transect was completed in order to obtain depth and current profiling data, and each transect was completed by guiding the boat along the pre-determined transects across the lake toward the end point at a rate no greater than 6 knots. Data acquisition began by estimating the distance from shore and recording the transect starting point location using GPS. The distance from shore and GPS location were determined at the transect endpoint. The locations of some of the transects were changed based on field conditions, observations, or lack of accessibility by boat, and the geographic positioning system (GPS) coordinates for both the starting and end points of each transect were recorded. Following data acquisition, the ADCP was turned off, pulled out of the water, and data were checked for quality and accuracy prior to moving to the next transect. A temperature profile of the lake was then obtained at the deepest point of each transect, and temperature measurements were recorded at ten-foot intervals from the surface to the bottom of the lake. ADCP survey activities were halted if the boat and ADCP equipment operators determined that wave and wind action was unsafe or would prevent the collection of representative and accurate data.

A boat was used to complete the ADCP transects across Lake Belton, as shown above on **Figure 5-35**. The following equipment was utilized to perform the ADCP survey:

- 600 kHz Rio Grande or Sentinel ADCP Workhorse with bottom-tracking capability
- Differential GPS (to be used if bottom-tracking is hindered due to high sediment conditions)
- Power supply and communications interface

- Velocity profiling and measurement software
- Manufacturer's documentation

Additional Equipment. The following ancillary equipment were utilized during the ADCP survey:

- Boat (non-steel hull)
- Mounting assembly for connecting ADCP and GPS equipment to the boat
- Laptop computer
- Handheld GPS
- Seabird SB19 CTD Profiler (temperature)

ADCP data were collected in blocks or ensembles across the entire cross-section as the boat traveled across each transect's length from the lake surface to the lake bottom. After the ADCP instrument completed a single data collection, it sent a block of data called a data ensemble to the laptop. A data ensemble consists of the data collected and averaged during the ensemble interval. A data ensemble can contain header, leader, velocity, correlation magnitude, echo intensity, percent accuracy, status, and bottom-track data. The slower the boat traveled the more ensembles the ADCP was able to generate for each transect's cross-section. The ADCP collects current speed and direction at multiple depths through the water column, excluding the upper and lower one-meter. Profiles of current data with navigation fixes were collected every three seconds at vessel speeds of approximately 2.5 knots, and were logged directly to a laptop computer. Discharge measurements were calculated by the ADCP software, making assumptions for the near bank volumes and the areas of no measurement near the surface and near bottom. The boat speed, during the field surveys, was kept to a minimum but speeds were varied slightly, depending on wind speed to keep the boat on course, and to prevent drifting away from the pre-determined transect location. Ensemble size changes can be seen in **Appendix Q**, which show differing sizes of data ensembles for the same transect for the spring, summer, fall, and winter surveys. The ADCP data generated (the combined ensembles) produced current velocity profiles across each transect.

5.1.4.2.2 Data

Current and temperature profiling of Lake Belton were carried out on March 17, 2003 (Spring), June 16 and 17, 2003 (Summer), September 22 and 23, 2003 (Fall), and December 10, 11, 15, 17 and 18, 2003 (Winter).

Navigation software provided spatial positioning for the vessel operator during the transect crossings using real time navigation software receiving Wide Area Augmentation Satellite Differential GPS fixes. Current data with navigation fixes were logged directly to a laptop computer. Current values were collected at 0.5-m vertical intervals.

Profiles of current data with 0.5-m vertical resolution were recorded every 3 seconds at vessel speeds of approximately 2.5 knots, which generated velocity contour plots included in **Appendix Q**. Discharge measurements or core flows were calculated by the

ADCP software, making assumptions for the near bank volumes and the areas of no measurement near the surface and near bottom.

Temperature profiles were developed at the deepest part of each transect by collecting temperature data using a Seabird SB19 CTD profiler. This instrument records temperature data at a frequency of 2 hertz. Temperature profiles were collected by lowering the SB19 to the lakebed and retrieving it to the surface while it logged temperature data internally. Recorded profiles were downloaded to a laptop computer and converted to degrees Celsius. Temperature profiles for the different transect locations in Lake Belton are included in **Appendix R**.

Using the velocity contour plots generated by the ADCP, core flows were generated across each transect. These core flows are depicted in **Plate 2** through **Plate 5**. The length of the arrow indicates the magnitude of the flow, while the arrowhead itself indicates the direction of flow.

Data Limitations

Currents. Current data collected with acoustic tools are derived from measurements of particulate velocities in the water column. The final reported current velocity is a statistical product of numerous samples of water particulate velocity. As a result, the error of a current measurement decreases as the sample volume and number of samples averaged together increases.

The cost of averaging is a loss of resolution both vertically in the water column and horizontally across the transect. The goal of locating core flows in thalwegs of several meters width dictates a maximum number of consecutive profiles that may be averaged together before the core flow data become "smeared" into the adjacent flow values. To achieve the highest level of horizontal resolution, the number of profiles can be increased (by traveling very slowly across the transect) and the vertical resolution can be decreased (by extending the sample bin size).

Vessel speeds during the survey were kept to the minimum possible to maintain steerage across the transect. Vertical sample bins were set to 0.5 meters. Having observed little vertical flow structure in the transect flows of the initial survey, the vertical bin size could be increased during subsequent surveys but with the risk of missing the thalweg by over-averaging the bathymetry near bottom. The thalweg of transect 16 (spring) was observed with 0.5-meter vertical resolution as approximately 1 meter deep below the streambed. If the vertical resolution were decreased to 1 meter, the thalweg would not appear as an obvious feature, frustrating the goal of locating thalweg flow patterns.

The operator recognizes inaccurate data as blank profiles or individual profiles of anomalous high or low value. The key to observing a realistic current feature is to collect multiple profiles with a meaningful trend in a contiguous section of the channel. Wide variations in current direction in low flow conditions should not be considered an indicator of bad data. As velocities approach zero, direction becomes expectedly less meaningful.

Discharge. The ADCP incrementally calculates discharge by summing volumes of flow as the vessel crosses the channel. While the summation process averages the entire velocity field of a transect, reducing the effects of single profile variability, other sources of error affect discharge calculations when flow assumptions are not met.

In areas of very low flow, discharge values are subject to errors due to variations in the circulation on a transect of the same temporal scale as the length of time to make the transect. In some cases, circulation variations, such as eddies, can form and dissipate, or translate across a channel during the time it takes a vessel to cross. This condition can cause large errors in the discharge measurements reported by the ADCP because the condition of steady flow for the period of the transect measurement is not met. Several discharge measurements in the southern area of the survey between adjacent transects corroborate well, with a few obvious bad values. Discharge values in the shallow, low-flow transects of the north transects are less reliable. Discharge values that are not supported by adjacent values are labeled "not supported" on the contour plots.

Temperature. The temperature system of the Seabird SBE19 profiler is extremely robust and has very few sources of data error aside from obvious sensor failure.

5.1.4.2.3 Discussion

The discussion of the ADCP-Lake Flow section is divided into five separate sub-sections to provide information and explanation of the data collected, allow individual discussion of the current velocity profiles, and discuss the temperature profiles collected during each of the four seasonal surveys that were conducted. The five discussion topics are as follows:

- Spring Data (spring survey)
- Summer Data (summer survey)
- Fall Data (fall survey)
- Winter Data (winter survey), and
- Discussion Summary

5.1.4.2.3.1 Spring Data

Currents

Extremely small currents hampered the understanding of the circulation of Lake Belton during the spring survey. Maximum flow cores were measured near 0.3 m/s. While stationary bottom mount ADCPs looking up from the lake bed can resolve extremely small currents, vessel mount surveys are limited by the need to progress across a transect at a reasonable speed. Transect 18 was too shallow to provide current data. It did provide a very short CTD profile.

Several transects provide evidence of slightly increased flow in the submerged thalweg **(Figure 5-36)**. Many transects show uniform magnitude fields of non-directional flow **(Figure 5-37)**. In these areas, flow may be dominated by non-riverine influences such as density variations, and surface winds. At least one transect showed a distinct flow core in mid-channel, outside the thalweg **(Figure 5-38)**.

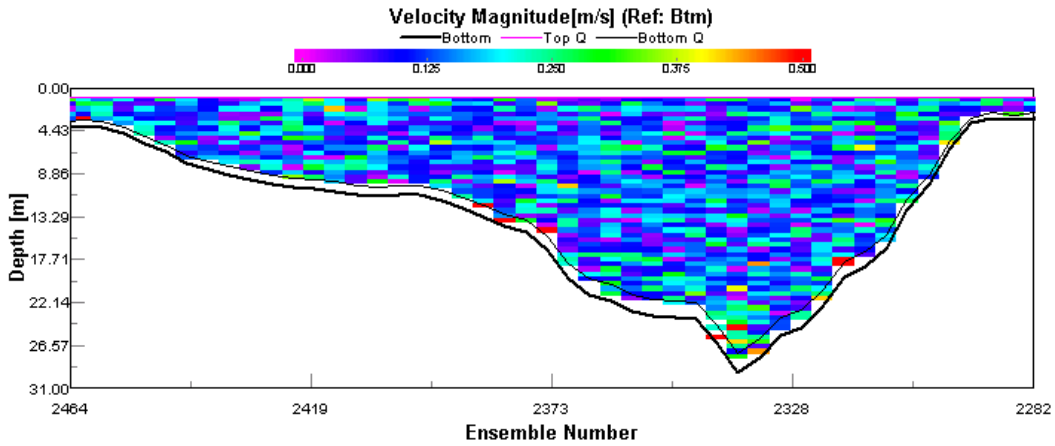


Figure 5-36
Increased Flow in the Bottom of a Thalweg, Transect 6

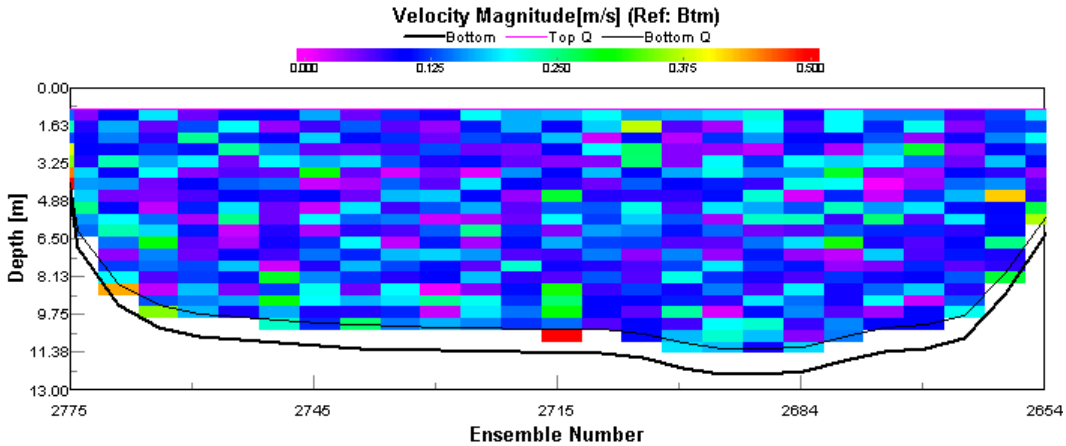


Figure 5-37
Uniform Flow, Transect 12

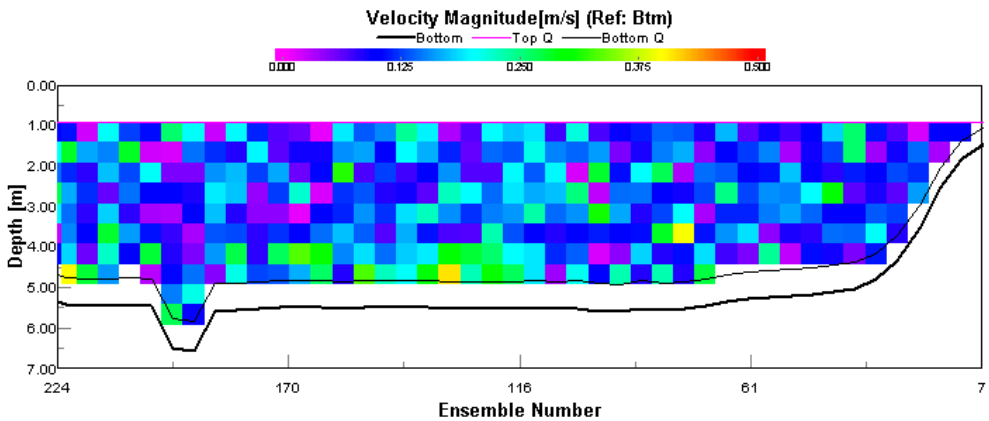


Figure 5-38
Channel Center Flow Adjacent to Thalweg, Transect 16

The following regional trends of core flow location were observed:

- Southern portions of Lake Belton (transects 1, 2) and the Cowhouse Creek branch (transects 3, 4, 5) are most likely to have thalweg flow.
- Central channel flow in the central section of Lake Belton (transects 6 to 13) may have slight thalweg flow.
- Lake channels north of transect 13 show very slight flows unconfined to the thalweg and often nondirectional. Where flow cores are observed they may be channel centered or adjacent to a bank.

Discharge
While it would be unwise to use collected discharge values for volumetric calculations, it is possible to observe the following area trends:

- The Cowhouse Creek branch (transects 3, 4, 5) provides nearly half the flow into the southern portion of Lake Belton (south of transects 3 and 6) with values near 60 m^3/s.
- The central channel of Lake Belton at transect 6 provides a similar flow into the southern portion of Lake Belton south of transects 3 and 6.

Temperature
Temperature profiles in the lake channels typically show a well-mixed surface layer with a distinct thermocline at 2 – 5m deep (Figure 5). By 12m depth, temperature becomes isothermal, near 10 °C.

5.1.4.2.3.2 Summer Data

Currents
Current data for the June survey cruise showed two primary flow regimes. In the southern lake transects, wind dominant currents appeared in the upper levels of the lake. These wind driven currents tended toward the up-channel direction, often confined within the

thermocline layer and against the core flows. Few concentrated core flows were observed in the south.

In the northern channels where river flows were observed, two-layer flows dominate with surface waters moving down-channel and deeper currents flowing up-channel.

Few areas of core flow were confined in the channel thalweg. Obvious instances of thalweg flow were up-channel as part of a circulation cell.

Measured discharge values are not likely to be accurate due to several factors including:

- Variations in wind driven circulation on the same time scale as the period of the transect
- Extremely low flow conditions
- Eddy conditions with shifting centers of circulation on the same time scale as the period of the transect

Temperature data show strong thermoclines in areas of vertical multi-layer flow in the southern channels. Northern channels that exhibit horizontal flow layers show constant temperatures with depth in the channel thalwegs.

Wind Effects

Maximum current velocities in the southern transects were found in the surface wind dominated layers **(Figure 5-39)**. These currents are frequently opposite in direction to the channel flow.

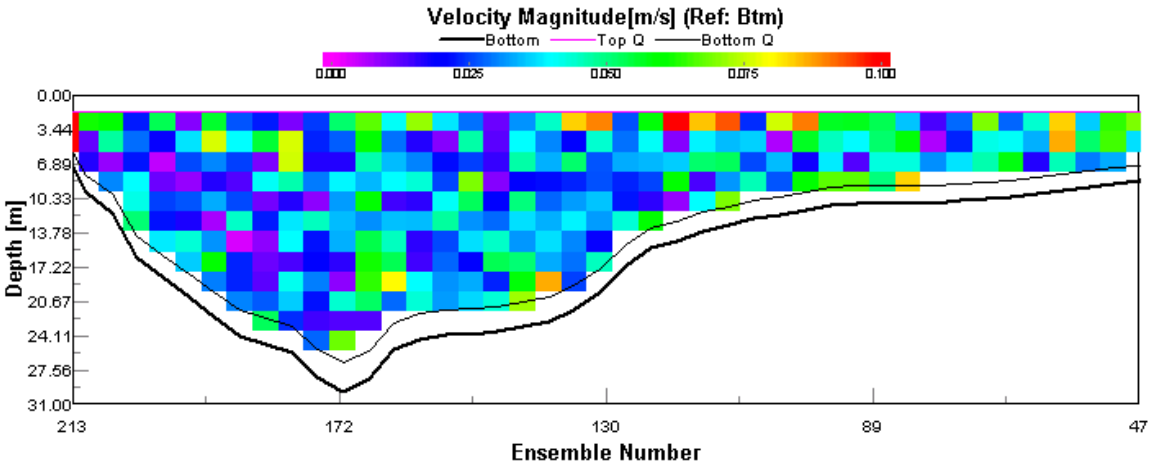


Figure 5-39
High Velocity Wind Drive Currents Above 5-m Depth, Transect 6

Wind speeds and direction varied during the survey. Gusty conditions of approximately 15 knots endured for short periods. Wind directions varied with location possibly due to the effects of the high channel banks on the prevailing southwest breeze.

Eddy Conditions

Reverse channel flows were observed in a number of transects. These features may be combinations of the wind effect, basin geometry, and core flow. In southern lake channels, wind driven currents appear near the surface with higher magnitudes while slow lake water below the thermocline flowed in the opposite direction.

Closer to the Leon River, reverse channel flows were observed along several transects. These reverse flows were deeper and on one side of the channel while an opposing river flow appears shallower on the opposite side **(Figure 5-40, Figure 5-41)**.

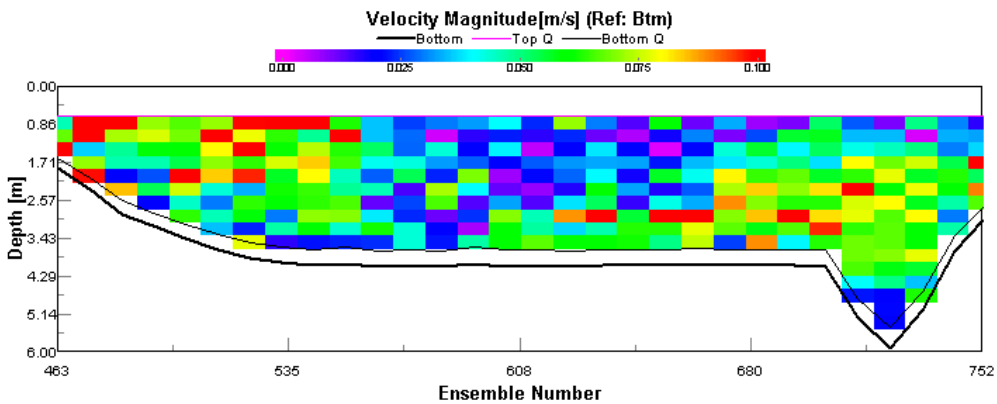


Figure 5-40
Eddy Flow, Two Core Magnitudes, Transect 17

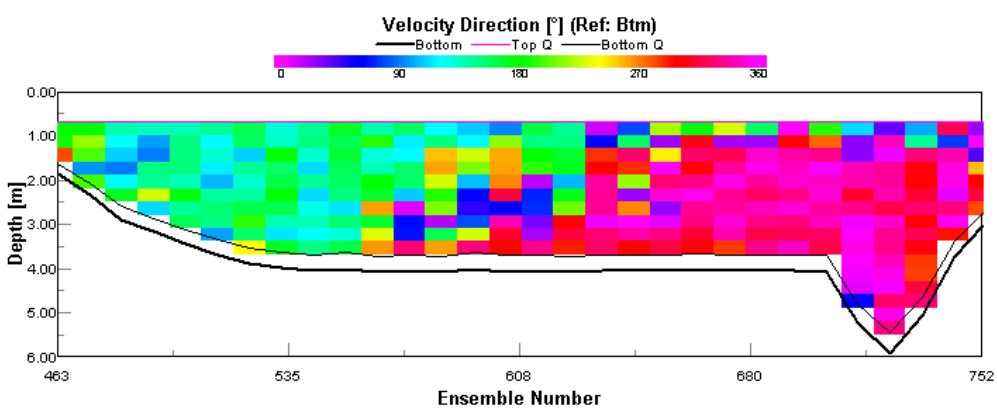


Figure 5-41
Eddy Flow, Southward (Green) Northward (Red), Transect 17

Discharge

Of all the collected transects, only the final measurement in the Leon River should be considered as an accurate measure of discharge. This transect showed strong laminar stream flow without effects of wind or eddies **(Figure 5-42)**.

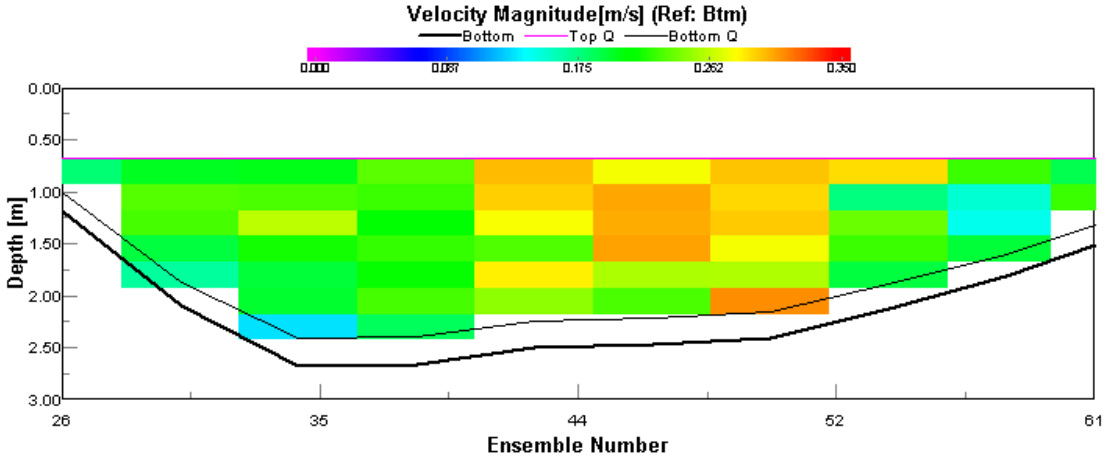


Figure 5-42
Laminar Stream Flow Discharge of 16.3 m^3/s, Transect 22 Leon River Section

Eddy circulation has a negative effect on discharge measurements. The value recorded for transect 17 **(Figure 5-40, Figure 5-41)** was 1.8 m^3/s. An accurate discharge measurement upstream from transect 22 in the Leon River showed 16.3 m^3/s. It seems clear that the Leon River flow should be conserved downstream at transect 17, suggesting that eddies can affect discharge measurements in the area by close to a factor of 10.

Thalweg Flow

Bottom flow was only apparent in a few transects **(Figure 5-43)**. Transects 15, 16, and 17 showed the bottom flow conditions characteristic of northern lake sections as the deeper half of a two-layer flow **(Figure 5-44)**. In these cases, flow was observed within the thalweg but flowing up-channel. Surface currents in the northern lake sections may represent core flows. The Leon River temperature sample showed the river to be one degree warmer than lake surface water suggesting that river water may override the lake water while the temperature difference endures.

These conditions are a departure from the observations of the spring survey. In March 2003, different wind directions, lake stages, and temperature structures in the river and lake created conditions of intuitive channel flow where maximum current magnitudes were more likely to be observed in the thalwegs as high velocity cores flowing down channel. The June 2003 conditions are characterized by wind effects, eddies, and uniform-slow channel flows below the thermocline.

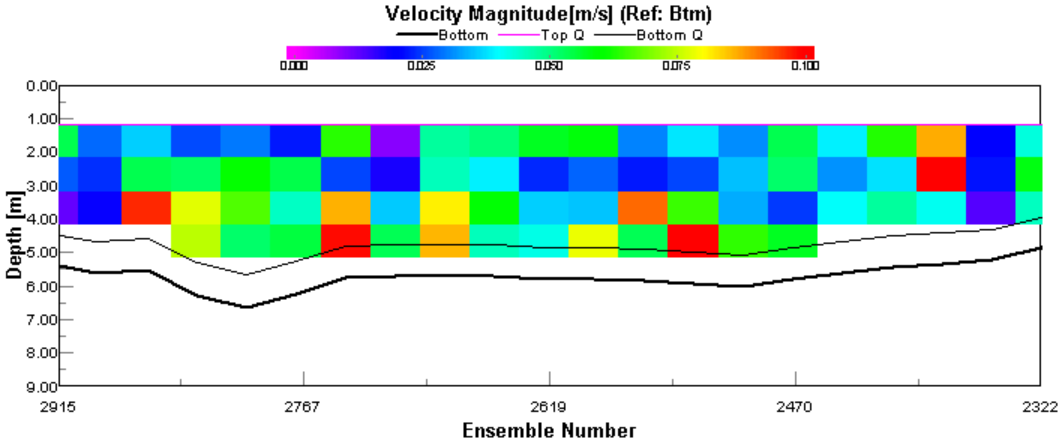


Figure 5-43
Bottom Flow Magnitude, Transect 16

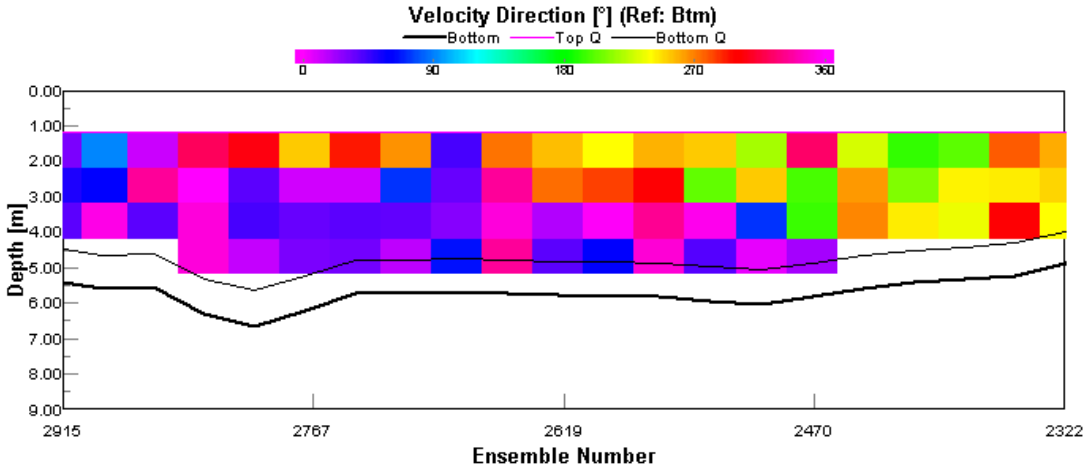


Figure 5-44
Bottom Flow Direction Up Channel (Purple/Blue) and Down Channel (Green/Yellow), Transect 16

Temperature

Lake and river temperatures were nearly 22 °C warmer in the summer (June 2003) than during the spring survey. Temperature ranges were much reduced in June, spanning only one degree. Surface water ranges in the spring (March 2003) were near 4 °C. Strong thermoclines were observed at all southern channel transects, which occurred near 8-m depths. Measurements north of transect 15 are well mixed with no thermoclines evident. Current data suggest that a temperature profile taken in the thalweg would be uniform. In these northern transects, temperature stratification in the channel may be horizontal across the channel and not vertical with depth.

5.1.4.2.3.3 Fall Data

Currents

Currents measured during the fall (September) survey were extremely low. The lake water level was the lowest observed during the study. Areas of measurable flow in June, such as the Leon River transect 22, were impassable in September. Transect 18 was too shallow to collect usable data. Measurements at transect 21 appear to be unrealistically out of range. A computer crash required the software to be reloaded just prior to this transect.

Similar complex trends of wind driven surface flow, channel eddies, and reverse currents were observed but appeared less vigorous than in earlier surveys. Few instances of significant core flow were observed. Several of these slightly higher flows were observed near the thalwegs, often above the submerged banks but centered on the thalweg **(Figure 5-45)**.

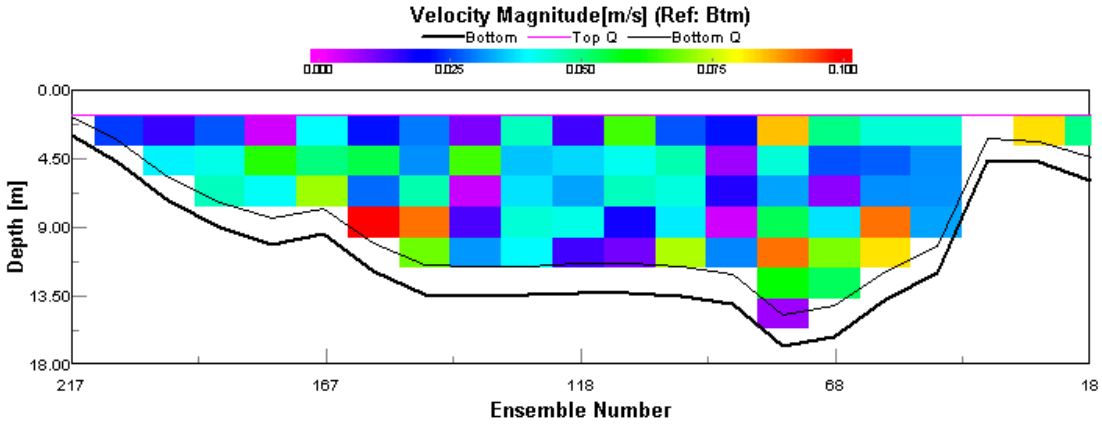


Figure 5-45
Core Flow Near a Thalweg, Transect 11

Measured discharge values are not likely to be accurate due to several factors including:

- Variations in wind driven circulation on the same time scale as the period of the transect.
- Extremely low flow conditions.
- Eddy conditions with shifting centers of circulation on the same time scale as the period of the transect.

Temperature data show strong thermoclines in areas of vertical multi-layer flow in the southern channels.

Wind Effects

Wind speeds were generally light during the survey period. Some instances of wind effect correlate to periods of building breeze during the transect **(Figure 5-46)**. Wind directions varied with location possibly due to the effects of the high channel banks on the breeze. These effects seem to cause shore-parallel wind directions regardless of the wind direction at elevations above the channel banks.

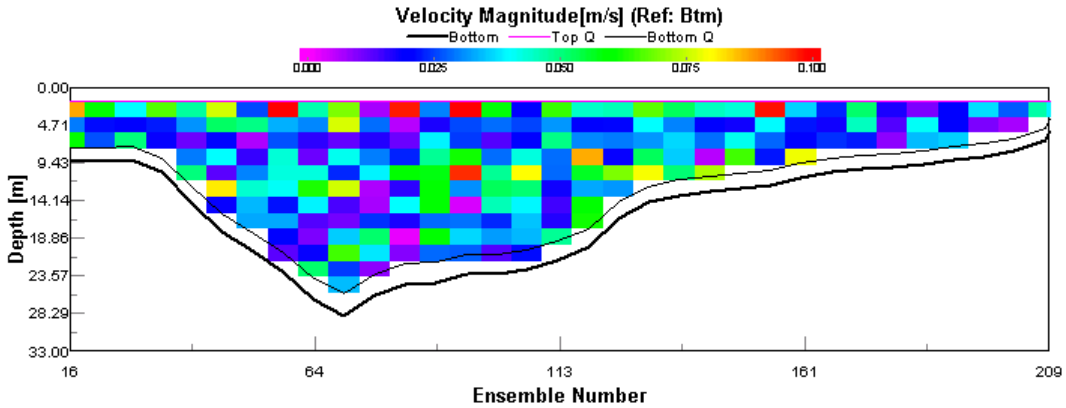


Figure 5-46
Surface Wind Current Effects During Breeze, Transect 6

Eddy Conditions

Good examples of diagonal shear layers across the channel were collected in spite of extremely low flow conditions. Reverse channel flows were observed in a number of transects **(Figure 5-47**, **Figure 5-48)**.

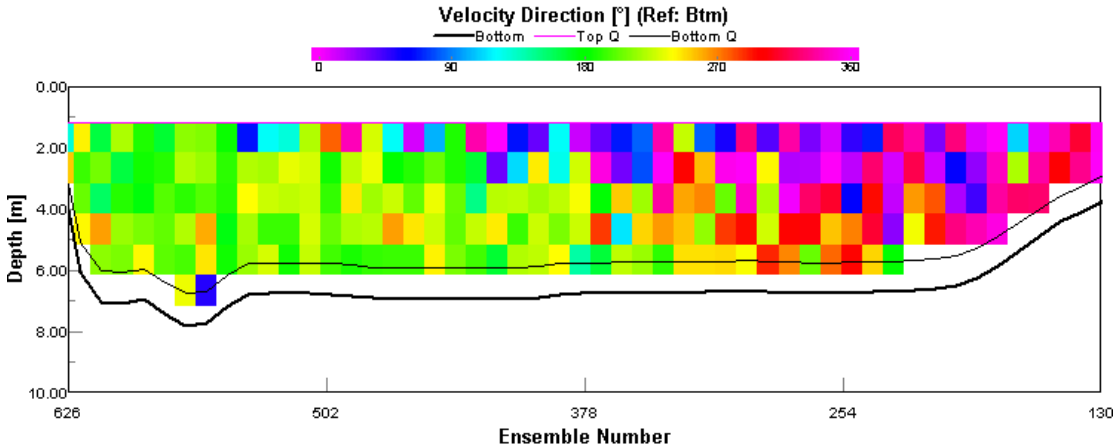


Figure 5-47
Diagonal Shear with Reverse Flow on Upper Right and Lower Left, Transect 15

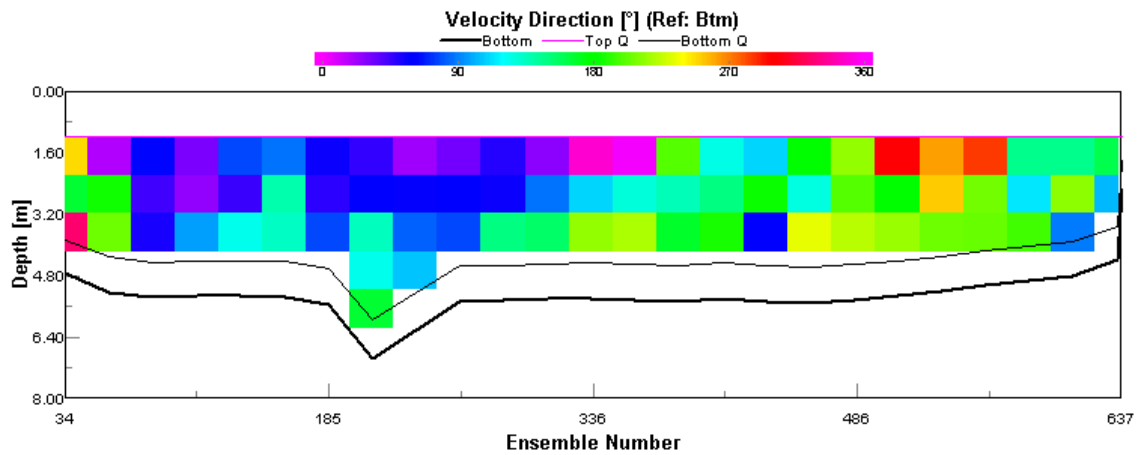


Figure 5-48
Diagonal Shear with Reverse Flow on Upper Right and Lower Left, Transect 16

Other examples show eddy circulation at a single depth **(Figure 5-49)**.

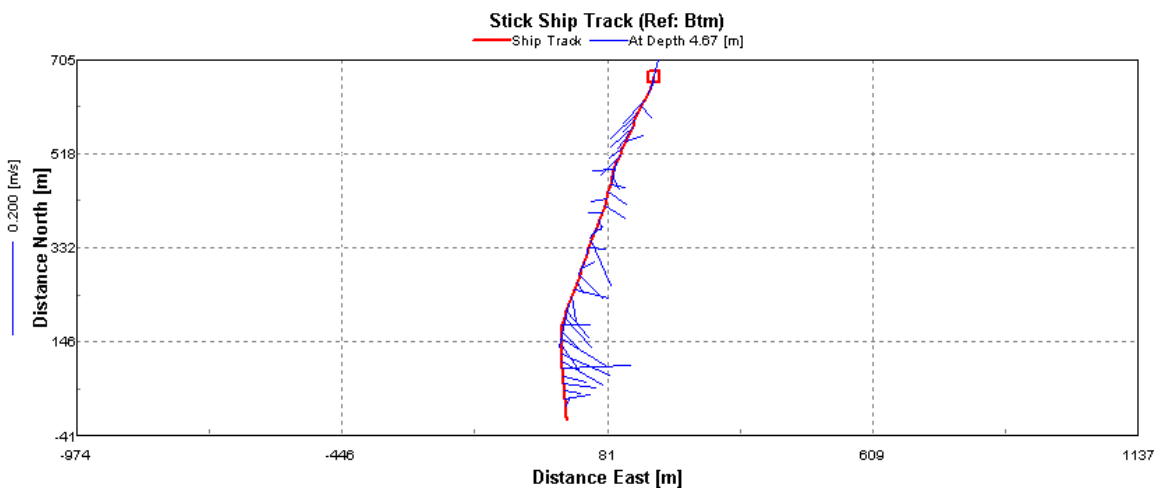


Figure 5-49
Vector Flow Diagram of Eddy Flow at 4.67m Depth, Transect 14

Discharge

Discharge measurements were frustrated by the extreme low flows. Multiple measurements were made at transect 23 with some success. Currents were too slow to observe "by eye" during collection of these transect data, but were recorded as laminar channel flow by the Rio Grande ADCP **(Figure 5-50)**.

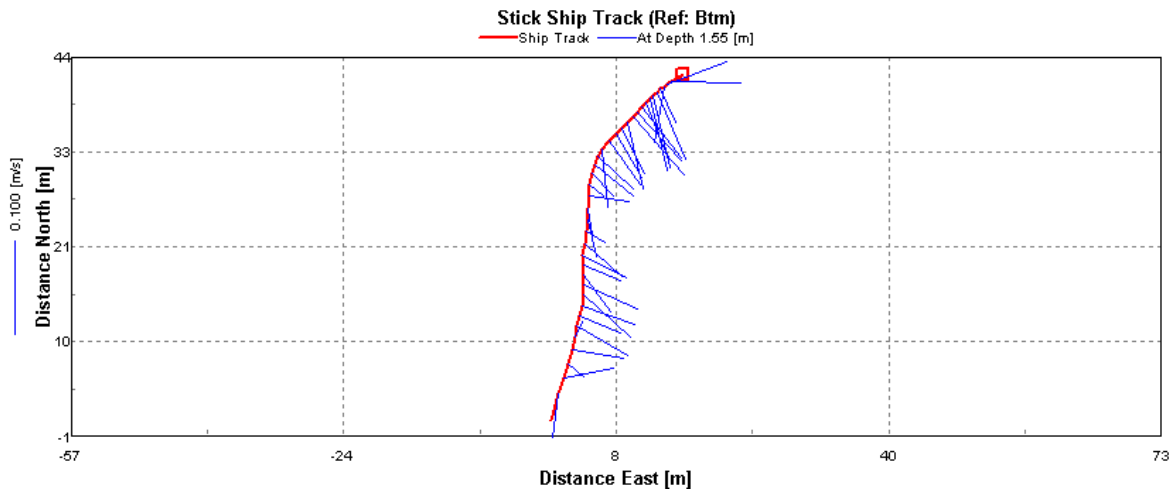


Figure 5-50
Vector Flow Diagram of Laminar Channel Flow at 1.55m Depth, Transect 23

Discharge values measured in the lake channels follow some plausible trends with a number of obviously bad discharge values. These include unlikely values at transects 1, 2, 10 and 11. Use of these data for summation of discharge rates for any purpose other than very general trend observations is not advisable. One additional source of discharge to be considered is the possible existence of submerged springs and seeps.

Thalweg Flow

Thalweg flows, although not obviously apparent and at very low velocities, are detected in a few transects. As with the previous survey, flow near the thalweg is often the deeper half of a two-layer flow **(Figure 5-51, Figure 5-52)**.

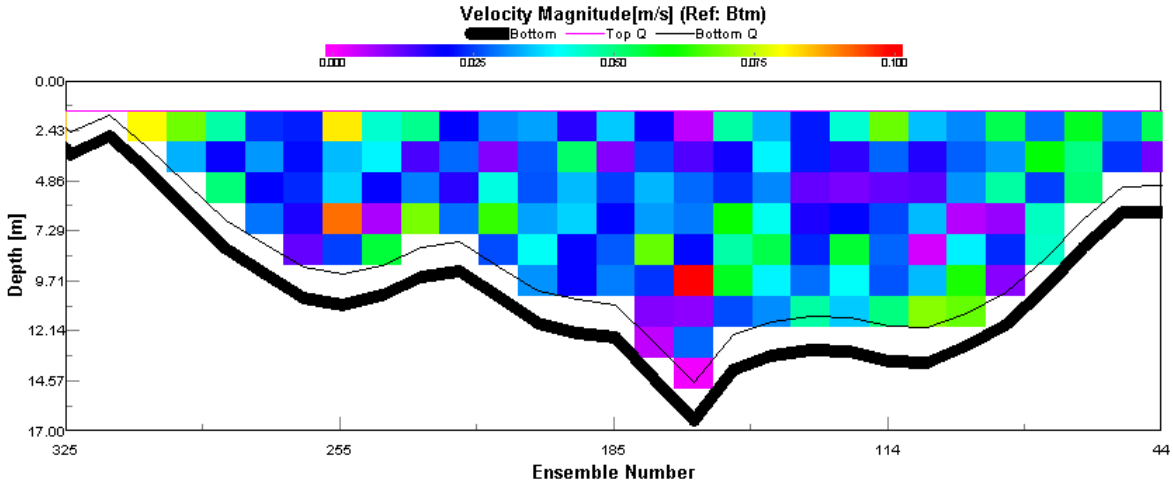


Figure 5-51
Thalweg Flow Velocity, Transect 4

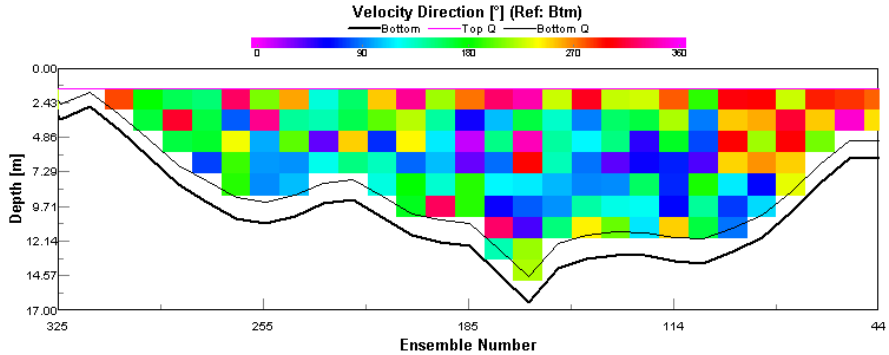


Figure 5-52
Opposite Surface Flow Direction Above Thalweg, Transect 4

Temperature
Southern lake channel surface temperatures are nearly identical to the June survey. Lake surface water temperature ranges are more consistent with this survey, spanning only 1 °C, and remain warmer to a deeper more abrupt thermocline at 11m. Measurements north of transect 15 are again consistent to depth and present no evidence of thermocline.

5.1.4.2.3.4 Winter Data

Currents
Currents measured during the December 2003 survey were again extremely low and chaotic. ADCP transects collected on December 17th, during calm weather, produced excellent data. All other transects were performed in windy conditions with wave chop affecting the upper water column data. Through averaging of ensembles and observance of data in Velocity Direction as well as Velocity Magnitude contour plots, core flow data were extracted from each transect. As in **Figure 5-53** where surface data are "noisy" and lack directional continuity, yet the data near the lake bottom produced ensembles of common direction.

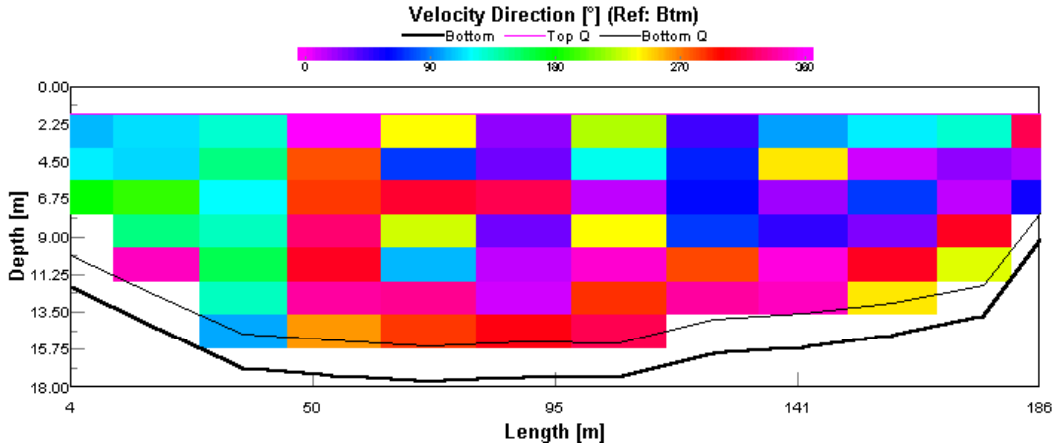


Figure 5-53
Transect 20, Velocity Direction Contour Plot

From these types of data, producing regions of common flow direction, many core flow criteria were met, even though a single ensemble in another region of the transect may have produced a higher velocity.

Transect 18 was again too shallow to collect usable data. Transect 23 was not surveyed.

Wind Effects

Wind speeds were strong to severe during two of the three days of data collection on Lake Belton. This created a wave chop, which produced chaotic flow patterns, as in **Figure 5-54**, in the shallower lake sections.

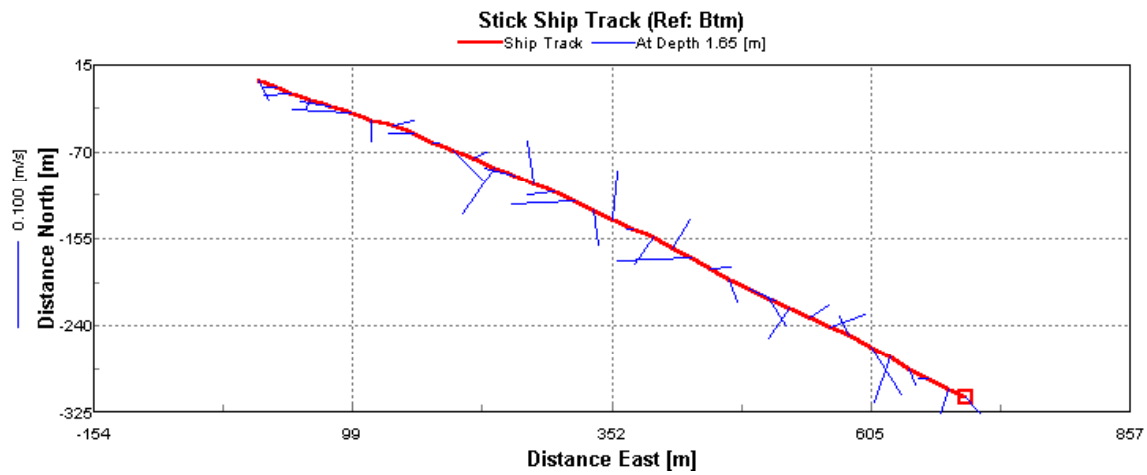


Figure 5-54
Surface Wind and Wave Chop Effects, Transect 16

Eddy Conditions

Complex eddy conditions were observed in several transects. Often, more than one reverse (eddy) flow pattern was recorded across a transect. In **Figure 5-55**, nearly the entire transect is an eddy feature, with similar velocity near each bank.

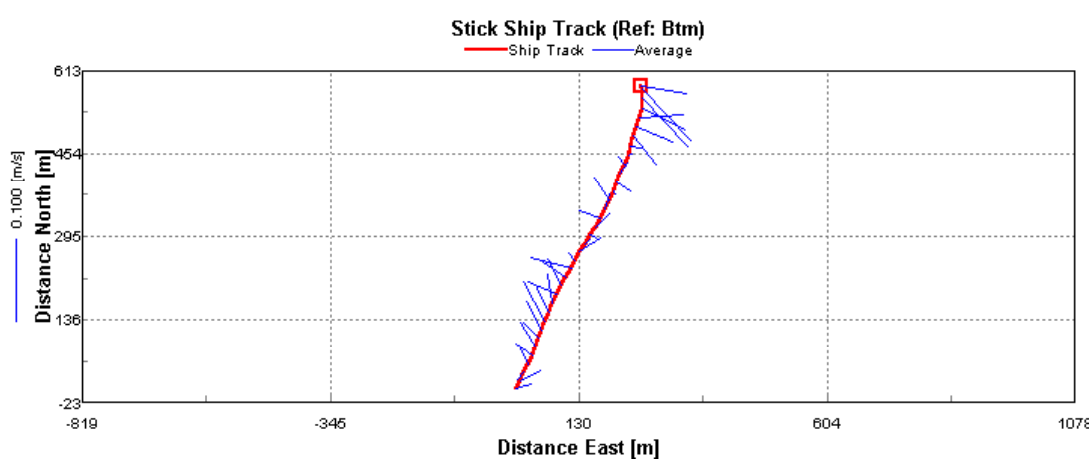


Figure 5-55
Large Eddy Feature Spanning Lake Channel, Transect 14

The following data examples of Transect 13 illustrate a diagonal shear associated with an eddy flow pattern (**Figure 5-56**, **Figure 5-57**).

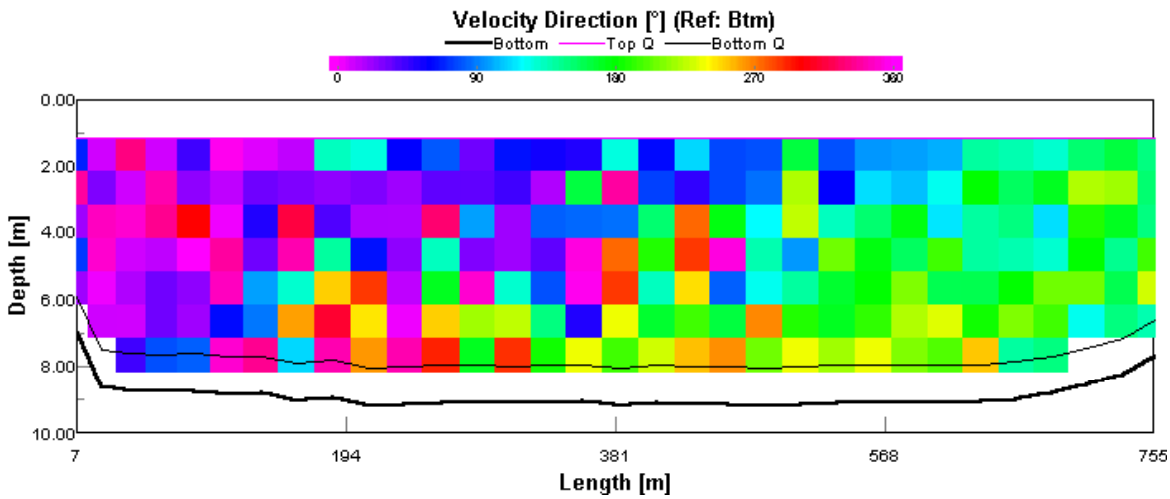


Figure 5-56
Diagonal Shear with Reverse Flow on Upper Left and Lower Right, Transect 13

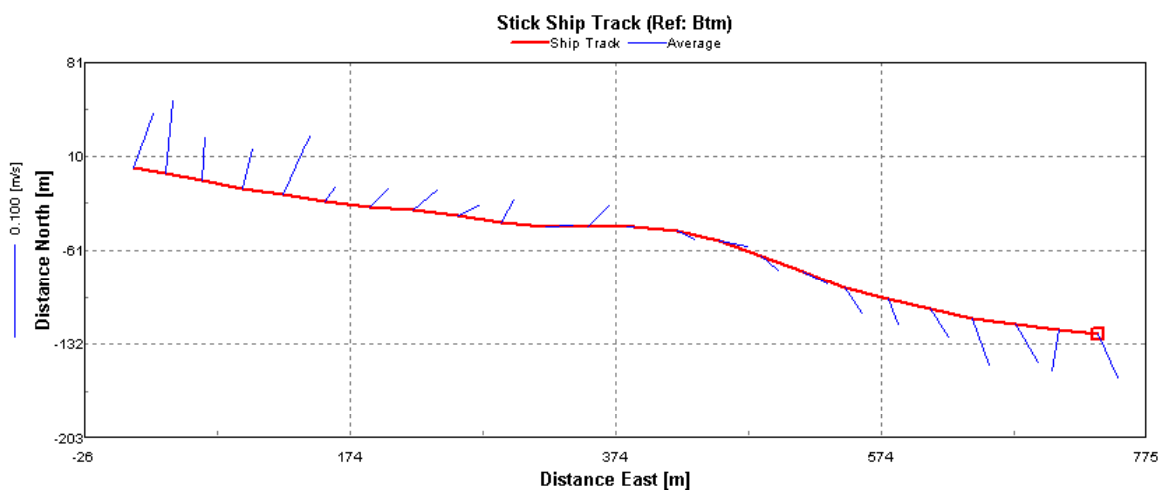


Figure 5-57
Vector Flow Diagram of Eddy Flow, Transect 13

Discharge

Discharge measurements varied greatly from transect to transect and should not be considered an indication of true lake flow. The discharge values present the general trend for the moments of the transects, at that location, and may be dominated by factors outlined below.

As with the previous surveys, measured discharge values are not likely to be accurate due to several factors including:

- Variations in wind driven circulation on the same time scale as the period of the transect.
- Extremely low flow conditions.
- Eddy conditions with shifting centers of circulation on the same time scale as the period of the transect.
- Wind driven surface currents greatly exceeding core flow, which may indicate lake discharge.

Discharge values are derived from data across the entire transect, bi-layer flow could produce low discharge values due to the canceling effect of opposing flow directions.

An area of laminar flow, possibly indicating discharge, is apparent on Transect 1, in the deepest section of the water column (**Figure 5-58**).

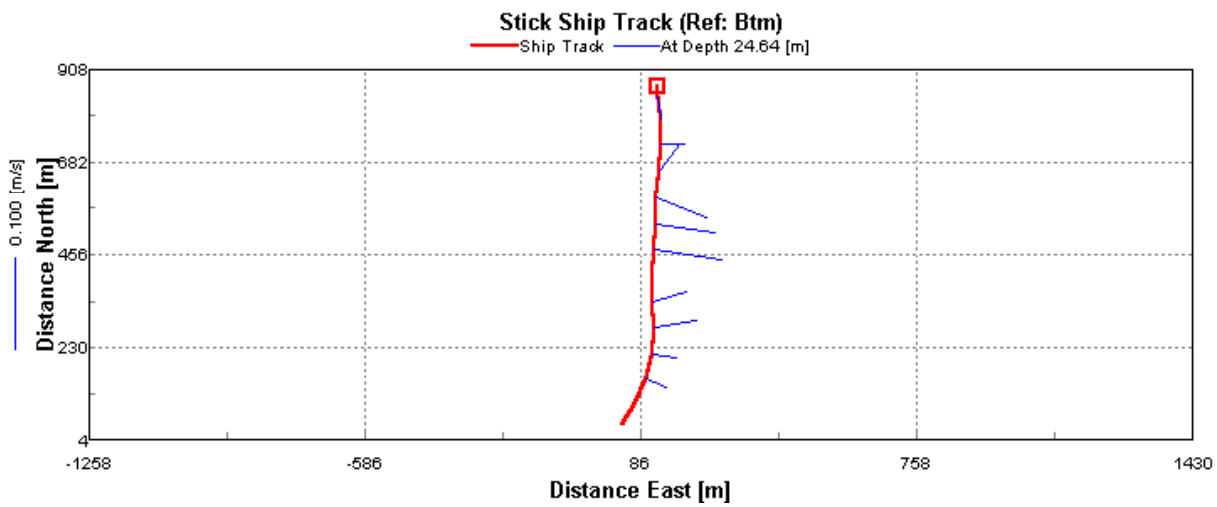


Figure 5-58
Vector Flow Diagram of Laminar Channel Flow at 24.64m Depth, Transect 1

Thalweg Flow

Thalweg flow, although at very low velocity, was detected in transects 6 and 7. Transect 6 reveals low velocity thalweg flow beneath very chaotic upper water flow (**Figure 5-59**); while Transect 7 produced thalweg flow as the lower half a nearly consolidated two layer flow pattern (**Figure 5-60**).

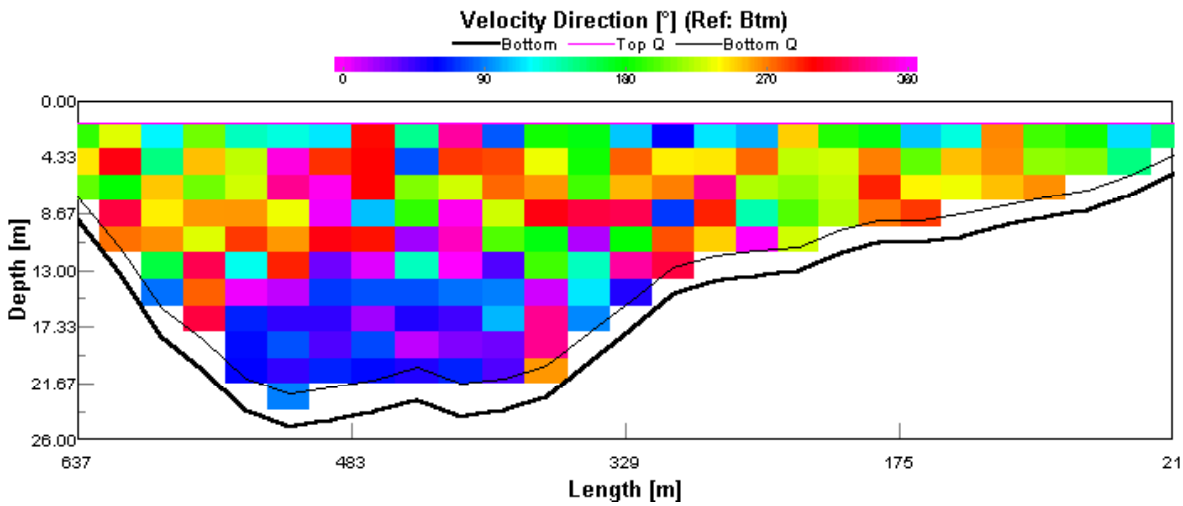


Figure 5-59
Thalweg Flow, Transect 6

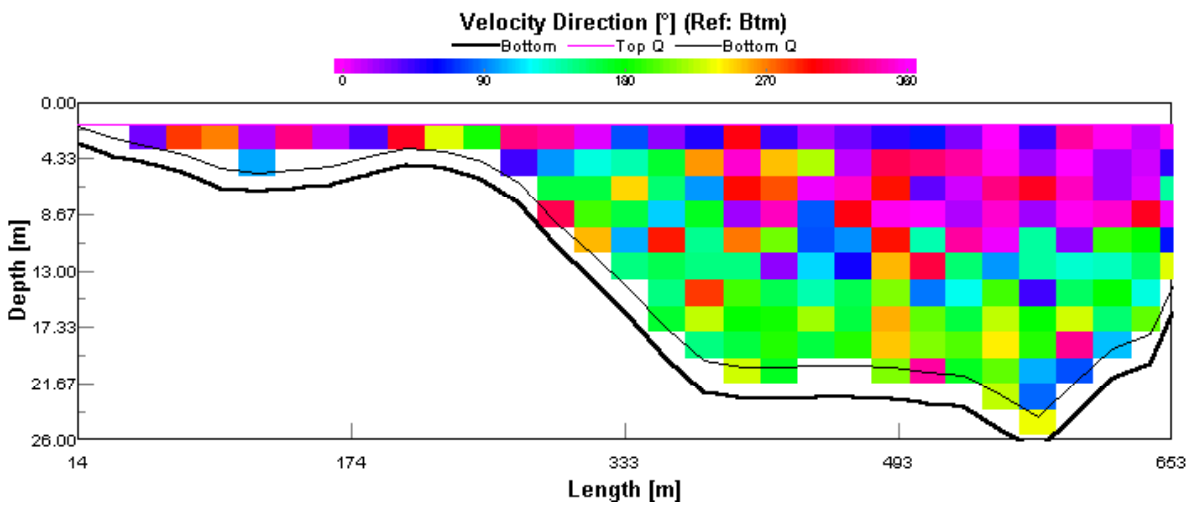


Figure 5-60
Thalweg Flow, Transect 7

Temperature
Temperature profiles at all sites indicate that the lake channels were well mixed, and that a thermocline was not present. Temperature measurements south of Transect 15 ranged from 12 ^{o} C to 14.5 ^{o} C. Measurements north of Transect 15 were slightly lower, ranging from 10.5^{o} C to just above 13 ^{o} C.

5.1.4.2.3.5 Overall Evaluation

Currents
Currents measured in Lake Belton were consistently low throughout the year, with maximum core flows not exceeding 0.3 m/s. Lake water levels were the highest during the June 2003 survey, and were the lowest during the September 2003 survey, leading to difficulties collecting data in shallow areas. Areas of measurable flow in June, such as the

Leon River Transect 22, were nearly impassable in December 2003 and produced data of limited value. Transect 18 was too shallow to collect usable data during all but the June survey.

Laminar Flow. During the June, 2003 survey, transect 22 showed strong laminar stream flow without effects of wind or eddies (**Figure 5-61**).

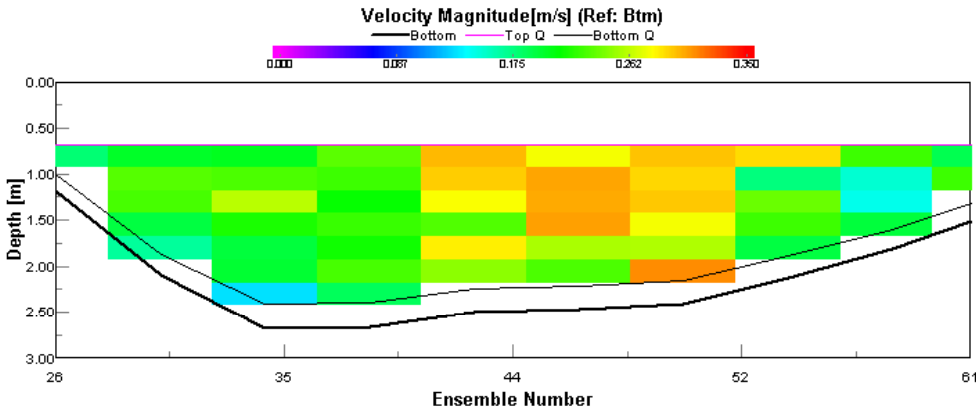


Figure 5-61
Laminar Stream Flow Discharge of 16.3 m^3/s. June 2003 Survey, Transect 22 Leon River Section

Eddy Conditions. Complex eddy conditions with diagonal shear layers across the channel were observed during the June, September, and December, 2003 surveys, even in spite of extremely low flow conditions during the September, 2003 survey. Often, more than one reverse (eddy) flow pattern was recorded across a transect. In the example below (**Figure 5-62**), nearly the entire transect is an eddy feature, with similar velocity near each bank. These reverse channel flows were observed in a number of transects, and three examples are shown below (**Figure 5-63**, **Figure 5-64**, and **Figure 5-65**).

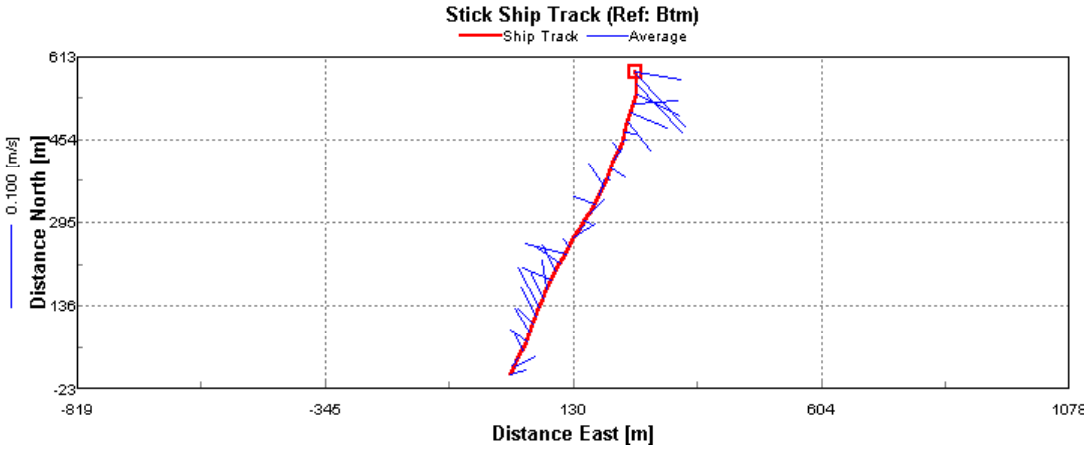


Figure 5-62
Large Eddy Feature Spanning Lake Channel. December 2003 Survey, Transect 14

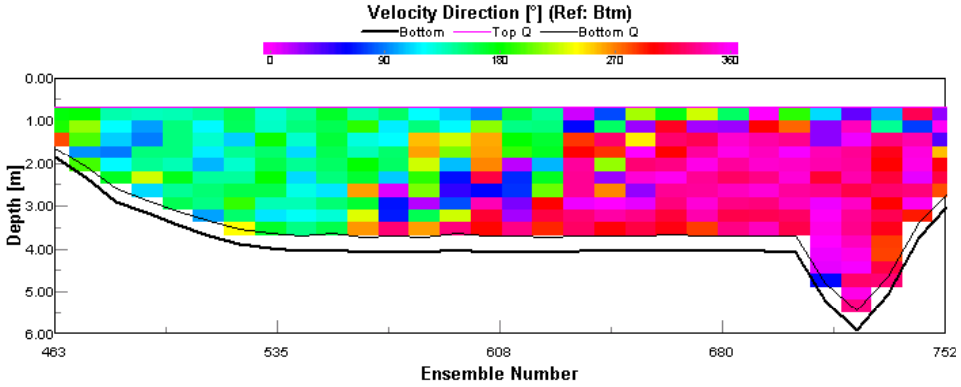


Figure 5-63
Eddy Flow. Diagonal Shear with Reverse Flow on Upper Left and Lower Right. Southward (Green) Northward (Red). June 2003 Survey, Transect 17

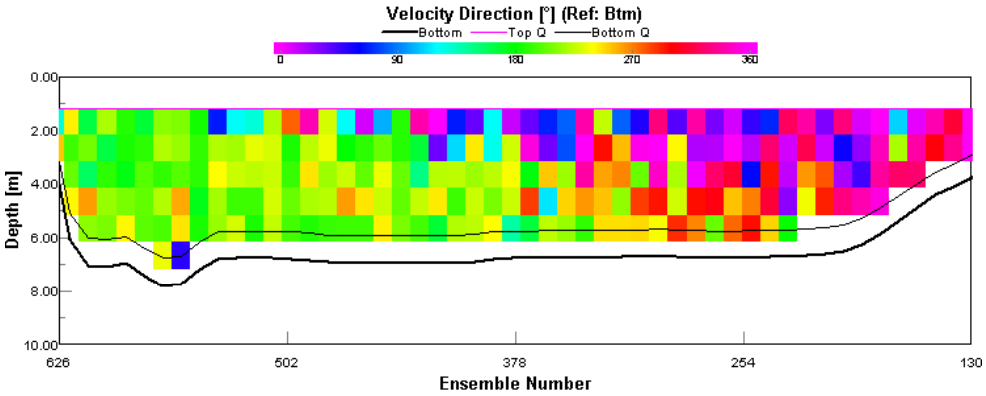


Figure 5-64
Diagonal Shear with Reverse Flow on Upper Right and Lower Left. Southward (Green) Northward (Red). September 2003 Survey, Transect 15.

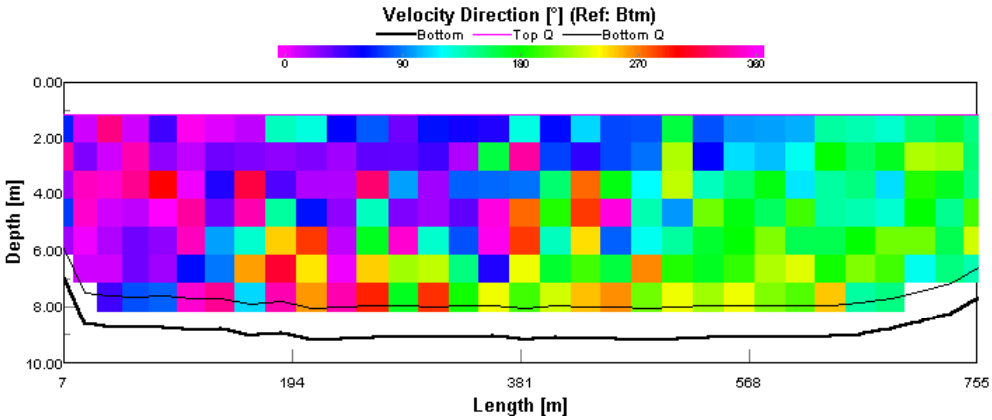


Figure 5-65
Diagonal Shear with Reverse Flow on Upper Left and Lower Right. Southward (Green) Northward (Red). December 2003, Transect 13

Thalweg Flow. Several examples of thalweg flow, or slightly increased flow in the submerged thalweg, were identified during each of the four seasonal current surveys. Two examples are shown below (**Figure 5-66** and **Figure 5-67**)

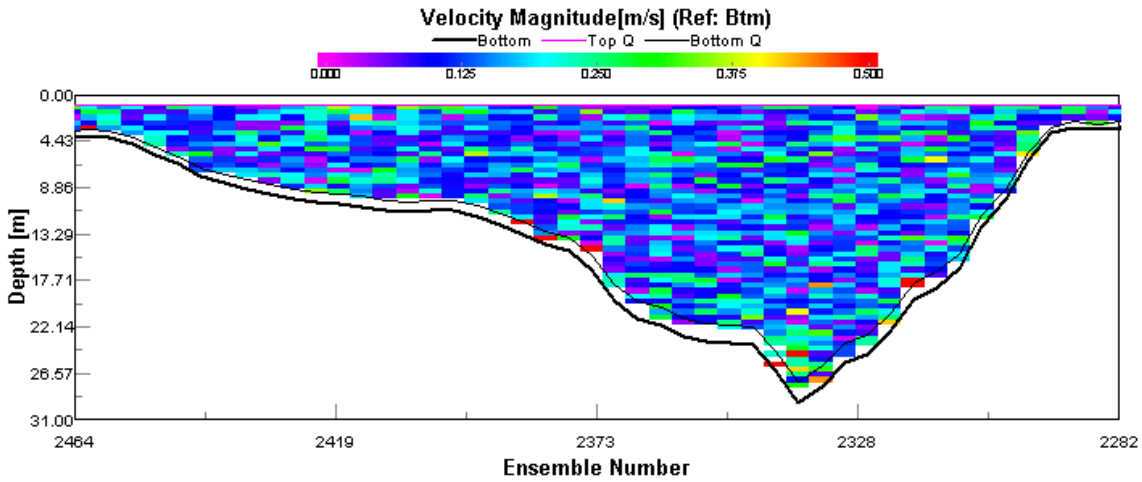


Figure 5-66
Increase Flow in the Bottom of a Thalweg. March 2003 Survey, Transect 6

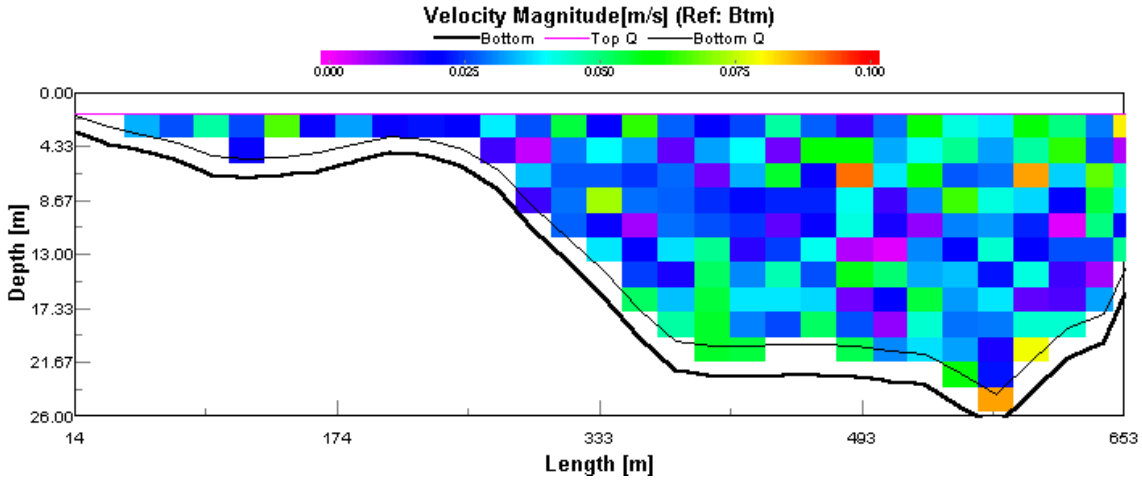


Figure 5-67
Increased Flow in the Bottom of a Thalweg. December 2003 Survey, Transect 7

Core Flow Outside Thalweg. Some transects showed a distinct flow core in mid-channel, outside the thalweg (**Figure 5-68** and **Figure 5-69**).

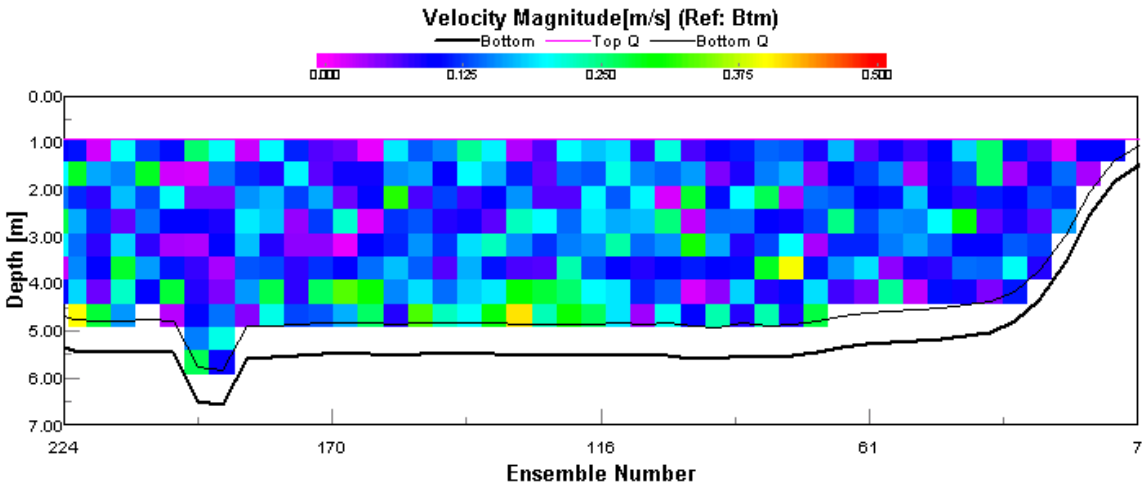


Figure 5-68
Channel Center Flow Adjacent to Thalweg. March 2003 Survey, Transect 16

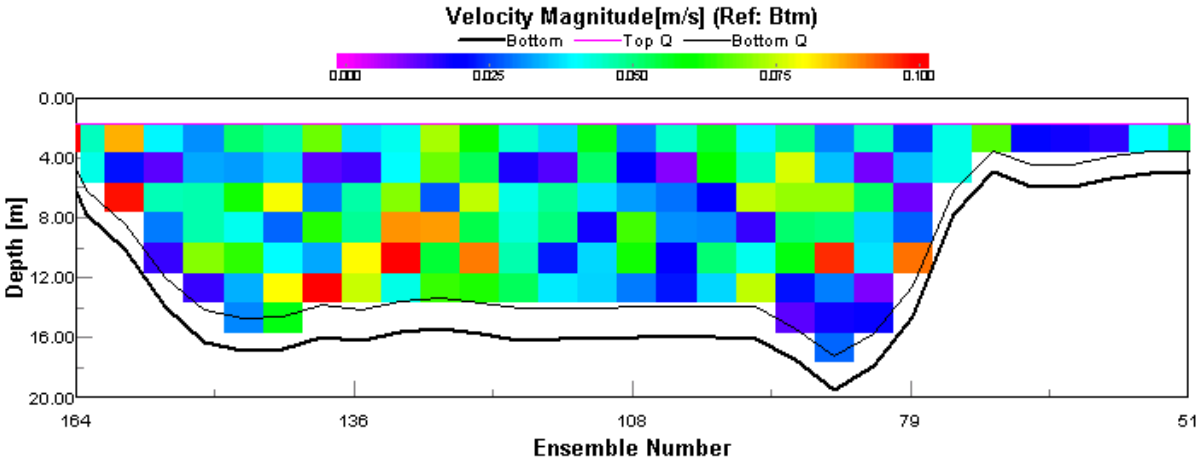


Figure 5-69
Channel Center Flow Adjacent to Thalweg. September 2003 Survey, Transect 10

Uniform Flow. Many transects show uniform magnitude fields of weak and variable flow (**Figure 5-70**). This was a consistent feature in each of the four surveys.

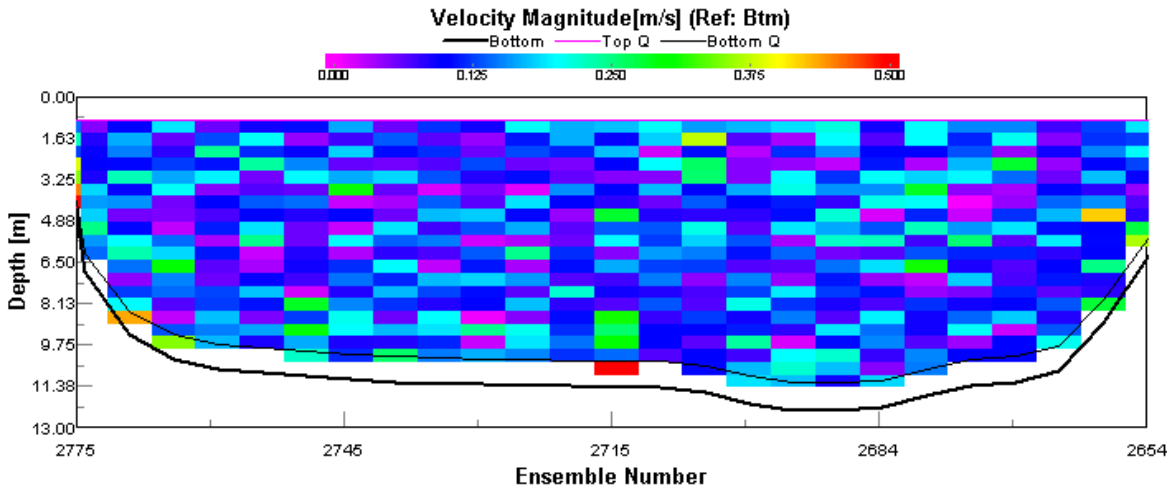


Figure 5-70
Weak and Variable Uniform Flow. March 2003 Survey, Transect 12

Seasonal Flow Comparison. Data were examined for seasonal trends, and little to no correlation was found between the four surveys. Due to extremely low current velocities found throughout the year, data indicate that external forces such as the adjacent landmass and wind speed and direction are the primary influences on the currents in Lake Belton.

An example of the influences of adjacent landmasses on the flow patterns of parts of the lake was seen at Transect 15 during each of the four surveys. At this transect, velocity fields were generally weak and inconsistent, however the direction fields reflected consistent eddy formation, evident by a diagonal shear with reverse flow on the upper right and lower left sides of the transect (**Figure 5-64**).

An example of the lack of seasonal correlation was seen at Transect 12. Velocity magnitude and direction profiles from each survey at Transect 12 are shown below for comparison (**Figure 5-71** through **Figure 5-78**). In the example below, it can be seen that trends in the velocity fields were generally weak and inconsistent between surveys (**Figure 5-71**, **Figure 5-73**, **Figure 5-75**, and **Figure 5-77**). Trends in the directional fields showed a tendency for the surface flow to follow the general shape of the surrounding landmass under the influence of the wind, however the overall direction of the flow was not consistent throughout the year (**Figure 5-72**, **Figure 5-74**, **Figure 5-76**, and **Figure 5-78**).

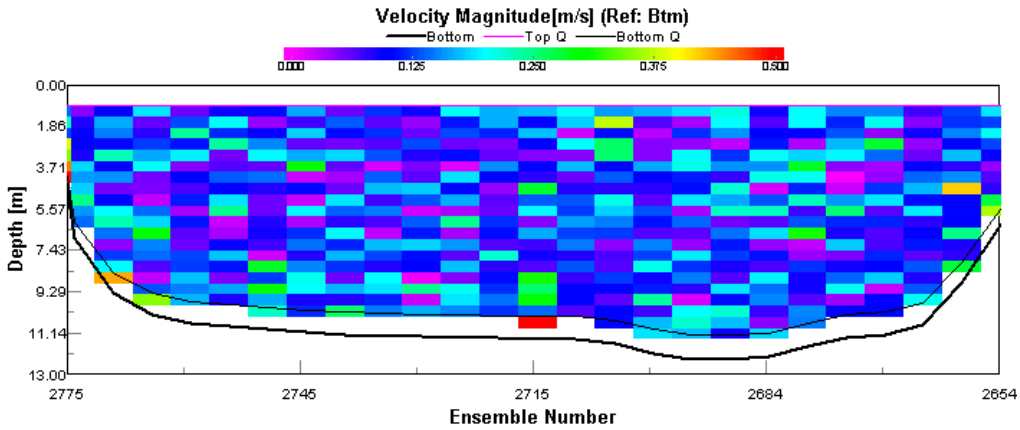


Figure 5-71
Current Velocity During the March 2003 Survey, Transect 12

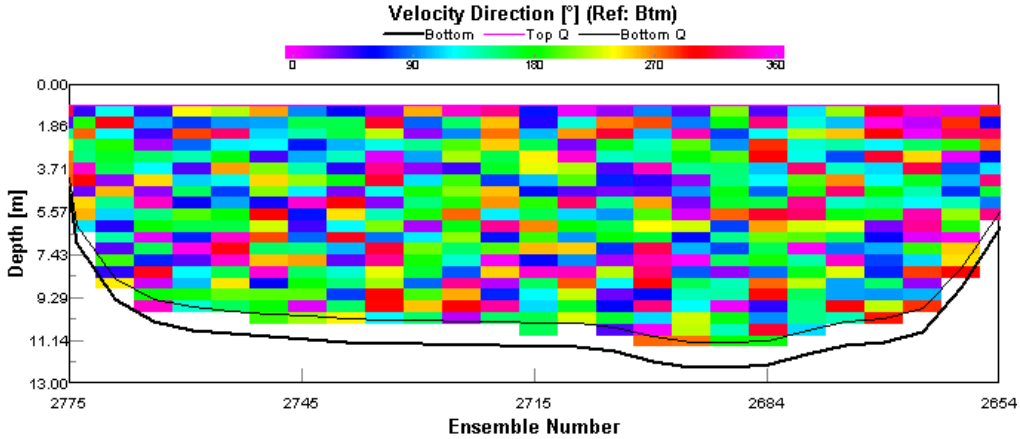


Figure 5-72
Current Direction During the March 2003 Survey, Transect 12

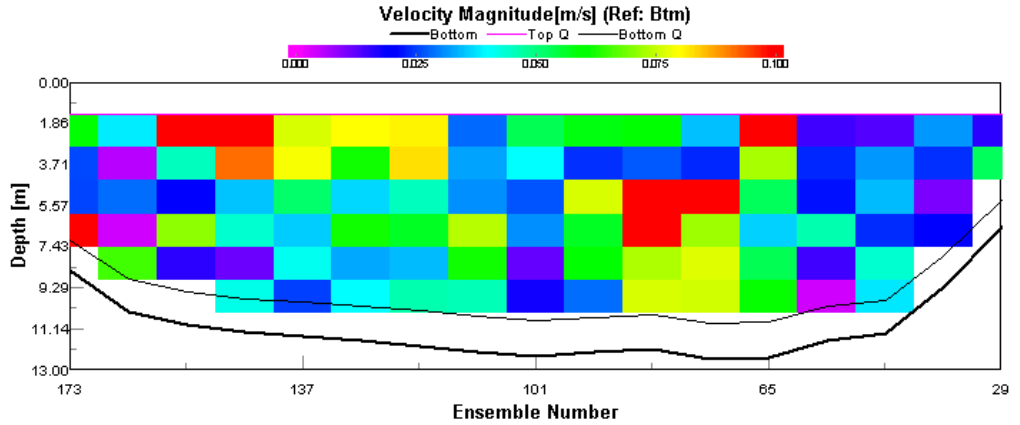


Figure 5-73
Current Velocity During the June 2003 Survey, Transect 12

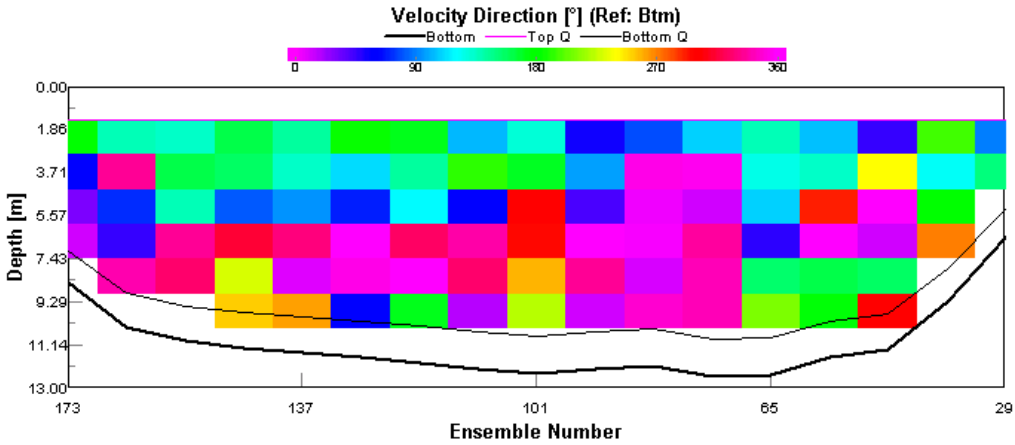


Figure 5-74
Current Direction During the June 2003 Survey, Transect 12

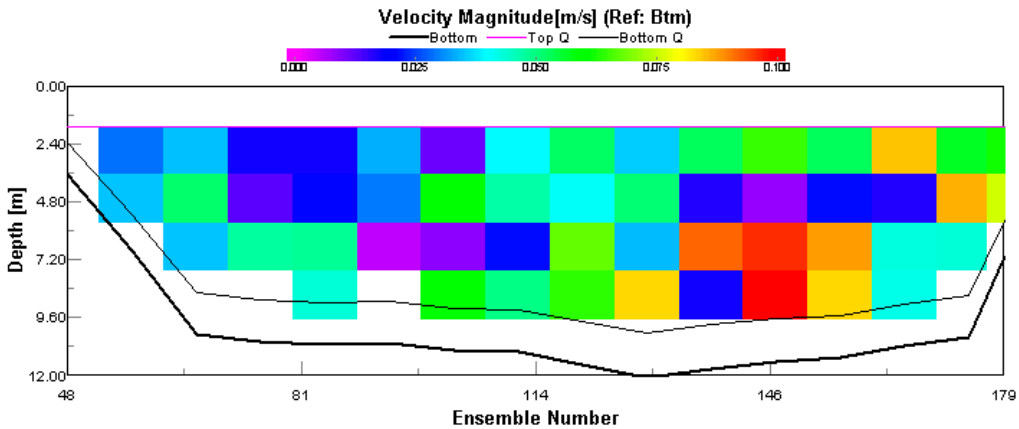


Figure 5-75
Current Velocity During the September 2003 Survey, Transect 12

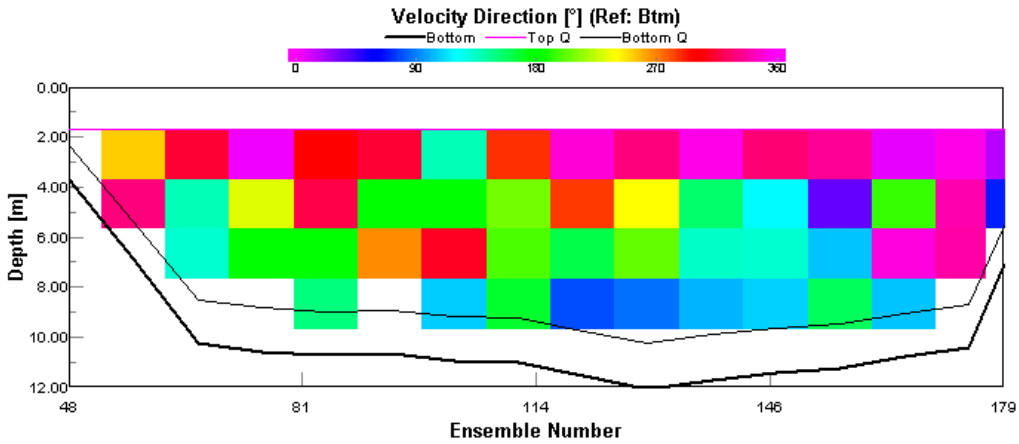


Figure 5-76
Current Direction During the September 2003 Survey, Transect 12

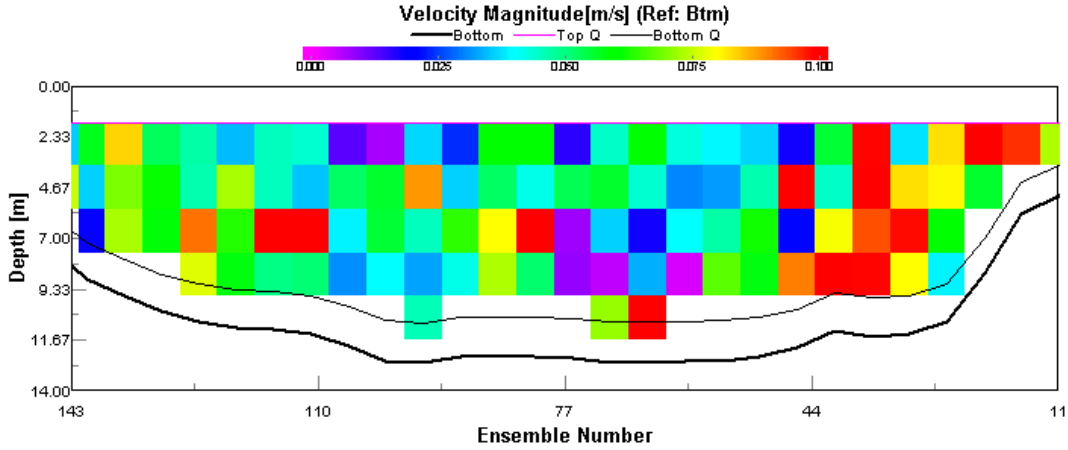


Figure 5-77
Current Velocity During the December 2003 Survey, Transect 12

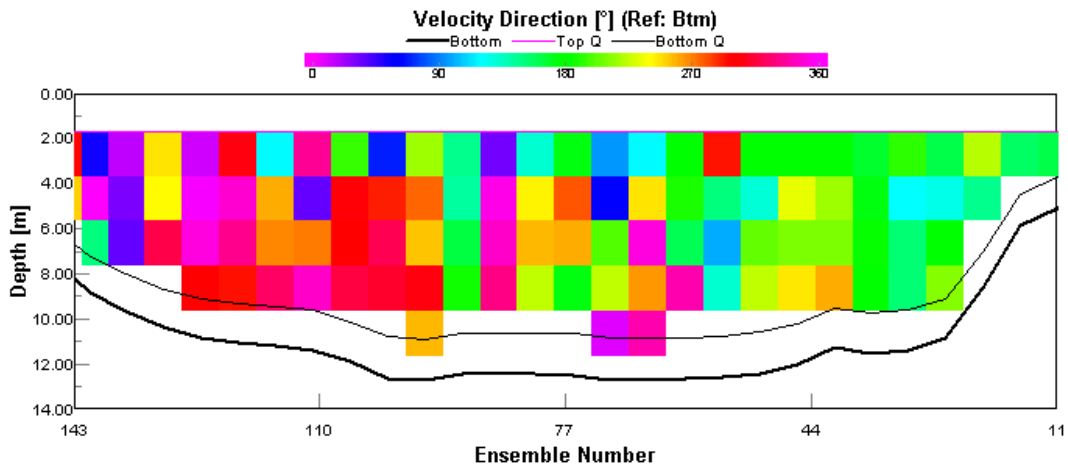


Figure 5-78
Current Direction During the December 2003 Survey, Transect 12

Wind Effects. Wind speeds were generally light during the survey period. Wind speeds were strong to severe during two days of the December 2003 survey period leading to noisy surface data. Wind speeds were generally light during the September 2003 survey period. Some instances of wind effect correlate to periods of building breeze during a given transect (**Figure 5-79**). Wind directions varied with location possibly due to the effects of the high channel banks on the breeze. These effects seem to cause shore-parallel wind directions regardless of the wind direction at elevations above the channel banks.

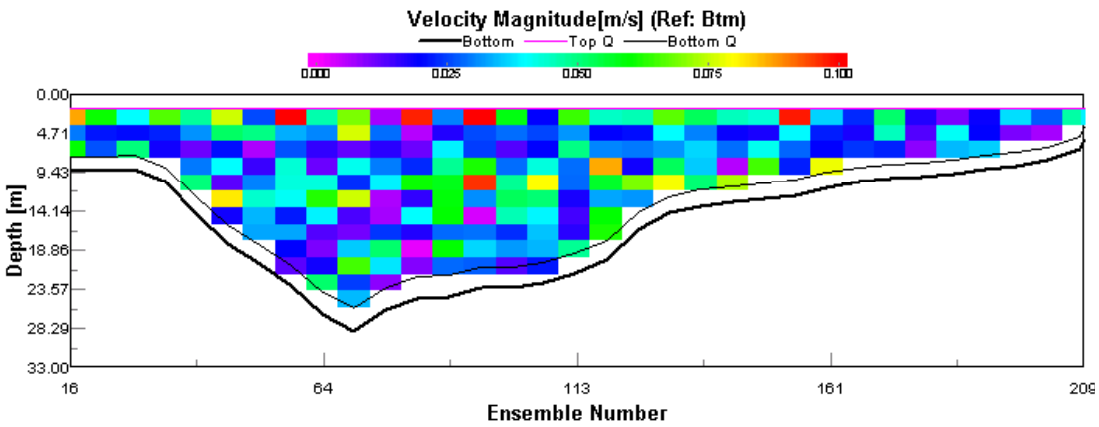


Figure 5-79
Surface Wind Current Effects During Breeze. September 2003 Survey, Transect 6

Discharge
Discharge measurements were frustrated by the extreme low flows. Discharge values measured in the lake channels follow some plausible trends. One additional source of discharge to be considered is the possible existence of submerged springs and seeps.

Summation of this discharge data for any purpose other than very general trend observations with this data is not advisable. Measured discharge values are not likely to be accurate due to several factors including:

- Variations in wind driven circulation on the same time scale as the period of the transect.
- Extremely low flow conditions.
- Eddy conditions with shifting centers of circulation on the same time scale as the period of the transect.

Temperature
Temperature data during the March, June, and September 2003 surveys showed strong thermoclines in areas of vertical multi-layer flow in the southern channels (**Figure 5-80**). Temperature profiles during the December survey, however, indicated that the lake channels were well mixed at all sites, and that a thermocline was not present (**Figure 5-81**).

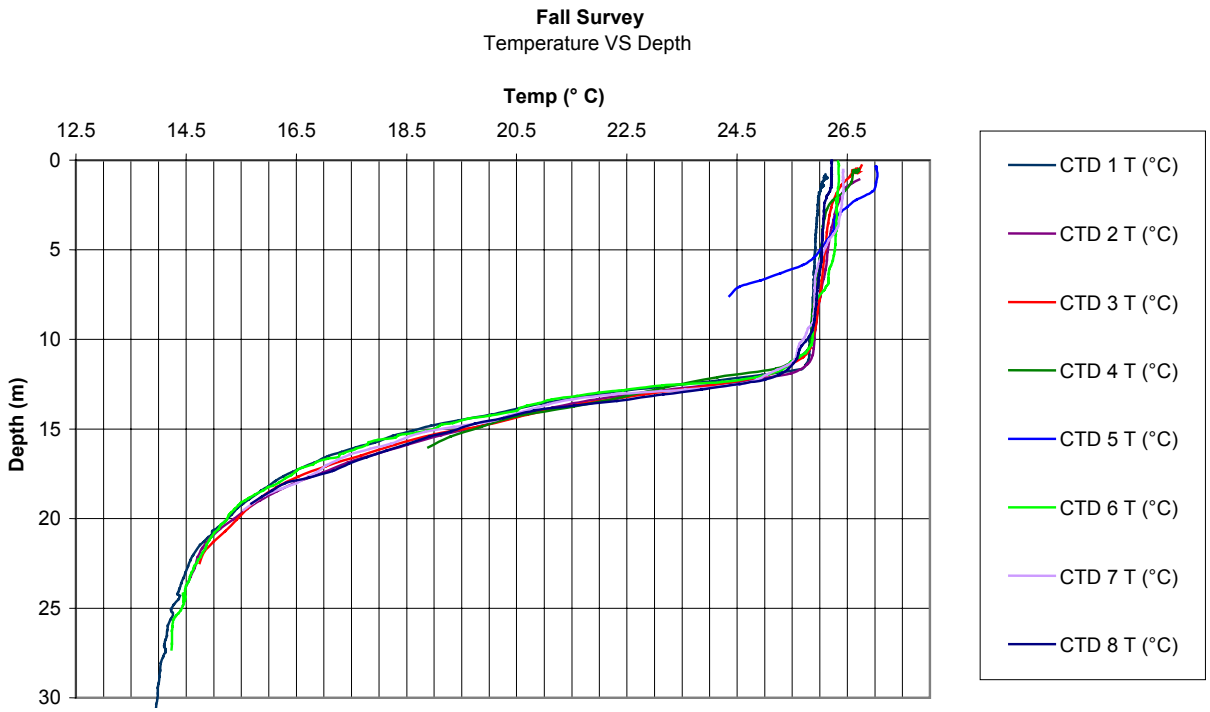


Figure 5-80
Temperature Profiles During the September 2003 Survey

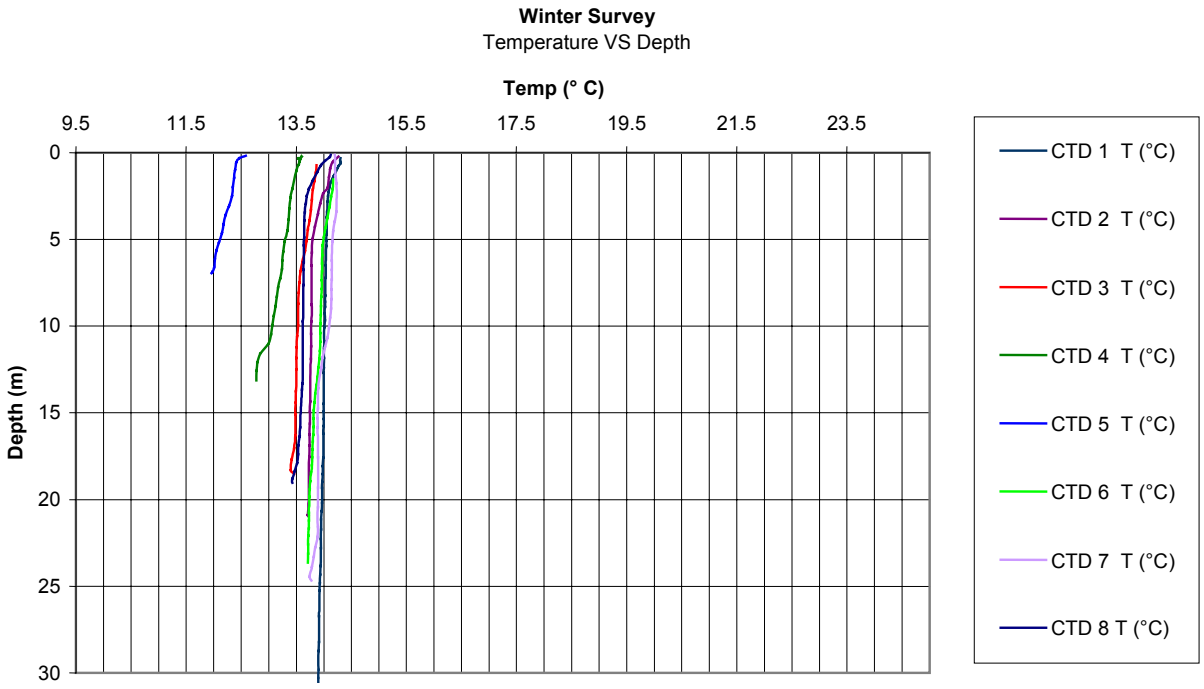


Figure 5-81
Temperature Profiles During the December 2003 Survey

Discussion Summary

For the ADCP Lake-Flow surveys, there is a limitation on the precision of accuracy for the data collected depending on natural lake conditions such as extremely low flows, eddy currents, wind conditions, and small sample volumes throughout a transect cross-section.

The seasonal surveys (spring, summer, fall, and winter) show the varying lake parameters (flow and temperature) throughout the year. Consistent preferential flow and current profiles were not identified during the study. Preferential flow and current profiles were more evident in the spring and summer surveys. Preferential flow and current profiles for the fall survey significantly decreased in comparison due to a lack of significant rain events throughout the summer. Adjacent landmasses were shown to cause the formation of numerous horizontally circulating eddies, which provides a mechanism for mixing the water column. The irregular shape and high banks of the lake tend to create wind-driven surface flow regardless of lakebed bathymetry or any suspected "down-stream" condition. Temperature profiles in the spring showed a well-mixed surface layer with a distinct thermocline between 2-m and 5-m deep at all transects. Summer surface temperatures for lakes and rivers were nearly 22° C warmer than during the spring. Strong thermoclines in the summer were observed at channel transects, at about an 8-m depth. Measurements north of transect 15 were well mixed with no thermoclines evident, possibly due to well-mixed thalwegs. Fall surface temperatures were nearly identical to the summer survey. The thermocline for the fall survey was deeper and more abrupt at 11-m. Measurements north of transect 15 in the fall were without evidence of a thermocline.

In conclusion, preferential flows along the old river channel have not been observed at all transects during any of the four surveys performed. Therefore, thalweg flow does not appear to be present as originally surmised in the CSM.

5.2 SEDIMENT

5.2.1 Perchlorate Occurrence in Sediment Pore Water

The project team sampled sediment pore water constituents to evaluate the presence and distribution of perchlorate in sediment pore water within streams and in delta areas of Lake Waco and Lake Belton. The various studies in which sediment pore water constituents were sampled are discussed in detail in the following sub-sections.

5.2.1.1 Stream Sediment Pore Water

5.2.1.1.1 Introduction

Pore water sampling in streams surrounding the NWIRP site is a vital facet of the overall exposure assessment. Pore water concentrations in sediment are both temporal and spatially variable. While bulk stream sampling is a vital means of exposure assessment, it represents a small snapshot of the overall exposure due to its dependence on source flow, dilution, and other variables. Further, it does not represent the exposure to sediment borne organisms or plants that derive their water from the sediment. Sediment pore water data collected can provide both high resolution vertical spatial analysis of perchlorate as well as seasonal variations in exposure. This information benefits the overall effort to determine the potential for exposure and trophic transfer.

5.2.1.1.2 Methodology

Seasonal sediment pore water sampling was conducted at multiple stream sites [Harris Creek at Highway 84 (T19,HW84 Mainstream), the spring on Oglesby Road (T18, HW84 Sidestream), Harris Creek at Highway 317 (T13, HW317), and S Creek at Highway 317 (T15, HW317/MN)] using peepers from May 2001 through October 2002. These locations are shown on **Figure 5-82**, and the sampling plan is summarized in **Table 5-14**. The specifically designed peepers were multi-chambered equilibrium dialysis samplers, each with length of 40-60 cm containing 13-22 chamber cells (**Figure 5-83**). Each chamber cell can hold 9 mL of water, with a center-to-center separation of 2 cm. The design is a modification of the original Hesslein peeper (Hesslein, 1976). Peepers were filled with de-ionized water, then covered with 0.45-µm pore size Tuffryn membrane (Pall Corporation).

The peepers were inserted into the saturated media by directly driving the peepers using a hammer to the desired depth with minimum disturbance to the sediment layer and allowed to equilibrate for a given period of time (2-4 weeks). In general, samplers were deployed to monitor up to 10 cm above the sediment-water interface and up to 20 cm below the sediment-water interface at 2-cm intervals. After the chambers equilibrated with the surrounding pore water, the peepers were removed and sampled by piercing the membrane with a clean syringe needle and withdrawing the water. Bulk water samples were taken at the time of peeper insertion and retrieval. Water samples were put on ice and then transferred to the laboratory for determination of a variety of anions including ClO_4^-, Cl^-, NO_3^-, NO_2^-, and SO_4^{2-}.

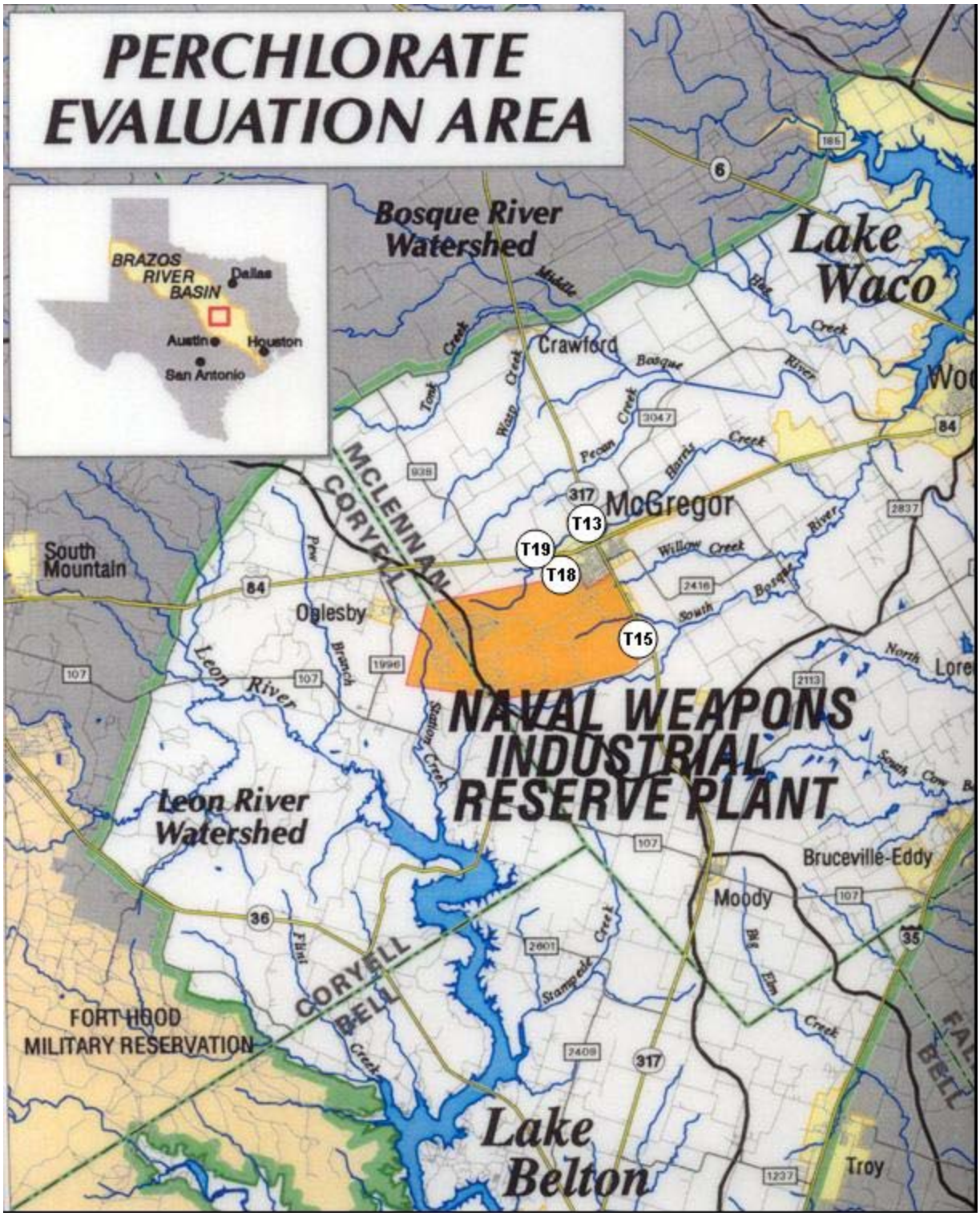


Figure 5-82
Map of Study Area Illustrating the Approximate Locations Where Sediment Pore Water Samples Were Collected

Table 5-14
Seasonal Sediment Pore-Water Sampling Plan at Multiple Sites Near NWIRP

Site	May	Aug.	Oct.		Jan.	April	June	Aug.	Oct.
	2001				2002				
HW84 Sidestream, T18	-	-	+		+	+	+	+	+
HW84 Mainstream, T19	+	-	+		+	*	+	+	+
HW317, T13	-	+	+		+	+	+	+	*
HW317/MN, T15	-	-	+		+	+	*	+	dry stream

+ sampled; - Not sampled; * Peeper was deployed but could not be found.

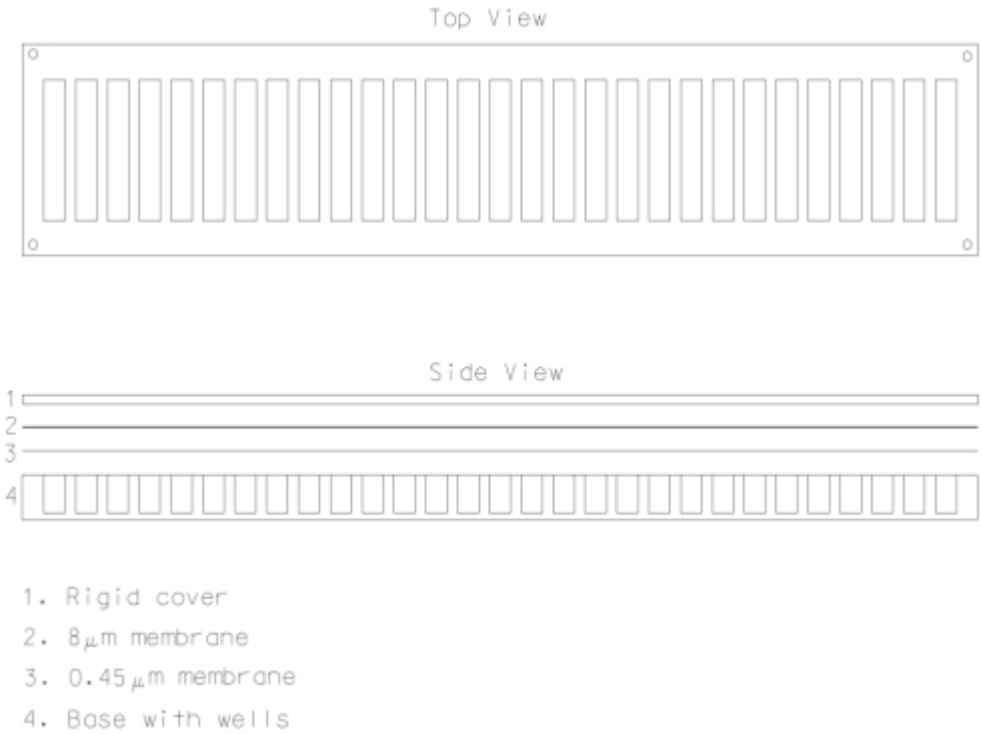


Figure 5-83
Schematic of the Peeper (Not to Scale)

A Dionex DX-500 ion chromatograph was used to determine perchlorate (ClO_4^-) and other anions (Cl^-, NO_3^-, NO_2^-, and SO_4^{2-}) in accordance with EPA Method 314.0 and 300.0, respectively. All samples were filtered with 0.45 µm syringe filter prior to the analysis. The method to determine perchlorate is described in **Appendix X**. The method to determine other anions (Cl^-, NO_2^-, NO_3^-, and SO_4^{2-}) uses IonPac AS14A column (4 x 250 mm), 8 mM Na_2CO_3/ 1mM $NaHCO_3$ eluent, 1.0 ml/min flow rate, and an anion atlas electrolytic suppressor (AAES) at 57 mA.

5.2.1.1.3 Data

HW84 Sidestream (T18) has a constant flowrate of approximately 1 gal/sec (324 m^3/day) year-around. This sampling location is approximately 200 m downstream from the head of the stream where there is a continuously flowing spring. This stream consistently contains perchlorate in the bulk stream water between 15 ppb and 30 ppb, with a high of 60 ppb observed in October, 2001 (**Figure 5-84**). In case of points not connected by the solid line in the figure, it means there are data missing either due to the broken cells or samples not tested. Results indicate that perchlorate was completely depleted at depths greater than 20 cm below the sediment-water surface for all months studied. Perchlorate and nitrate were degraded simultaneously. Chloride concentration was fairly constant in the sediment pore water, implying biodegradation instead of dilution was a major process resulting in the concentration decrease of other anions in the sediment pore water. Sulfate reduction did not start until preferential electron acceptors nitrate and perchlorate were depleted at depth -15 cm to -20 cm. There were large seasonal variations in profiles of all anions except chloride at this site.

To compare seasonal differences, perchlorate and nitrate concentrations, respectively, were plotted against sampling months in **Figure 5-85**. Bulk water nitrate concentrations in this stream were in the range of about 10 to 20 ppm (reported as NO_3^--N). Perchlorate penetration into bed sediments ranged from 0-20 cm with minimum penetration in summer (June) and greatest penetration in cold weather-months (October, January, and April). The temporal variation of perchlorate penetration might be linked with the seasonal variation of bacterial activity affected by temperature. Perchlorate distribution closely mirrored nitrate distribution in sediment pore water, and perchlorate degradation and nitrate degradation were observed almost simultaneously in an active degradation zone (with a varying depth of 5-10 cm), from about 10 cm below to 20 cm below the sediment-water interface.

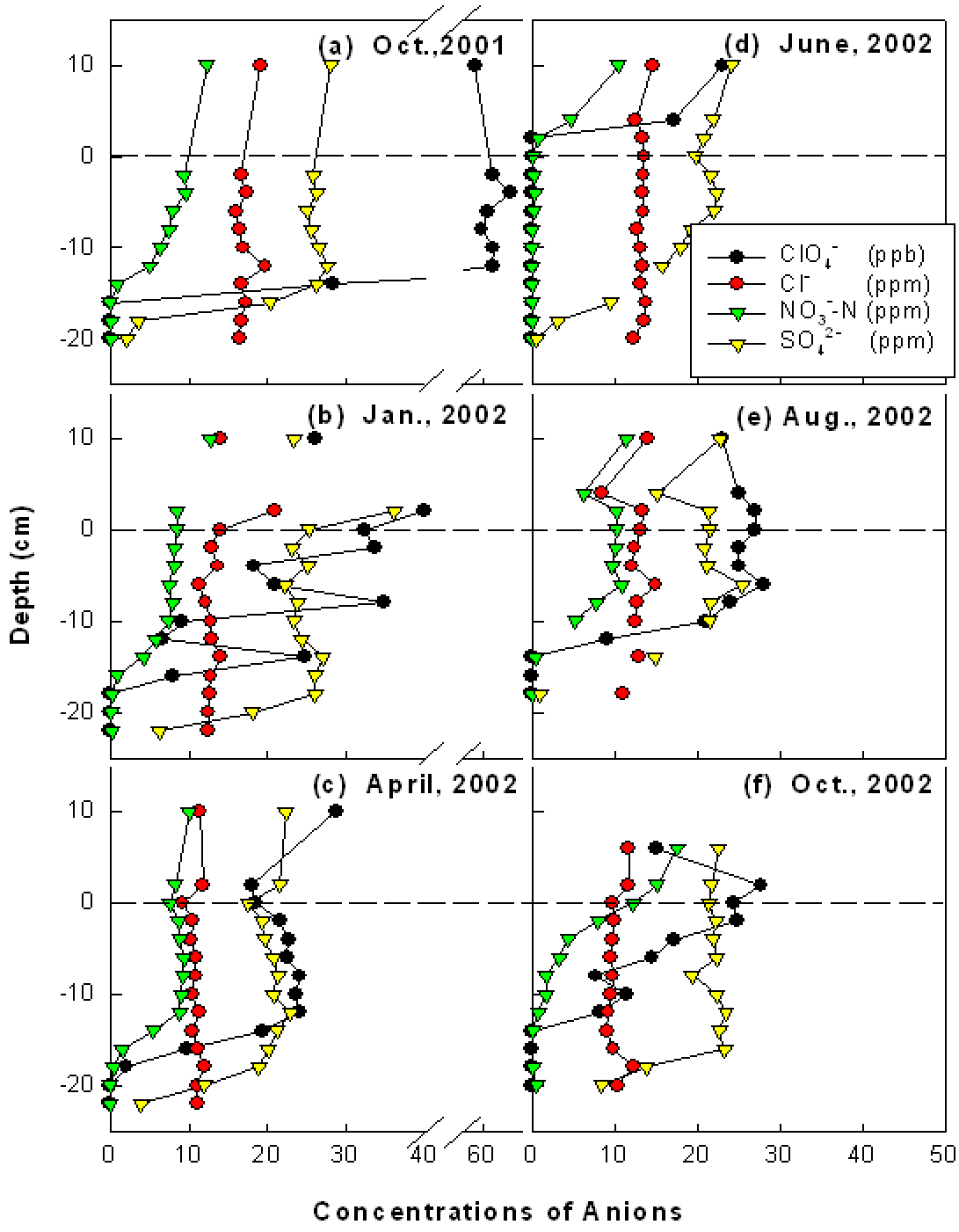


Figure 5-84
Profiles of Anions in Sediment Pore Water at HW84 Sidestream (T18) from October 2001 to October 2002

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

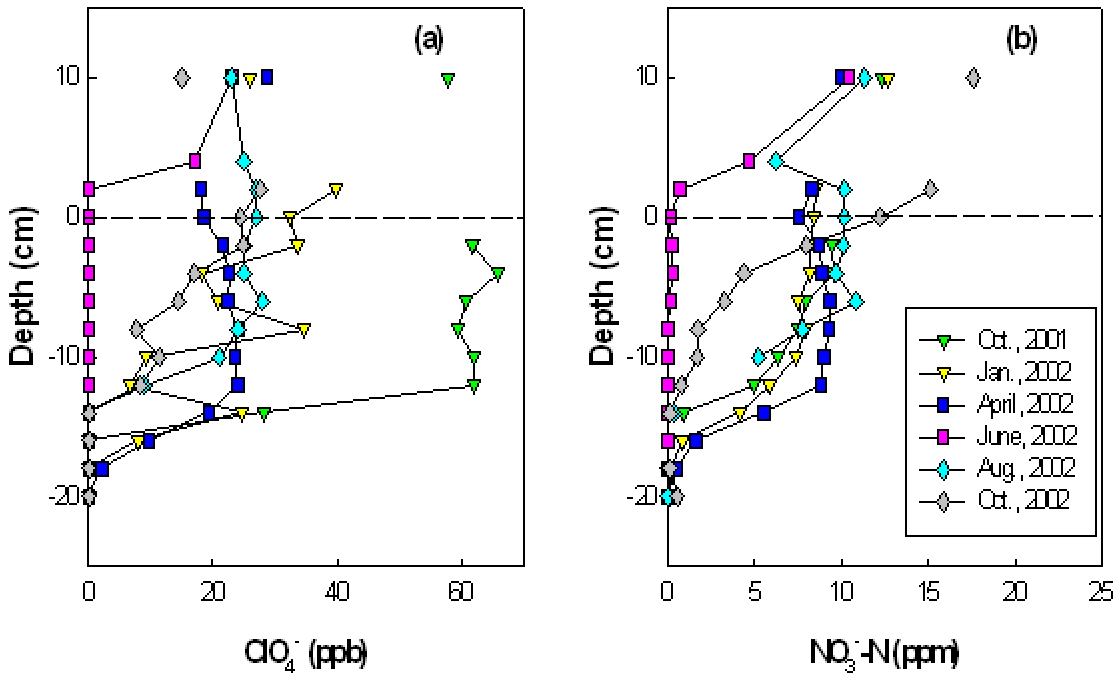


Figure 5-85
Seasonal Variation of Perchlorate and Nitrate Penetration in Sediment Pore Water at HW84 Sidestream (T18): (a) CLO_4^- vs. Depth; (b) NO_3^--N vs. Depth

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

The HW84 Mainstream (T19) site receives perchlorate-contaminated water from HW84 Sidestream. The water level in the stream varies dramatically following rainfall events or droughts (fluctuation 0.3-1.5 m yearly). Our results indicate rapid perchlorate degradation occurring in the sediment. Generally, perchlorate penetration in the sediment was not observed for all monitored months with the exception of the May, 2001 and January, 2002 sampling events (**Figure 5-86**). The reason for the perchlorate penetration in May, 2001 is not very clear, but may be linked with high concentration of groundwater discharge. Perchlorate was not degraded until a depth of 10 cm into the sediment in January 2002, which may have been due to slow bacterial degradation rate in cold weather conditions. Perchlorate and nitrate degradation followed similar patterns (**Figure 5-87**).

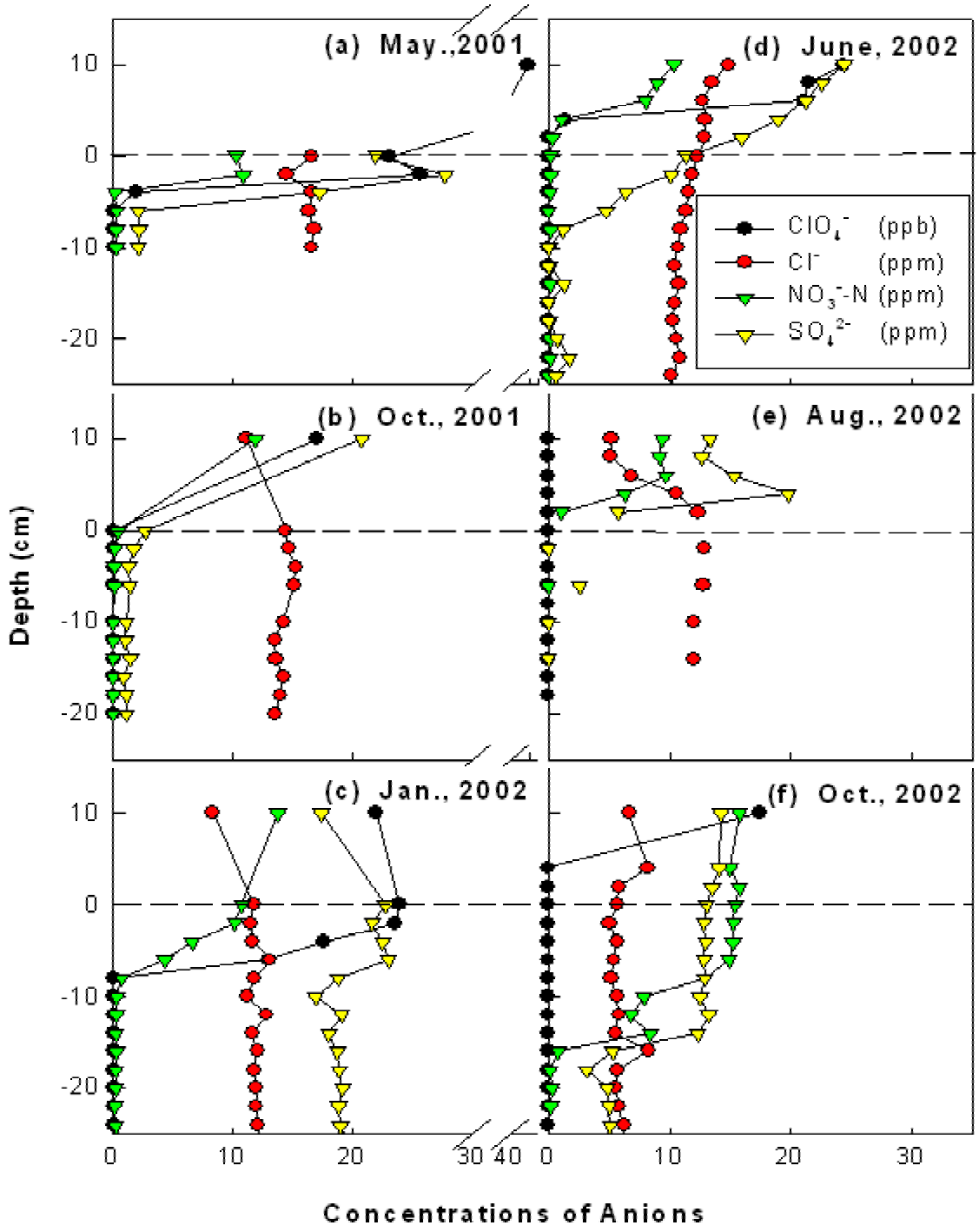


Figure 5-86
Profiles of Anions in Sediment Pore Water at HW84 Mainstream (T19) from May 2001 to October 2002

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

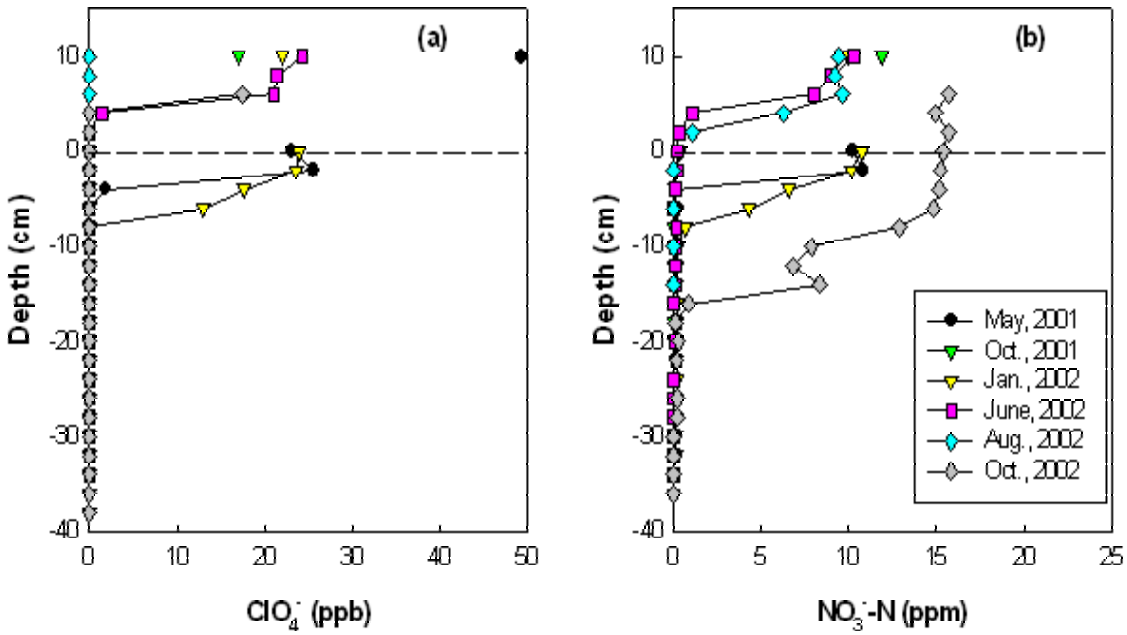


Figure 5-87
Seasonal Variation of Perchlorate and Nitrate Penetration in Sediment Pore Water at HW84 Mainstream (T19): (a) ClO_4^- vs. Depth; (b) NO_3^--N vs. Depth

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

The HW317 (T13) site is located downstream of HW84 Mainstream along Harris Creek. This site is a natural wetland habitat with vigorous growth of aquatic plants (smartweed and watercress). This natural wetland habitat is continuously flooded, with a water depth fluctuation of approximately 40-150 cm. Rapid perchlorate degradation occurred in all months monitored, probably resulting from enhanced rhizodegradation due to the availability of organic material in bottom sediments as well as uptake by aquatic plants.

Perchlorate bulk water concentration in the stream ranged from 0 to 15 ppb, which was slightly lower than that of HW84 Mainstream. Perchlorate penetration in sediment was not observed (**Figure 5-88**). Sulfate degradation occurred at depths of (2 to -2 cm), implying that this site had relatively higher organic substrate in the sediments compared to previous two sites, possibly due to rhizosphere exudates from the intensive plant growth at this site. Perchlorate degradation was also found to mirror nitrate degradation (**Figure 5-89**). Both perchlorate and nitrate degradation occurred at an active degradation zone (from 5 cm to 0 cm).

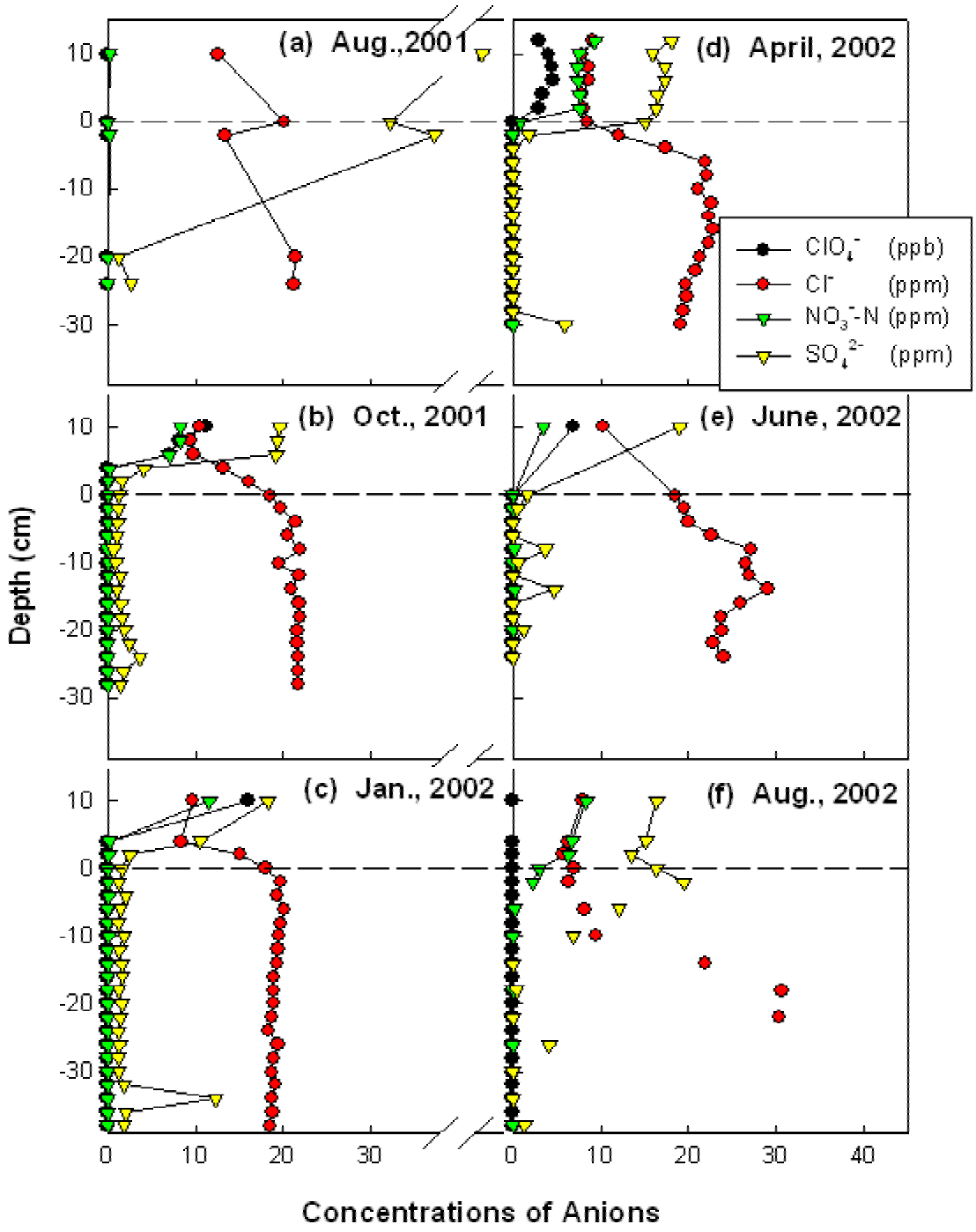


Figure 5-88
Profiles of Anions in Sediment Pore Water at HW317 (T13) from August 2001 to August 2002

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

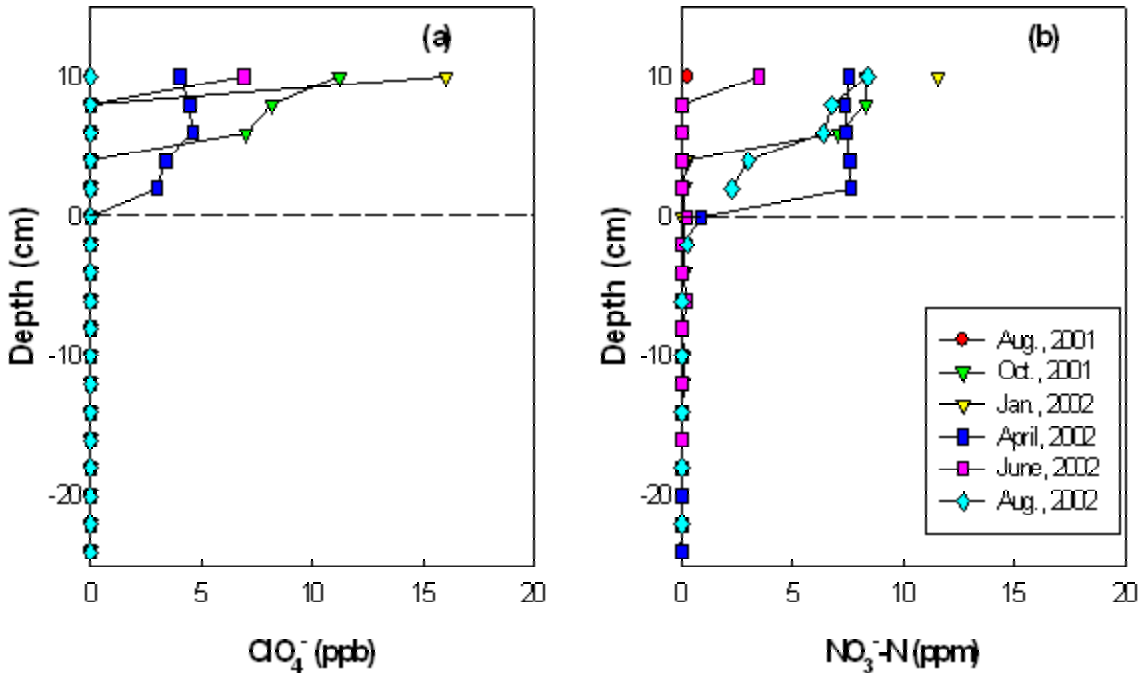


Figure 5-89
Seasonal Variation of Perchlorate and Nitrate Penetration in Sediment Pore Water at HW317 (T13): (a) ClO_4^- vs. Depth; (b) NO_3^--N vs. Depth

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

The HW317/MN (T15) site has the highest perchlorate bulk water concentration, with a range of 100 to 400 ppb. Perchlorate penetrated 10 cm below the sediment-water surface in most months monitored except in October 2001 (**Figure 5-90** and **Figure 5-91**). Perchlorate and nitrate degradation followed similar patterns as the HW84 Sidestream site (**Figure 5-91**). At depths from 10 cm above to 5 cm below the sediment-water interface, there was almost no obvious perchlorate degradation, When nitrate-N concentration was degraded to a level below approximately 10 mg/L (at about -10 cm), rapid perchlorate degradation occurred at an active zone of 1-10 cm, although the depth of this zone changed seasonally.

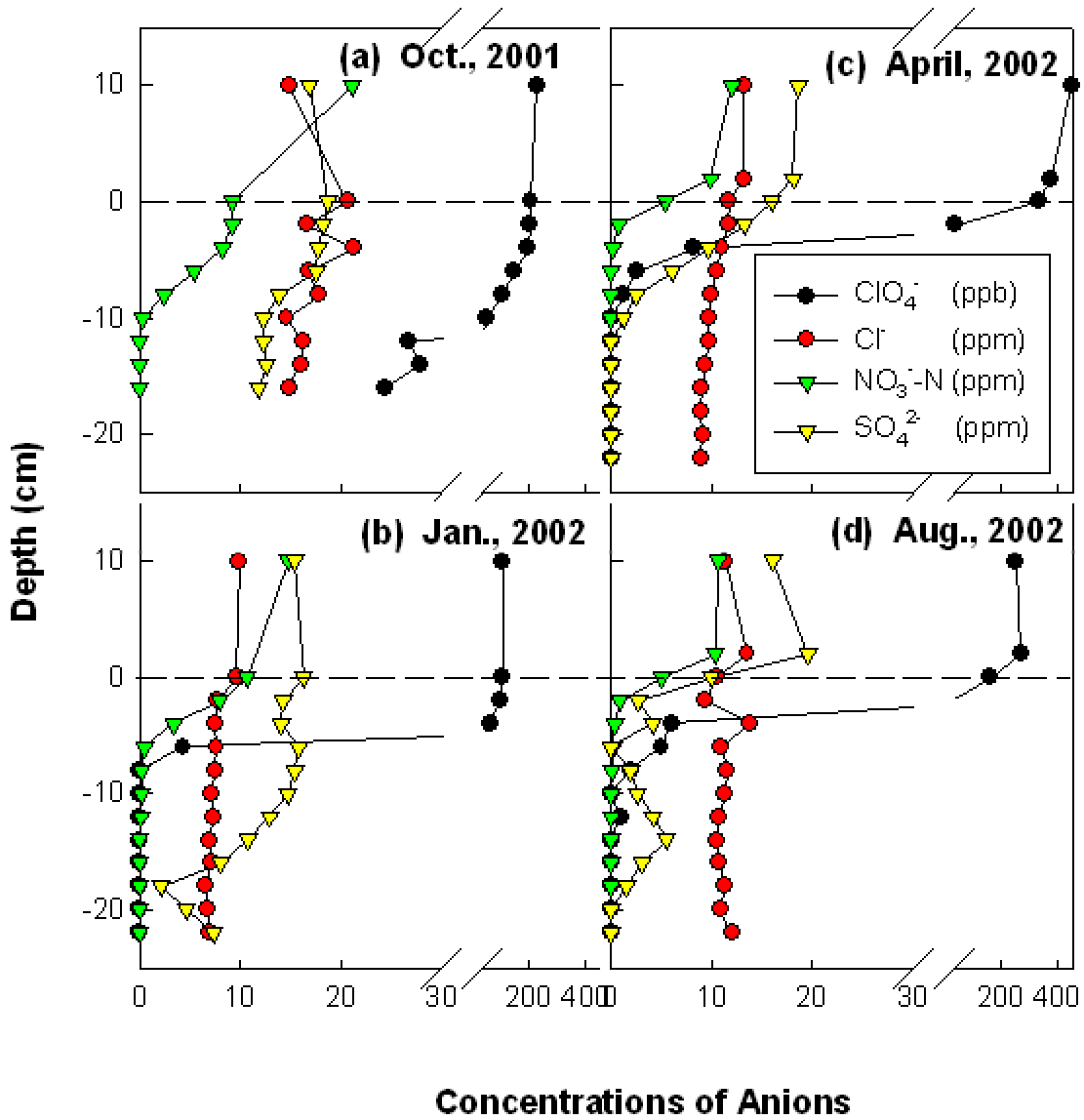


Figure 5-90
Profiles of Anions in Sediment Pore Water at HW317/MN (T15) from October 2001 to August 2002

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

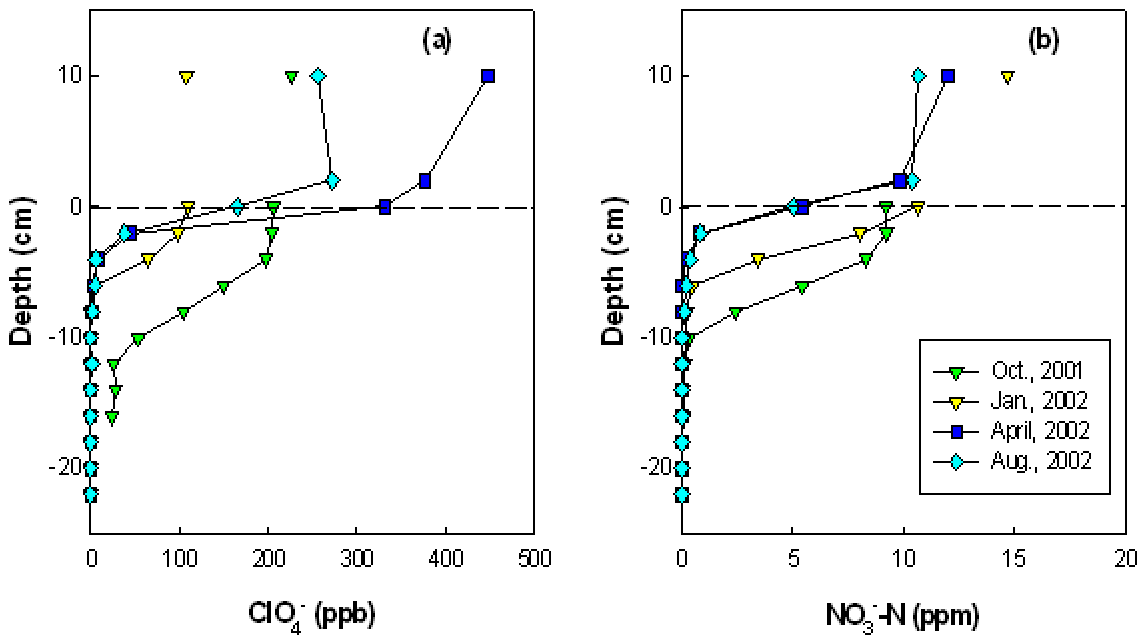


Figure 5-91
Seasonal Variation of Perchlorate and Nitrate Penetration in Sediment Pore Water at HW317/MN (T15): (a) ClO_4^- vs. Depth; (b) NO_3^--N vs. Depth.

(Dash lines represent the sediment-water interface. "+" represents depth above the sediment-water interface, and "-" represents depth below the sediment-water interface.)

5.2.1.1.4 Discussion

Perchlorate distribution and persistence in sediment pore water was spatially and temporally dependent, with variations for different streams. Generally, throughout the period of the one and half years of investigation, bulk water ClO_4^- concentration ranged from 0 µg/L to 30 µg/L for most streams, with up to approximately 400 µg/L in one of the streams. More fluctuation was observed for perchlorate concentration in bulk water than in sediment pore water. Significant seasonal variation was observed for those streams with fluctuating flowrate. Biodegradation of perchlorate can occur over a sediment depth of only 1-10 centimeters, although this active depth changes seasonally.

Perchlorate penetrated into sediments to a depth corresponding to the gradual depletion of nitrate, and faster perchlorate degradation was observed at sites with a deeper sediment layer and with vigorous wetland habitat. This study demonstrated a large capacity for natural attenuation in sediment pore water and the importance of nitrate as an indication of perchlorate degradation. It also highlights the need for spatial and seasonal studies involving exposure assessment. In general, perchlorate did not penetrate into bed sediments below 30 cm of the sediment-water surface due to the rapid microbial

degradation. However, perchlorate was generally present in stream water for most seasons. It suggests that exposure assessment should focus on the ecological receptors that may be exposed to stream water and sediment at a shallower depth. If proper environmental conditions exist for the proliferation of perchlorate-reducing bacteria, such as temperature, anaerobic condition, organic substrate, moisture, and nitrate levels, sediments (30 cm below the water surface) would not become a major concern for perchlorate contamination.

5.2.1.2 Delta Areas Sediment Pore Water

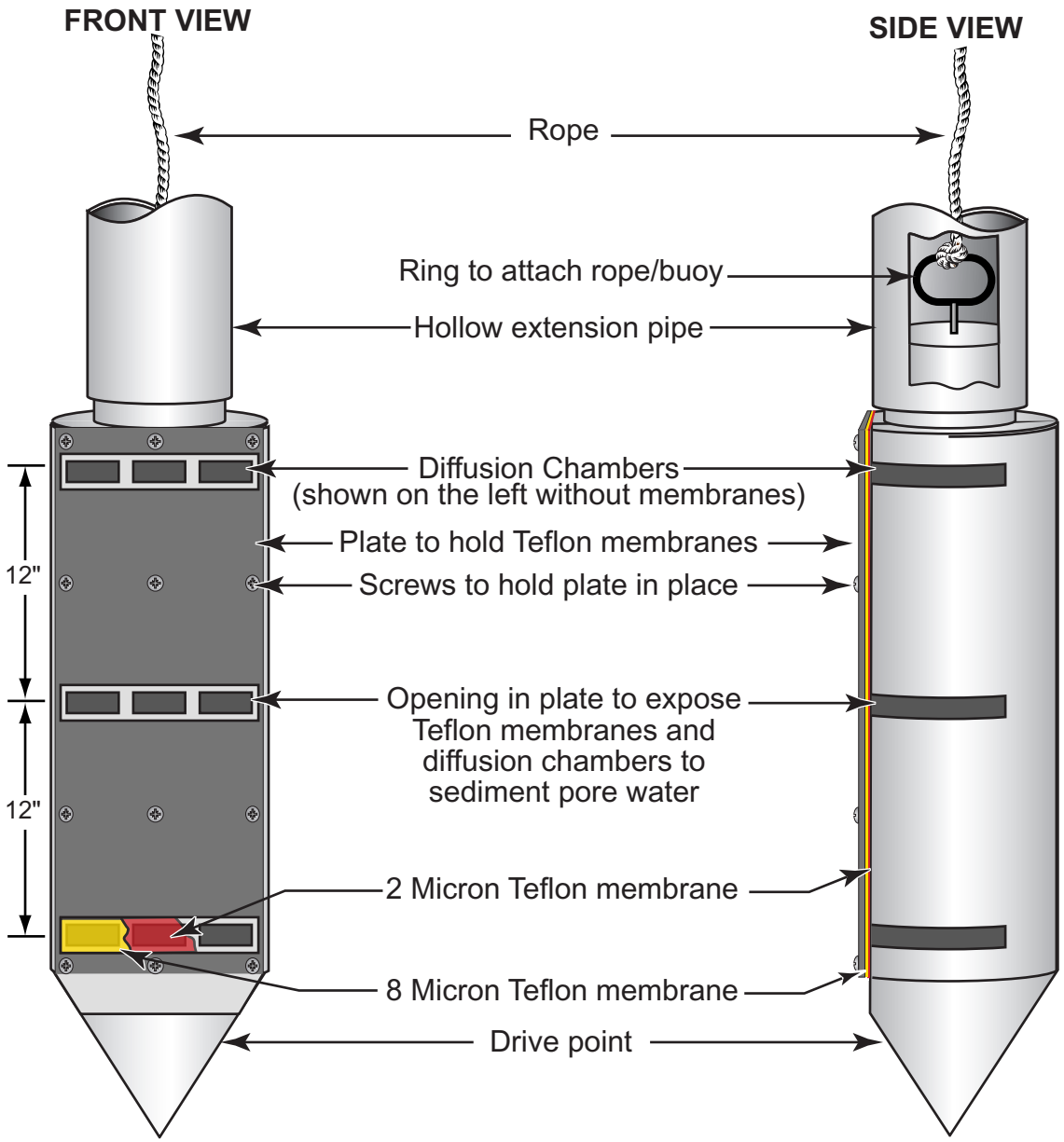
5.2.1.2.1 Introduction

Sediment pore water samples were collected from within the delta areas of Lake Waco and Lake Belton to evaluate the presence and distribution of perchlorate in sediment pore water. This sampling effort was conducted in conjunction with surface water sampling activities performed in these areas as previously described in Section 5.1.2.1, and sediment pore water samples were collected from the same delta area grid sampling locations established for the surface water sampling. The actual GPS coordinates of the sampling locations are documented in **Table 5-3** and actual sampling locations are shown in **Figure 5-18** and **Figure 5-19** in Section 5.1.2.1. This portion of the study was conducted as part of the Delta Areas Study. All of the methodologies followed are detailed in the *Final Lake Belton and Lake Waco Delta Areas Field Sampling Plan* (MWH, 2002c). Any deviations from this plan are discussed further below.

5.2.1.2.2 Methodology

Sediment pore water samples were collected using diffusion samplers (also known as peepers). TIEHH constructed the peepers that were used for this portion of the study. The peepers consisted of a stainless steel or Teflon drive stake with three columns and three rows of diffusion chambers or sample ports bored into the stake. The diffusion chambers were filled with de-ionized water and covered with two Teflon membranes (one 8 micrometers [µm] thick and the other 0.2 µm thick) to allow sediment pore water dissolved constituents to diffuse into the three chambers while keeping the sediments out. The Teflon membranes were held in place with a perforated plate that screws into the drive stake. A drawing showing a typical diffusion sampler is shown in **Figure 5-92**.

The sediment pore water samples were obtained by pushing the peepers into the lake bottom. The peepers were installed by attaching a rope to the top of the peeper and threading the rope through hollow extension pipes that have a diameter slightly smaller than the drive stake of the peeper. For the purposes of this study, the diffusion chambers were spaced along the drive stake such that samples were obtained at three different depths for each sample location: 1) just below the lake water/ sediment interface, 2) one foot below the lake water/sediment interface, and 3) two feet below the lake water/sediment interface. Tension was maintained on the rope to keep the peeper in contact with the lead extension pipe. The extension pipes were approximately 5 feet in length and either had threaded ends or quick-connects to allow additional lengths of pipe to be attached as the sampler was lowered through the water column. Once resistance was felt as a result of the peeper contacting the lake bottom, the peeper was pushed or driven with a slide hammer until the bottom diffusion



U.S. Army Corps of Engineers	
PROJECT: BOSQUE AND LEON RIVER WATERSHEDS STUDY	
DRAWING TITLE: SEDIMENT PORE WATER SAMPLER (PEEPER)	
	Sheet 1 of 1 sheets
	SCALE: No Scale / FIGURE: 5-92

chamber reached the desired sampling depth (i.e., 2 feet below the sediment/lake water interface). The field team tested the depth to sediment at each sample location with the extension pipes or a weighted probe prior to actually setting the peeper to assure that the samplers were properly installed.

After the peepers were pushed or driven to the desired depth, the extension pipes were retracted, leaving the peeper in place. The rope extending from the top of the drive stake was attached to a buoy to allow for locating and retrieving the peeper. The de-ionized water in the peepers was allowed to equilibrate with the adjacent sediment pore water for a period of two weeks. After the peepers were retrieved, the samples were extracted from the diffusion chambers with a clean syringe and transferred into an appropriate sample container. Each diffusion chamber was capable of holding a minimum of 20 milliliters of de-ionized water, which provided an adequate volume of water for the laboratory to perform the perchlorate analyses. Photographs of installation of peepers and extraction of the sample using a syringe are shown in **Figure 5-93**, **Figure 5-94**, and **Figure 5-95**.

Figure 5-93
Installation of Sediment Pore Water Sampler

Figure 5-94
Installation of Sediment Pore Water Sampler

Figure 5-95
Retrieval of a Sediment Pore Water Sample

Duplicate Samples. Blind duplicate and equipment blank samples were collected at a frequency to represent 10% of the environmental samples collected, and MS/MSD samples were collected at a frequency to represent 5% of the environmental samples collected. The samples were labeled, handled, and shipped according to the procedures described in the QAPP (MWH, 2002e).

The locations of the sampling grid points were documented using a Garmin GPS 76 instrument. The GPS information recorded at each grid point included latitude (degrees and minutes) and longitude (degrees and minutes).

Sample Designation. The sediment pore water samples were designated similarly to the surface water samples collected at the delta area grid locations except that the lake name abbreviation and grid point number was followed by SPW to indicate a sediment pore water sample, and finally the depth from which the sample was obtained. For example the sediment pore water sample collected from just below the lake water/sediment interface at Lake Waco grid point number 1 was designated "LW1-SPW-0' ".

Blind duplicate sediment pore water samples were designated with a fictitious number so the laboratory would not know where the sample was collected. For example, the first sediment pore water blind duplicate sample was designated "SPW-1001". The field crew kept careful records of the designations given to the blind duplicate samples and their corresponding environmental sample so that the analytical results could be correlated with the field locations. Each MS/MSD sample had the same designation as its associated environmental sample except that "MS" or "MSD" followed the sample designation (e.g., "LW1-SPW-0' MS" and "LW1-SPW-0' MSD"). Each equipment blank sample had the same designation as its associated sampling location except that "EB" followed the sample designation (e.g., "LW1-SPW-0'-EB").

Sample Analysis. All sediment pore water samples were analyzed for perchlorate by USEPA Method 314.0 at the USACE Engineer Research and Development Center Environmental Laboratory at the Environmental Chemistry Branch in Omaha, Nebraska (See **Appendix V**). The USACE laboratory conformed to the analytical method requirements, analytical quality control requirements, instrument calibration frequency, and the laboratory quality control requirements presented in the QAPP (MWH, 2002e). The data verification report for samples analyzed by the USACE laboratory is included in **Appendix W**.

5.2.1.2.3 Data

A total of 63 sediment pore water samples including six duplicate samples were collected from 19 sample locations within Lake Belton. Sixty-five sediment pore water samples including six duplicate samples were collected from 20 sample locations within Lake Waco. All of the water quality and temperature data collected from the lake delta areas are included for reference in **Appendix H**. Perchlorate was not detected in any of the sediment pore water samples collected from the lake delta areas (method detection limit

of 1 µg/L). The sediment pore water perchlorate sampling results for each lake are included in **Appendix S**.

Perchlorate was not detected in the equipment blank samples analyzed for either lake. Perchlorate was also not detected in the investigations derived waste (IDW) samples collected from Lake Belton. No IDW was generated from the sediment pore water sampling in Lake Waco, since the sediment pore water samplers were not reused. These results are also included in **Appendix S**.

5.2.1.3 Historical Data

5.2.1.3.1 Introduction

As part of the Phase II Groundwater Investigation at NWIRP McGregor, the U.S. Navy assessed water quality within Lake Belton and Lake Waco as previously described in Section 5.1.2.4. The lake assessment approach is presented in the *Lake Water Quality Assessment Work Plan* (EnSafe, 2000a). This investigation was designed to complete a thorough, yet expedited, environmental assessment of perchlorate occurrence in the lakes and produce data that could be used to assess risk to human health and the environment. EnSafe conducted two sampling events, one in spring 2000 and the other in summer 2000. The spring 2000 sampling event was conducted to assess the lakes under cool weather conditions, while the summer 2000 sampling event was conducted to assess the lakes under warm weather conditions. It should be noted that U.S. Navy sampled bulk sediment, not sediment pore water directly. Therefore, historical results from the U.S. Navy are not necessarily comparable to the sediment pore water samples discussed above.

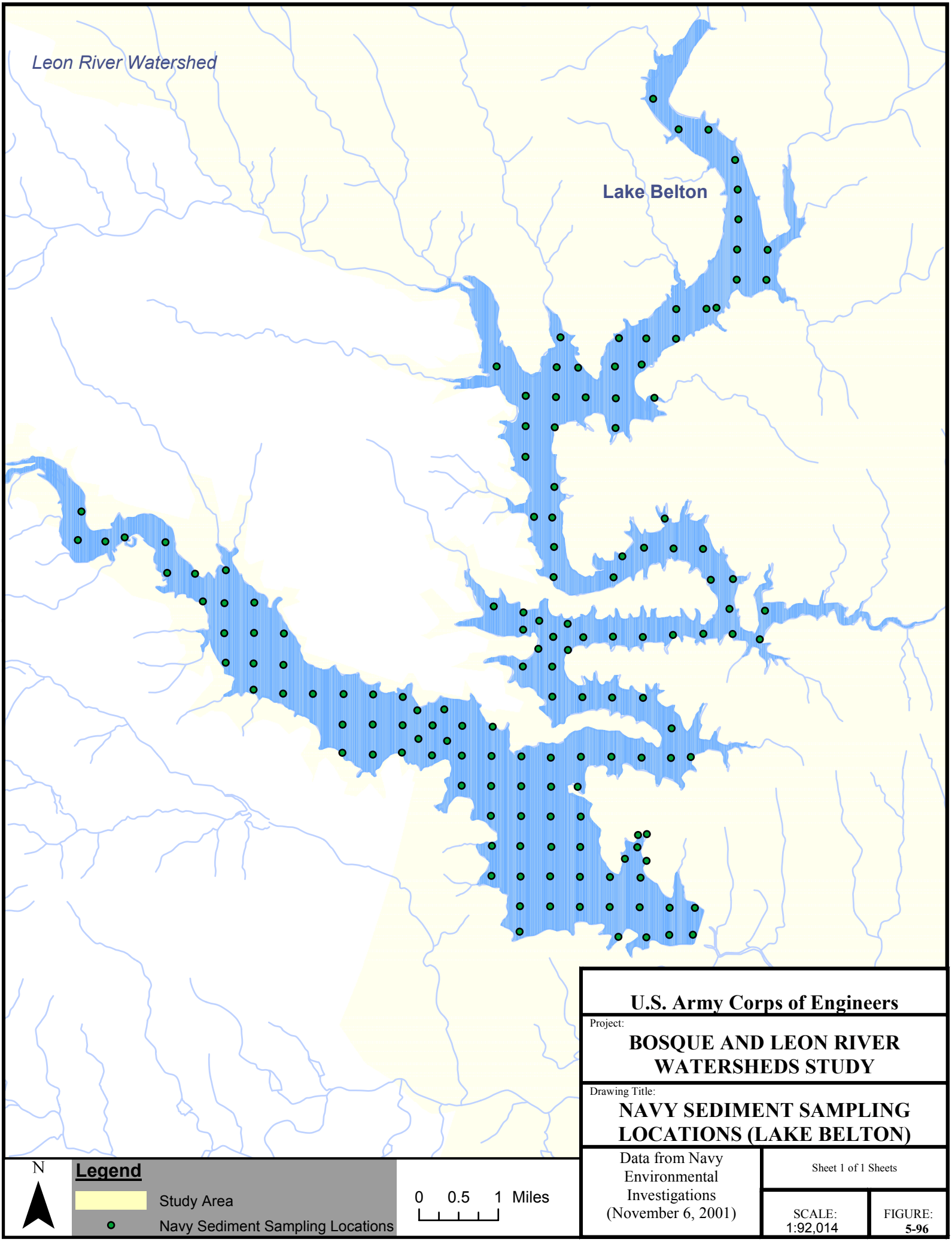
5.2.1.3.2 Methodology

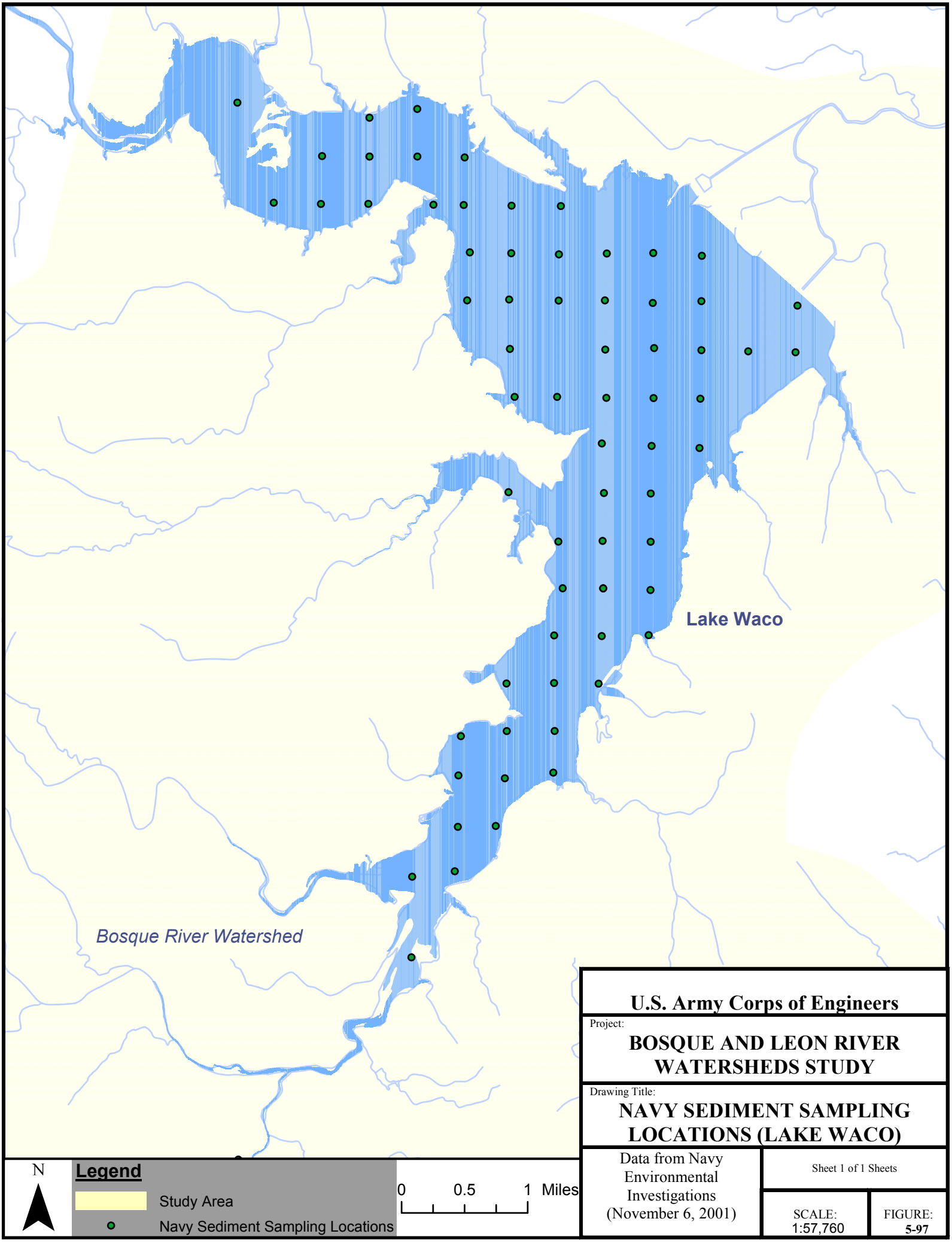
The overall approach, sampling techniques, and methods used for lake assessments are described in detail in the *NWIRP McGregor Final Groundwater Investigation Work Plan* (EnSafe, 1998b). Both the spring and summer sampling events utilized the same field protocols.

A 2,000-foot by 2,000-foot sampling grid was established in each lake as presented in **Figure 5-96** and **Figure 5-97**.

During the spring 2000 sampling event, one sediment sample was collected at each of the grid sampling locations. TCEQ authorized the reduction of lake-wide sediment sampling during the Summer 2000 sampling event due to the absence of perchlorate in nearly all cool water sediment samples, and sampling was conducted only at locations where perchlorate was previously detected.

All the sediment samples were collected using a petite ponar clam-shell grab sampler developed by a deck-mounted davit, pulley, and power windlass winch system, following the methodology outline in American Society for Testing and Materials (ASTM) D 4342-84:1993 (ASTM, 1997).


Leon River Watershed
Lake Belton
N
Legend
Study Area
Navy Sediment Sampling Locations
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SEDIMENT SAMPLING LOCATIONS (LAKE BELTON)
Data from Navy Environmental Investigations (November 6, 2001)
Sheet 1 of 1 Sheets
SCALE: 1:92,014
FIGURE: 5-96


Lake Waco
Bosque River Watershed
N
Legend
Study Area
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U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SEDIMENT SAMPLING LOCATIONS (LAKE WACO)
Data from Navy Environmental Investigations (November 6, 2001)
Sheet 1 of 1 Sheets
SCALE:
1:57,760
FIGURE:
5-97

5.2.1.3.3 Data

Detailed observations and findings made during both the sampling events are provided in the *Final Draft Report Lake Water Quality Assessment* (EnSafe, 2000b). A summary discussion of these findings is presented below.

A total of 138 sediment samples were collected from 141 sample locations in Lake Belton during the spring 2000 sample collection event. In the summer event, a total of 15 sediment samples were submitted from 15 locations. A total of 69 sediment samples were collected from 78 sample locations in Lake Waco during the spring 2000 sample collection event. No sediments were collected from Lake Waco during the summer event.

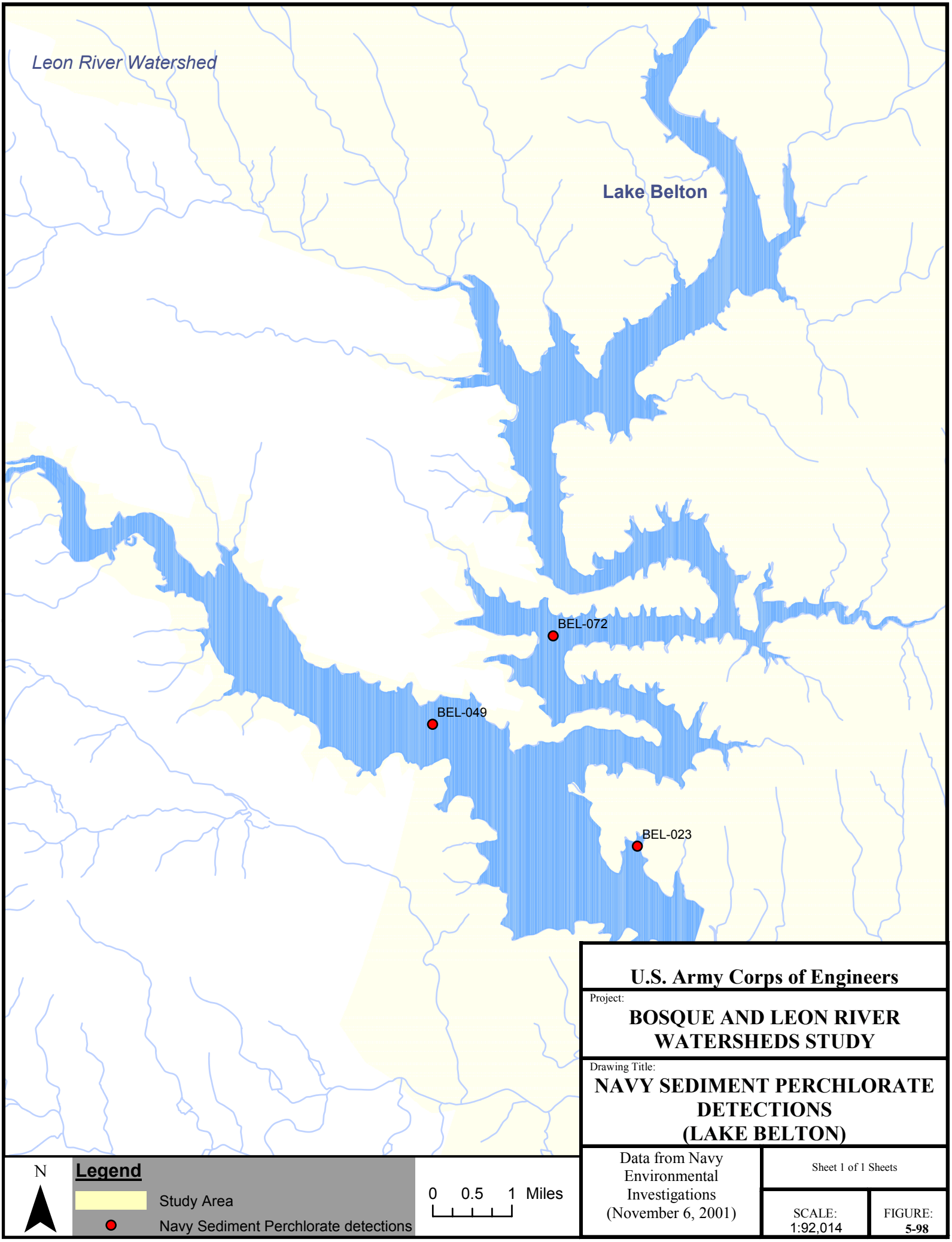
The following briefly describes the laboratory analytical results for both the lakes.

- **Spring 2000 (cool water sediment)** – No perchlorate was detected in any of the 69 sediment samples collected from Lake Waco. The laboratory detection limits for sediment samples ranged from 0.097milligrams/kilogram (mg/kg) to 0.28 mg/kg, averaging 0.20 mg/kg. Perchlorate was detected in three sediment samples in Lake Belton (See **Figure 5-98**). Concentrations ranged from 0.13J mg/kg to 0.54 mg/kg (J = laboratory code from estimated concentration).
- **Summer 2000 (warm water sediment)** – No perchlorate was detected in the 15 confirmatory sediment samples from Lake Belton. Laboratory detection limits for the sediments ranged from 0.10 mg/kg to 0.34 mg/kg, averaging 0.20 mg/kg.

Of all the 222 lake sediment samples collected during both the events, only three had very low detects, which represents 1.3% of the samples. They were non-detect on re-sampling. The analytical data produced by this investigation are included in *the Final Draft Report Lake Water Quality Assessment* (EnSafe, 2000b).

The U.S. Navy concluded that there was no apparent distribution pattern for either the detected perchlorate concentrations in Lake Belton sediment during the spring sampling event or the Station Creek/NWIRP McGregor area of the Leon River. Spatially, the perchlorate detects were separated by miles with numerous non-detect samples between them. There was one 0.54 mg/kg perchlorate detect one-half mile east of the City of Gatesville raw water intake.

The U.S. Navy also conducted sediment sampling for perchlorate within NWIRP McGregor in 2000 and in tributaries adjacent to the facility. Locations of sediment sample collection are shown in **Figure 5-99**. Concentrations of perchlorate below 1 mg/kg were detected in one sediment sample collected from Tributary M (SDST-3), in two sediment samples collected from the South Bosque River (SDSB-2 and SDSB-3) and within NWIRP (SD8PAS-5). The locations of these samples are shown in **Figure 5-100**. Reporting limits were below 0.25 mg/kg in the majority of sediment samples collected within the streams.



U.S. Army Corps of Engineers

Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY

Drawing Title:
NAVY SEDIMENT PERCHLORATE DETECTIONS (LAKE BELTON)

Data from Navy Environmental Investigations (November 6, 2001)

Sheet 1 of 1 Sheets

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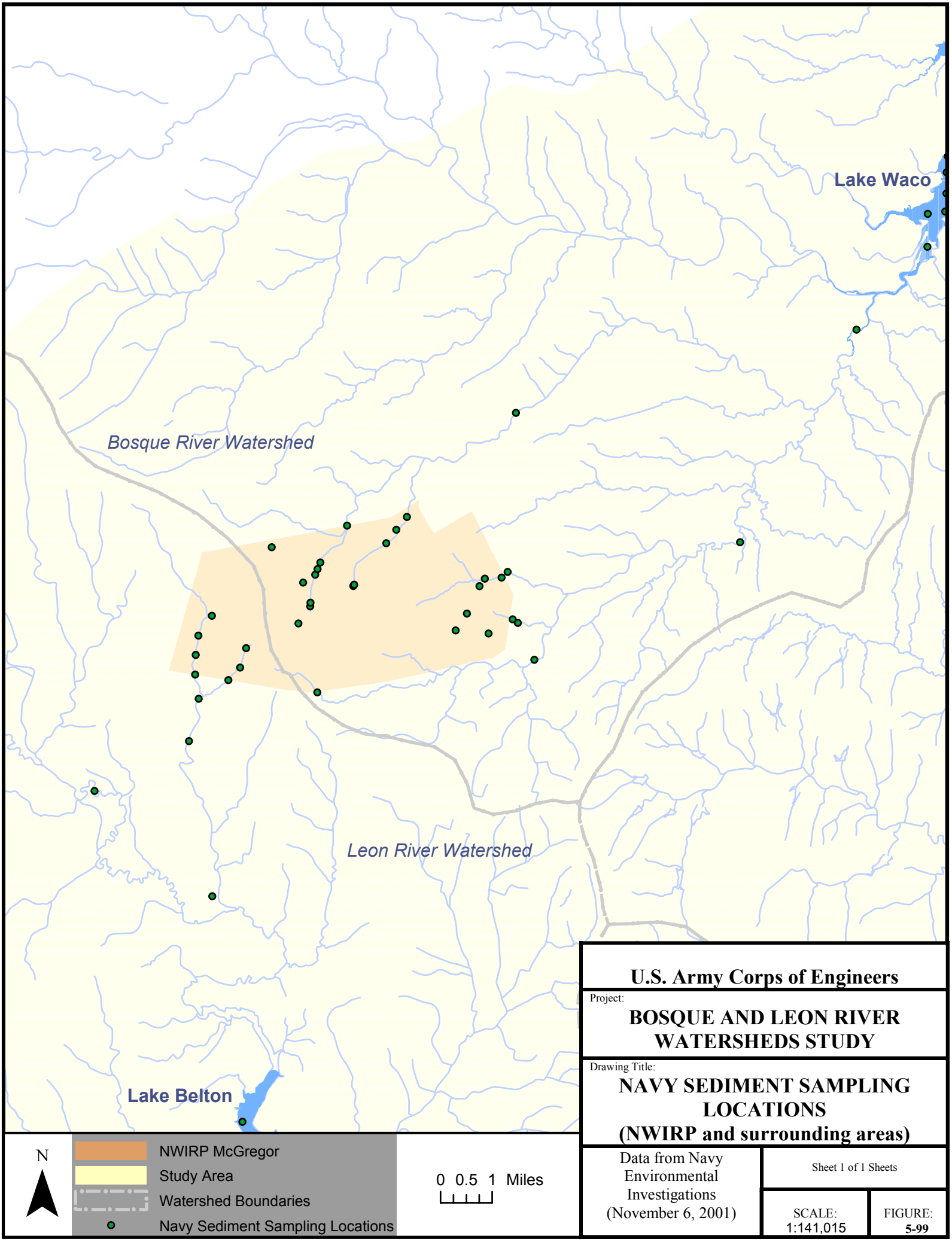
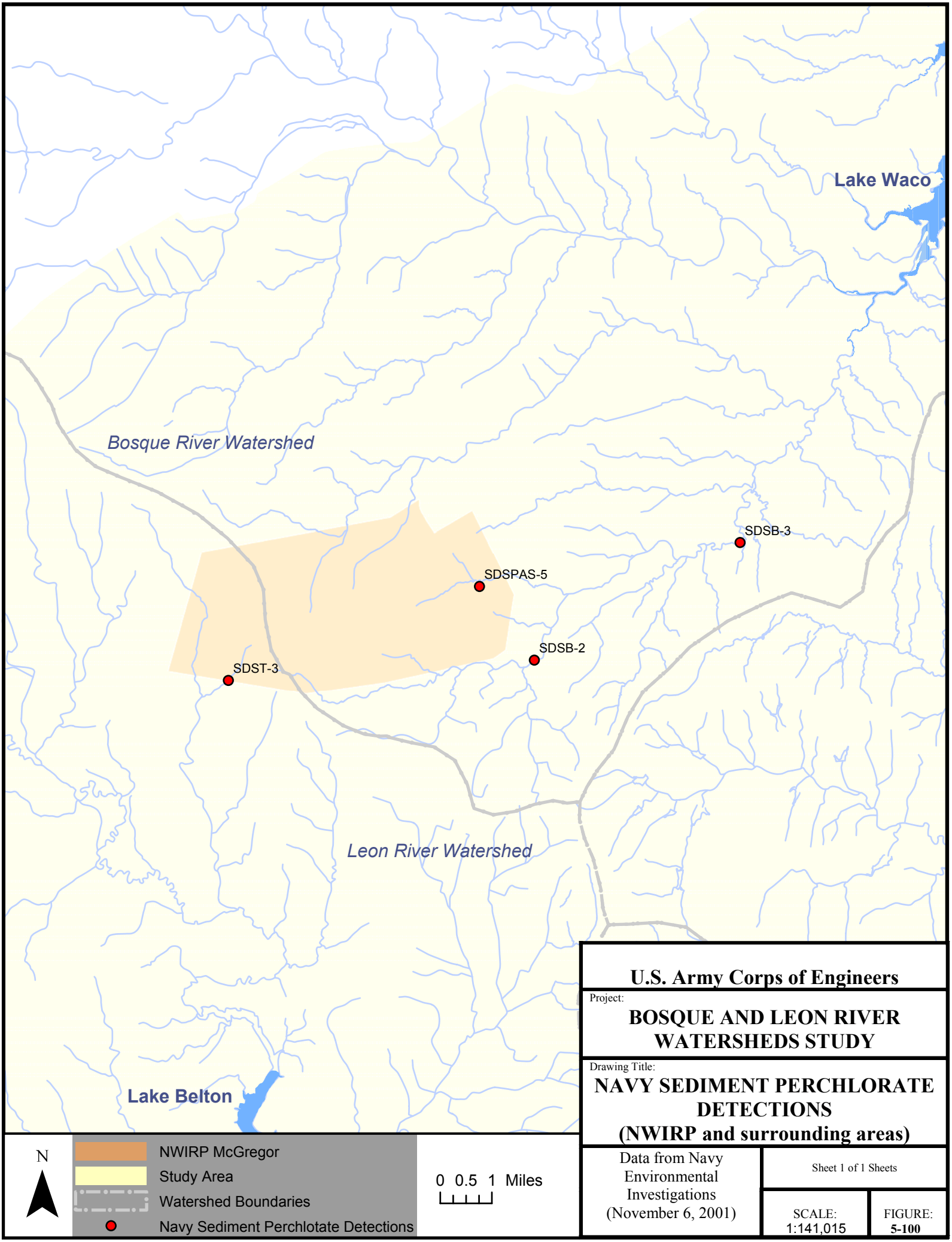
FIGURE: 5-98


Lake Waco
Bosque River Watershed
Leon River Watershed
Lake Belton
N
NWIRP McGregor
Study Area
Watershed Boundaries
Navy Sediment Sampling Locations
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SEDIMENT SAMPLING LOCATIONS
(NWIRP and surrounding areas)
Data from Navy Environmental Investigations (November 6, 2001)
Sheet 1 of 1 Sheets
SCALE:
1:141,015
FIGURE:
5-99


Lake Waco
Bosque River Watershed
SDSB-3
SDSPAS-5
SDSB-2
SDST-3
Leon River Watershed
Lake Belton
N
NWIRP McGregor
Study Area
Watershed Boundaries
Navy Sediment Perchlotate Detections
0 0.5 1 Miles
U.S. Army Corps of Engineers
Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY
Drawing Title:
NAVY SEDIMENT PERCHLORATE DETECTIONS
(NWIRP and surrounding areas)
Data from Navy Environmental Investigations (November 6, 2001)
Sheet 1 of 1 Sheets
SCALE: 1:141,015
FIGURE: 5-100

5.2.1.4 Discussion

Perchlorate, if it were reaching the lakes, would likely be found in the delta areas, as these areas receive direct discharge from the Bosque and Leon River watersheds and have the greatest sediment deposition in each lake. However, no perchlorate was detected in the delta area sediment pore water sampling conducted by the project team. In addition to all the results discussed above, the U.S. Navy had a detailed 2,000-foot by 2,000-foot sample grid. Three sediment samples collected by the U.S. Navy on the Lake Belton grid had perchlorate detections, but according to the U.S. Navy, these three samples had no apparent distribution pattern. It should also be noted that the U.S. Navy collected and analyzed bulk sediment samples, as opposed to sediment pore water samples. Although the collection of bulk sediment samples is valid, pore water concentrations are generally more sensitive for highly water-soluble chemicals (USEPA, 1999). Sediment criteria or screening benchmarks are not currently available for perchlorate.

5.2.2 Anoxic Study

5.2.2.1 Introduction

In an effort to assist the USACE in developing a better understanding of the possibility of natural perchlorate reduction within Lake Belton, the project team performed a series of microecology experiments to determine the potential for natural perchlorate bioreduction within the lake. These tests explored the potential for the microorganisms (i.e.. bacteria) naturally present in both the water column and sediment at the water/sediment interface to metabolize perchlorate.

The ability for dissimilatory perchlorate and chlorate reduction is widespread among bacteria in the natural environment. Bacteria that practice dissimilatory perchlorate reduction do so as part of the process to gain energy that they can use for growth and maintenance. In this process, oxygen ions are successively stripped from perchlorate until only chloride remains in solution. By-products of this process include the development of biomass and the production of carbon dioxide. A simplified conceptual equation for the biological reduction of perchlorate using acetic acid as the electron donor is:

$$ClO_4^- + CH_3COOH \rightarrow Cl^- + 2CO_2 + 2H_2O$$

In the above reaction, perchlorate is the electron acceptor, gaining eight electrons, as it is reduced to chlorate (ClO_3^-), chlorite (ClO_2^-), and finally to chloride (Cl^-). In this same reaction, acetic acid is the electron donor, supplying eight electrons, as it is oxidized to carbon dioxide.

Oxygen, nitrate and sulfate may also be present in natural waters that contain perchlorate. If all these electron acceptors are present, the prevailing order of biological preference is:

Oxygen > Nitrate > Perchlorate > Sulfate

In other words, if oxygen is present, the rates of biological reduction of nitrate and perchlorate are usually reduced. Once oxygen is depleted, the biological reductions of nitrate (i.e., denitrification) and perchlorate are greatly improved. However, the reduction of perchlorate to chloride is typically impaired by the presence of nitrate. Once these electron acceptors have been depleted, biological reduction of sulfate is generally initiated.

The actual order that the electron acceptors are reduced and the rate at which this reduction occurs depends on a number of variables. Some of these variables include:

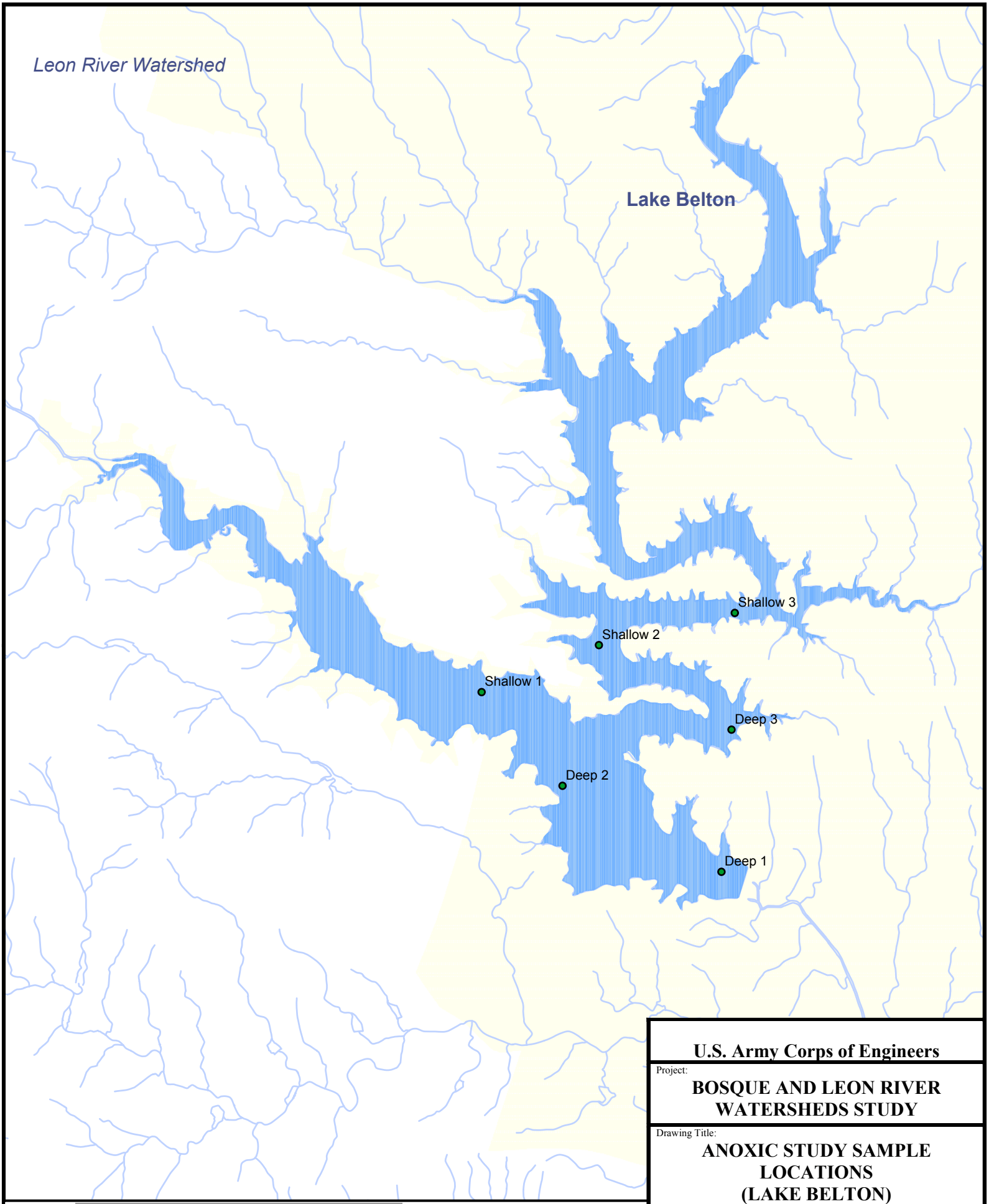
- Biologically active microorganisms (presence/quantity and type)
- Electron acceptors (relative concentrations)
- Electron donors (quantity and type)
- Other biological nutrients (quantity and type, i.e. trace metals)
- Environmental conditions (i.e. temperature, pH, redox potential, etc.)
- Micro-environments (e.g. while both oxygen and nitrate might be present in a bulk fluid, they may be absent or low in portions of a large biological community enabling perchlorate reduction to occur in the micro-environment while in the bulk fluid perchlorate reduction is inhibited)

This portion of the Study was conducted as part of the Anoxic Study. All the methodologies and protocol followed are detailed in the *Final Lake Belton Perchlorate Bioreduction Bench-Scale Study Field Sampling Plan* (MWH, 2002d). Any deviations from the Field Sampling Plan are discussed further below.

5.2.2.2 Methodology

5.2.2.2.1 Field Activities

Sediment samples were collected from the deepest portions of Lake Belton, which were likely to have been under anoxic (oxygen deficient) conditions for the longest period of time, and from three shallower locations where the thermocline was close (1 meter [± 0.5 m] above) to the water/sediment interface. The deep and shallow sampling locations are shown on **Figure 5-101**. The deeper locations were expected to exhibit anoxic conditions because this lake is over 90 feet deep in this area and thermally stratifies. The survey coordinates of the sampling locations and a summary of the samples collected at each location are presented in **Table 5-15**. Samples were collected during the summer (August 2003) when the lake was thermally stratified and when anoxic conditions were expected to occur near the lake bottom and below the thermocline. The field team altered the locations of the three shallow sampling locations based on the thermal profile of the lake at the time of sampling. For example, if a thermocline was not detected at a proposed shallow sampling location, the field team moved to progressively deeper water until the thermocline existed 1 meter (± 0.5 meter) above the lake bottom. Likewise, if the lake bottom was greater than 1.5 meters below the thermocline at a proposed shallow sampling location, the field team moved to progressively shallower water until it was established that a thermocline existed 1 meter (± 0.5 meter) above the lake bottom.



U.S. Army Corps of Engineers

Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY

Drawing Title:
ANOXIC STUDY SAMPLE LOCATIONS (LAKE BELTON)

Data from Navy Environmental Investigations (November 6, 2001)

Sheet 1 of 1 Sheets

SCALE: 1:104,937

FIGURE: 5-101

Table 5-15
Anoxic Study Sample Collection Points

Location Identifier	Sample(s)	Latitude	Longitude
Deep† 1	Sediment, Water near bottom, Water close to (but below) thermocline, Water close to surface (above thermocline)	31.11070	-97.47860
Deep 2	Sediment	31.12770	-97.51448
Deep 3	Sediment	31.14068	-97.47661
Shallow 1 (Close to Thermocline‡)	Sediment, Water near bottom	31.14891	-97.53251
Shallow 2 (Close to Thermocline)	Sediment	31.19272	-97.50623
Shallow 3 (Close to Thermocline)	Sediment	31.18243	-97.50602

† - The deepest locations of Lake Belton that have presumably been anoxic the longest at the time of sampling.
‡ - These locations are selected such that the water/sediment interface is close to (but below) the thermocline.

The following field observations and water quality measurements collected at each sampling location were recorded in the field logbook:

Field Observations:

- Air temperature
- Wind speed and direction
- Water color
- Aquatic vegetation in percent cover (qualitative)
- Cloud cover (qualitative)

Water Quality Measurements:

- Secchi Disk transparency
- Temperature (measured every 5 feet to the lake bottom)
- Dissolved oxygen (measured every 5 feet to the bottom of the thermocline)
- pH (measured every 5 feet to the bottom of the thermocline)

Identification of Thermocline

Sample depths were determined based on the location of the thermocline. At each sampling location, the temperature profile was established prior to collecting the samples. A thermometer or multi-parameter instrument (Hydrolab Mini Sonde 4a) was lowered through the water column in order to develop the vertical temperature, dissolved oxygen (DO), and pH profiles for each sampling point. A representative temperature, dissolved oxygen, and pH profile for sample "Deep 1", which is similar for all sampled locations is shown in **Figure 5-102**. Based on these profiles, it can be seen that the lake temperature varied from 30 to 14 ^{o}C. Additionally, DO was not detected below approximately 10 meters below the surface at any of the locations. Data tables summarizing these results and water quality measurements for each location are provided in **Appendix T**. The

selected water quality meter was calibrated daily according to the manufacturer's instructions. The temperature data were collected at 5-foot increments from the lake surface to the lake bottom and recorded.

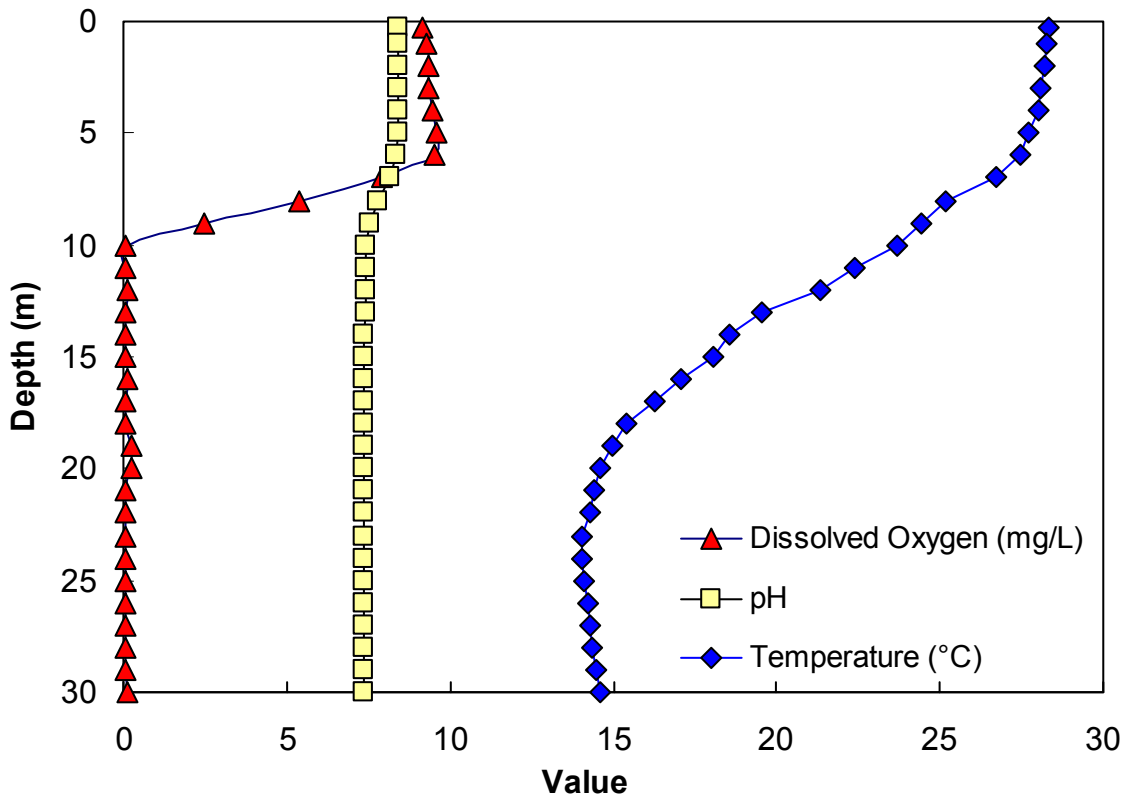


Figure 5-102
Temperature, pH, and Dissolved Oxygen Profile of Lake Belton (*Deep 1* Location)

Lake Water Sampling Equipment and Procedures
Once the appropriate sample depths were established based on the location of the thermocline, the water samples were collected with an Alpha thief sampler previously described in Section 5.1.2.1.2. A summary of the water samples collected is presented above in **Table 5-15**.

Sediment Sampling Equipment and Procedures
Sediment samples were collected using a gravity corer constructed of an outer rigid metal tube (core barrel) into which a polyethylene or acrylic liner fits with minimal clearance (refer to **Figure 5-103**, diagram A). Samples were obtained by allowing the sampler, which was attached to sufficient length of stainless-steel cable, to drop to the bottom. An opening exists above the liner to allow free flow of water through the corer as it moves vertically through the water and into the sediment. The weight of the sampler drives the core barrel into the sediment to varying depths depending on the characteristics of the sediments.

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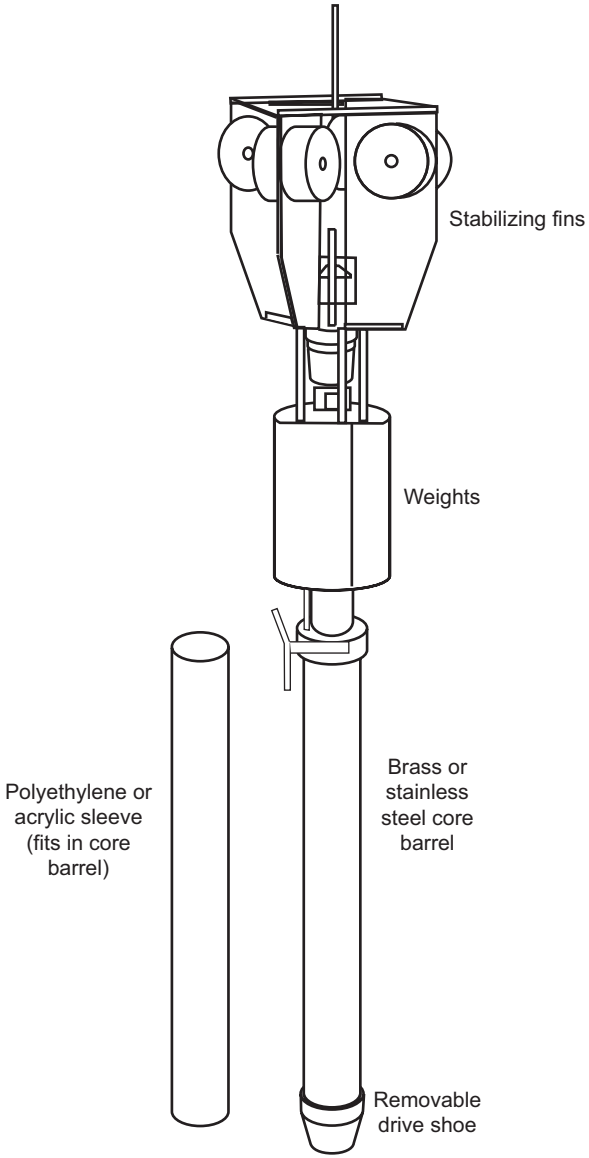
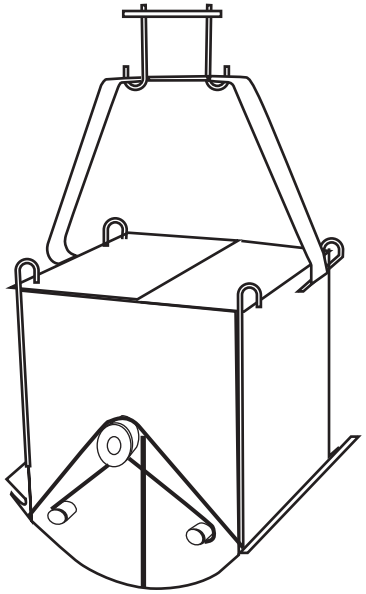
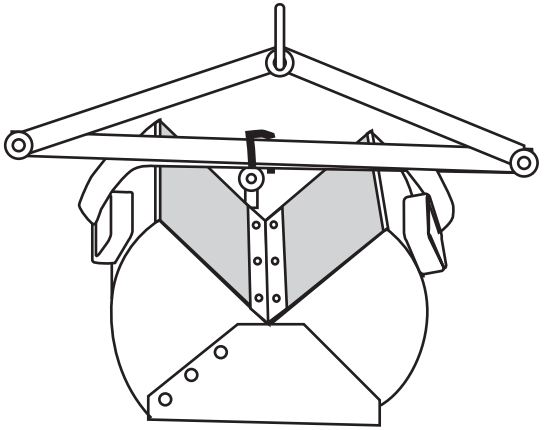


A. Gravity Corer



B. Ekman Dredge (shown closed)



C. Ponar Dredge (shown open)

U.S. Army Corps of Engineers		
PROJECT: **BOSQUE AND LEON RIVER WATERSHEDS STUDY**		
DRAWING TITLE: **GRAVITY CORER**		
	Sheet 1 of 1 sheets	
	SCALE No Scale	FIGURE **5-103**

The sampler has a messenger-activated valve assembly that seals the opening above the liner following sediment penetration, which creates a partial vacuum to assist in sample retention during retrieval. Upon retrieval, the liner was removed, trimmed such that the sediments inside the liner are flush with the ends of the liner, and capped with a sheet of Teflon held in place by a plastic cap. The caps were secured with tape on the outside of the liner. The gravity corer was raised and lowered with a stainless-steel cable attached to a boat-mounted winch. A summary of the sediment samples collected is presented in **Table 5-15**. At locations where both sediment and lake water samples had to be collected, the lake water samples were collected prior to collecting the sediment samples to prevent disturbed sediments from contaminating the lake water samples.

Photographs of field sampling activities are provided in **Figure 5-104**, **Figure 5-105**, and **Figure 5-106**.

Figure 5-104
Gravity Corer Photograph

Figure 5-105
Gravity Corer Dropped to Collect Sediment Sample

Figure 5-106
Sediment Sample Retrieval

Sample Frequency
One sediment sample was collected from each of the shallow and deep sampling locations for a total of six sediment samples. At sampling location "Shallow 1", one lake water sample was collected from just above the lake bottom, below the thermocline. At sampling location "Deep 1", three lake water samples were collected: 1) from just below the lake surface, 2) from just below the thermocline, and 3) from just above the lake bottom. The sampling locations were documented using a Garmin GPS 76 instrument, which recorded the latitude and longitude (degrees and minutes) at each grid point.

Sample Designation
Each sample was designated with an alphanumeric character string set apart by hyphens. The designation began with the lake name abbreviation (e.g., LB for Lake Belton), followed by LW to indicate a lake water sample or SED to indicate a sediment sample, "Deep" or "Shallow" to indicate the deep or shallow sampling location, and finally by the depth of collection. For example, a lake water sample collected from Lake Belton sampling location "Deep 1" at 1 foot depth (just below the lake surface) would be designated "LB-LW-Deep1-1." A sediment sample collected from Lake Belton sampling location "Shallow 3" from 60 feet deep would be designated "LB-SED-Shallow3-60."

5.2.2.2.2 Laboratory Activities

Sample Analysis
Aliquots of all lake water and sediment samples were analyzed for perchlorate by USEPA Method 314.0 at the USACE Engineer Research and Development Center Environmental Laboratory at the Environmental Chemistry Branch in Omaha, Nebraska (See **Appendix V**). The USACE laboratory conformed to the analytical method requirements, analytical quality control requirements, instrument calibration frequency, and the laboratory quality control requirements presented in the QAPP (MWH, 2002e). The data verification report for sample analysis is included in **Appendix W**.

Bioreduction Experiments
Bioreduction experiments on these field samples were designed to address several aspects of the potential for perchlorate biodegradation in Lake Belton. These aspects include:

- *Reductive Medium*. It was anticipated that biological reduction of perchlorate could occur in both the sediment and in the water column. Consequently, tests were performed separately on each of these mediums. Sterile controls were prepared for each of these mediums by autoclaving both the sediment and water column samples. These controls were used to establish that any measured reduction of perchlorate was a result of microbial metabolism and not chemical reduction or physical adsorption.
- *Spatial Variability*. Due to the inherent variability in nature, it could not be expected that the microbiological quality, quantity and composition would be homogeneous throughout the water column, sediment and in different locations in the lake. In order to evaluate how some of this natural variability might impact the perchlorate biodegradation potential; six locations in Lake Belton were selected. Three of the locations were selected in the deeper portions of the lake that were likely to have been under anoxic conditions for

the longest period of time at the time of sampling. The remaining three locations were chosen in shallower water where the thermocline was close to the water/sediment interface. Sediment was collected from each of these locations. Water samples were collected from one of the deep locations near the bottom, close to (but below) the thermocline, and close to the surface (above the thermocline). One additional water sample was collected near the bottom of one of the shallower water locations.

- *Nutrients*. While it is not possible to easily determine the limiting nutrient of these biological systems, experiments were performed to determine if perchlorate degradation in the water column would be enhanced by the addition of an electron donor (acetate) and/or minerals. The minerals were obtained from two sources: 1) sterilized sediment; and 2) laboratory prepared solution. For example, if an enhancement was observed, then the water column had the appropriate microorganisms, but was limited or lacking some nutrient.
- *Oxygen*. Oxygen has been shown to inhibit perchlorate reduction in the facultative reducers. In order to characterize how the oxic/anoxic conditions of the lake can impact perchlorate reduction, an anoxic water sample was oxygenated and provided sufficient electron donor to reduce the oxygen. This was to show if the culture could recover in the time allotted. This was then compared to an unoxygenated system. Additionally, a water sample from above the thermocline was tested for its ability to reduce perchlorate.
- *Temperature*. The temperature in the anoxic region of the lake can be substantially colder than close to the surface. It is likely that these colder temperatures will reduce the rate of any perchlorate biodegradation. To assess the impact of temperature, a set of microecology experiments was carried out at both room temperature (~20°C) and at 6°C.
- *Kinetics*. Determining the rate biodegradation *a priori*, based on the microecology of environmental mixed cultures and available micronutrients is not possible given the current state of knowledge. Consequently, samples were collected at regular intervals over the course of 14 days to establish how rapidly biodegradation took place for selected conditions and establish how different environmental factors may impact this observed rate.

A summary of the experimental matrix is presented in **Appendix U**. This summary outlines general experimental conditions, matrix modifications, incubation periods, number of samples, and quality control samples.

5.2.2.3 Data

Spatial Variability

To test the influence of spatial variability within the water column and around the lake on the perchlorate bioreduction potential, samples were collected at each of the locations previously discussed above using the protocols set forth in the Field Sampling Plan (April 2003):

Sediment Variability

Under temperature controlled conditions in an anaerobic chamber, a series of autoclaved 100 mL Amber Pyrex Media bottles were filled with 90 mL of a laboratory prepared stock solution consisting of sterilized water collected from Lake Belton and spiked with 50 µg/L of perchlorate. These bottles were inoculated with 10-mL aliquots of the six different sediments, purged with nitrogen to strip any dissolved oxygen and ensure the headspace in the bottle was oxygen-free, capped, and agitated (in triplicate). All six core sediment samples were processed in triplicate bottles to insure representative sampling of the core plug as a whole. Control samples were prepared, in triplicate, by autoclaving sample bottles inoculated with sediment. The samples were allowed to react for a 14-day period under constant, low-level agitation, provided by an orbital shaker, in a low-temperature incubator set at ambient conditions (20ºC). During processing and incubation, all sample exposure to light was minimized to reduce the chance of algal growth that could result in undesirable oxygen generation.

As shown in **Figure 5-107**, significant perchlorate reduction was observed within a 14-day incubation period for all six sediment samples. With the exception of the sediment sample from the *Deep 2* location, which achieved approximately 80% reduction, the initial 50 µg/L of perchlorate was reduced to below the reporting limit (<4 µg/L). Since the sediment samples were collected from six widely dispersed locations in the lake, these results indicate that indigenous perchlorate-reducing bacteria are likely to be present within sediment throughout Lake Belton. As complete reduction was observed in most of the samples, it was not possible to characterize the variability in microecologies.

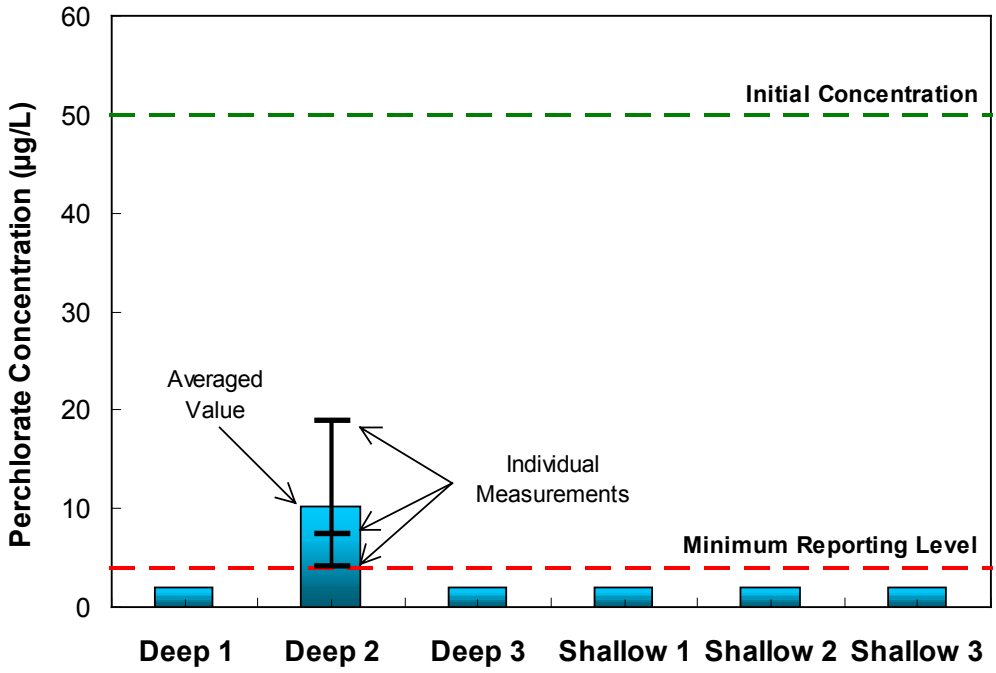


Figure 5-107
Spatial Variability of Perchlorate Reduction for Sediment Sample Cultures†

† Bars represent average values of the three replicates. "Error bars" represent individual values.

Water Column Variability

Under temperature controlled conditions in an anaerobic chamber, 50 mL aliquots of the three samples from the water column at the *Deep 1* location were volumetrically transferred into amber, 50 mL serum bottles and spiked with 50 µg/L perchlorate. The samples were then nitrogen purged, capped, agitated, and allowed to react for a 14-day period in a low-temperature incubator set at ambient conditions. All sample processes were carried out in triplicate, and exposure to light was minimized. Control samples also were prepared in triplicate by autoclaving sample bottles inoculated with an aliquot of the water column samples. At the end of the contact time, a portion of each sample was filter-sterilized (0.45 µm) and submitted to the USACE laboratory for perchlorate analysis.

Of the three aqueous samples incubated, only the *Deep 1 Bottom* sample achieved removals of perchlorate to below the reporting limit (< 4 µg/L) within 14 days (**Figure 5-108**). The *Deep 1 Thermocline* and *Surface* samples, however, did achieve 50% and 16% reduction, respectively. The difference in perchlorate reduction observed at the three different depths at the *Deep 1* location may be attributed to the anticipated inhibitory effect of dissolved oxygen on populations of perchlorate-reducing microorganisms. At the *Surface* (0.3 m) and *Thermocline* (10 m) depths, dissolved oxygen concentrations were 9.13 mg/L and 0.06 mg/L, whereas the *Bottom* (30 m) sample had 0 mg/L. This phenomenon was further investigated in the Impact of Oxygen experiments (see Oxygen section below). Results of this Water Column Variability experiment demonstrated that the perchlorate reducers are present throughout the water column. However, their activity or numbers appeared to be increasing with depth.

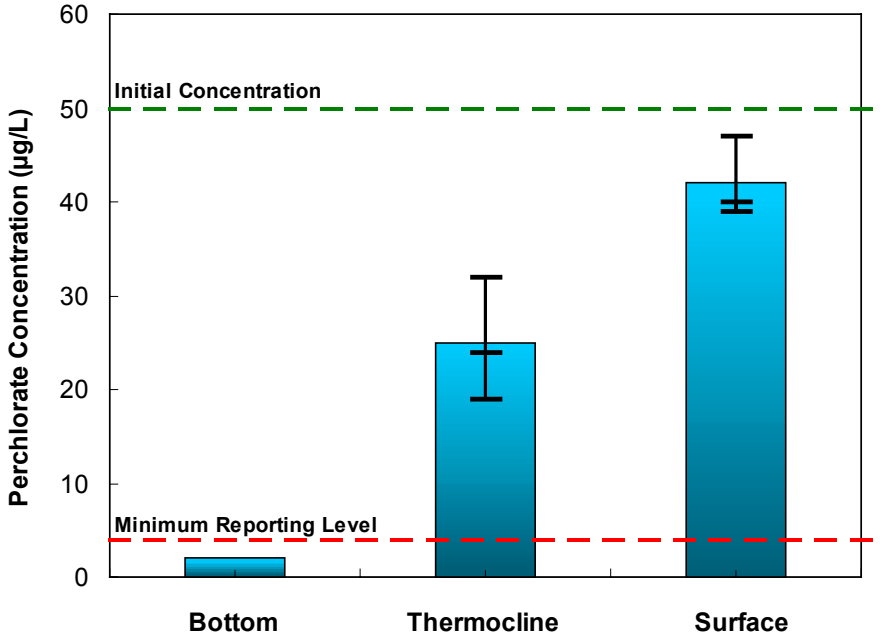


Figure 5-108
Spatial Variability of Perchlorate Reduction for *Deep 1* Aqueous Sample Cultures[†]

[†] Bars represent average values of the three replicates. "Error bars" represent individual values.

Kinetics

As the rate of biodegradation was not known, cultures were prepared using the aqueous *Deep 1* sample and the sediments from the *Deep 1* and *Shallow 1* locations. Samples were prepared so that they could be individually sacrificed at 1, 3, 5, 7, 10 and 14 days to characterize the rate of perchlorate degradation. **Figure 5-109** and **Figure 5-110** compare the rate of perchlorate degradation for both aqueous and sediment samples. For sediment samples, the rate of perchlorate reduction was more rapid for the *Shallow 1* location as compared to the *Deep 1* location with perchlorate reaching non-detect levels (< 4 µg/L) in 10 versus 14 days, respectively. It is hypothesized that the *Shallow 1* culture may have a greater diversity, have a higher density, or have a higher activity of microorganisms in this region.

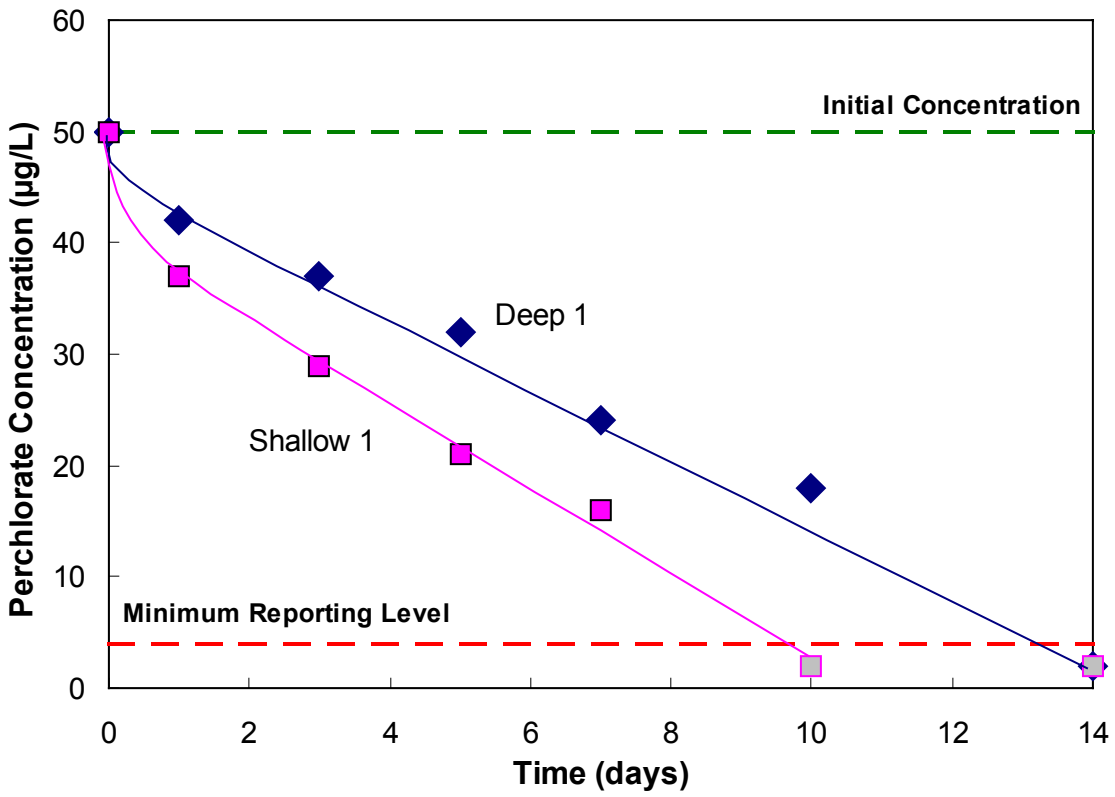


Figure 5-109
Rates of Perchlorate Degradation for Sediment Sample Cultures

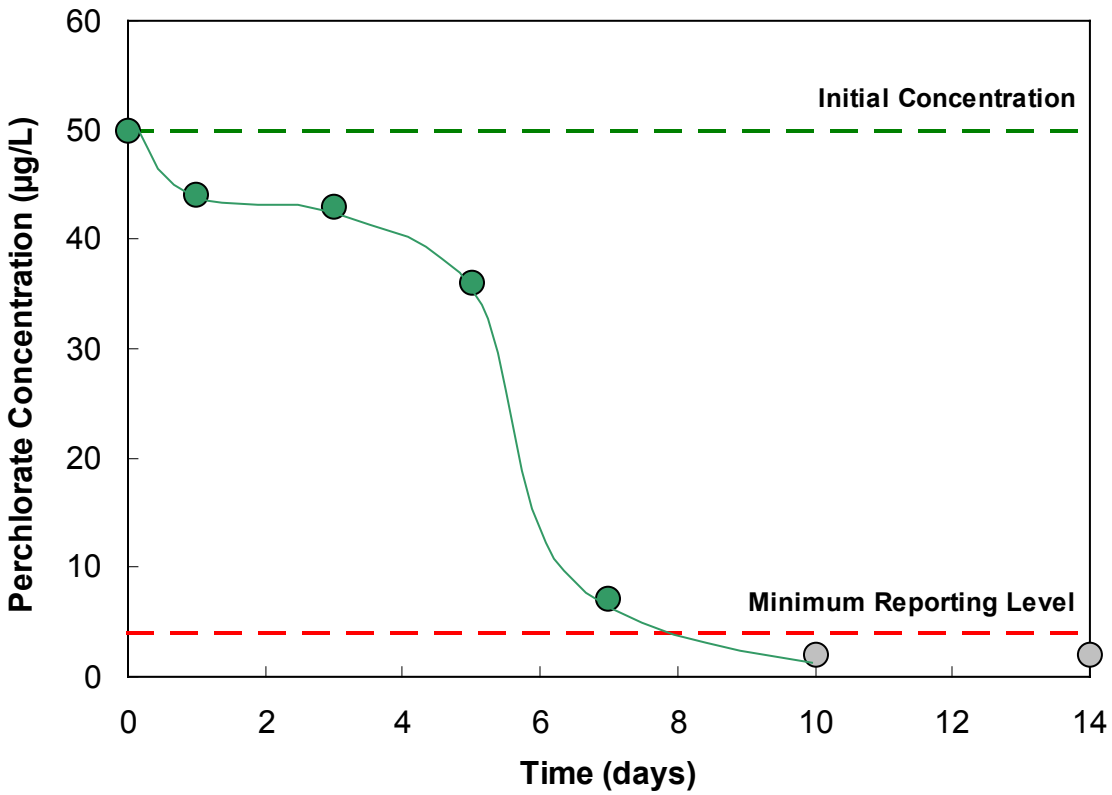


Figure 5-110
Rates of Perchlorate Degradation for Aqueous Sample Cultures (Deep 1)

The aqueous *Deep 1* samples reduced perchlorate to non-detect levels (< 4 µg/L) within 10 days. Perchlorate reduction, however, was slow for the first four days and then accelerated quickly thereafter. This slow initial response represented the lag time required for the perchlorate-reducing bacteria to begin the expression of (per)chlorate-reductase, whereas little to no lag was observed in the samples containing sediment-borne bacteria. The lag observed in aqueous cultures may be associated with (1) the possible exposure to dissolved oxygen during sampling, transportation, or handling of the sample which temporarily inhibited the expression of (per)chlorate-reductase, (2) temporary inhibition due to the presence of nitrate, (3) differing initial microbial composition – type of organisms and quantities of each organism and/or (4) the limited surface area available for attached growth (i.e., soil particles available in sediment inoculated cultures). However, additional and more detailed microecology experiments would be needed to further develop these hypotheses.

Nutrients

To determine if perchlorate reduction might be inhibited by a nutrient deficiency, samples of the sediment and water column were amended with an electron donor and/or metals.

Sediment Nutrients

Portions of the sediment samples collected were processed as described in the "Sediment Variability" section of this document. These samples were then spiked to 50-mg/L acetate; nitrogen purged, agitated and allowed to react for a 14-day period. At the end of the contact time, filter-sterilized (0.45 µm) samples were collected and submitted to the USACE laboratory for perchlorate analysis.

Comparing results of the amended and unamended cultures, it can be seen in **Figure 5-111** that perchlorate reduction to below reporting limits was observed in all acetate-amended cultures. Specifically for the *Deep 2* sediment culture, perchlorate reduction improved from approximately 80% to greater than 92% when amended with acetate. Although this marked improvement was noticeable for the *Deep 2* sediment culture, based on the range of cultures tested from Deep and Shallow sediments, it would appear that sufficient electron donor and carbon source exists for the indigenous perchlorate-reducing microorganisms to subsist. Additional perchlorate reduction tests were performed to evaluate if the addition of acetate improved the rate at which perchlorate was degraded.

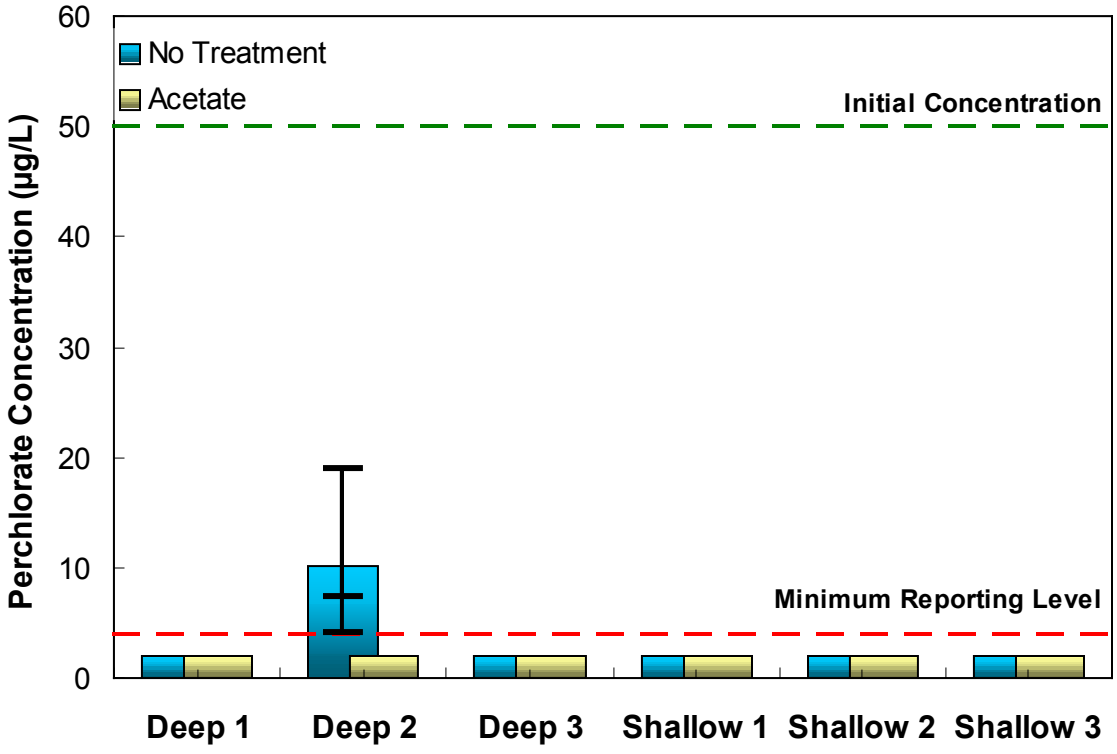


Figure 5-111
Effect of Acetate Addition on Perchlorate Reduction for Sediment Sample Cultures[†]

To determine if the addition of a readily accessible electron donor would alter the rate of biodegradation, additional cultures were prepared for two of the sediments. These

[†] Bars represent average values of the three replicates. "Error bars" represent individual values.

cultures were individually sacrificed at 1, 3, 5, 7, 10 and 14 days to characterize any potential impacts on perchlorate degradation.

As shown in **Figure 5-112** and **Figure 5-113**, the addition of acetate improved the rate of perchlorate biodegradation in both the *Deep 1* and *Shallow 1* sediment cultures. A significant improvement was observed with the *Deep 1* culture as perchlorate was reduced to below detection limits (< 4 µg/L) within 5 days, compared to the 14 days required for unamended cultures. A similar but slight improvement was noticed for *Shallow 1* cultures as perchlorate reduction improved from 10 to 7 days with the addition of acetate. These results revealed that while neither sediment sample was stoichiometrically limited by the electron donor, readily accessible excess electron donor could accelerate perchlorate reduction.

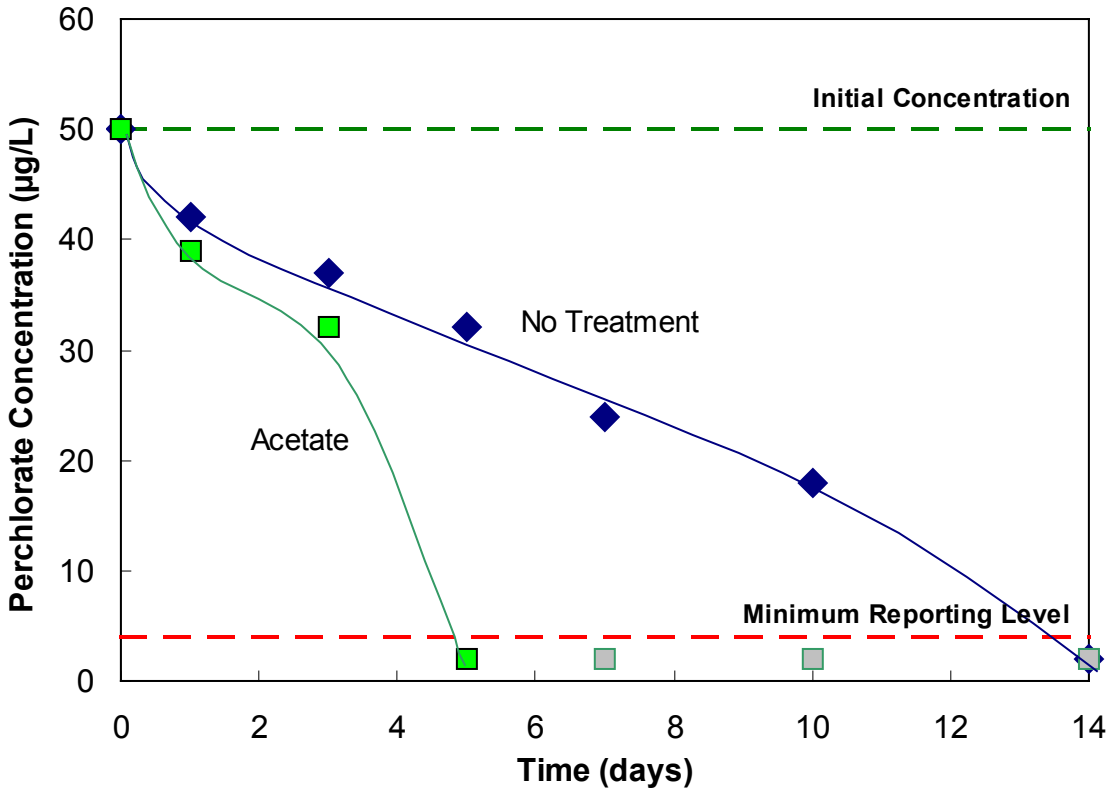


Figure 5-112
Impact of Acetate Addition on Perchlorate Reduction Rates for *Deep 1* Sediment Cultures

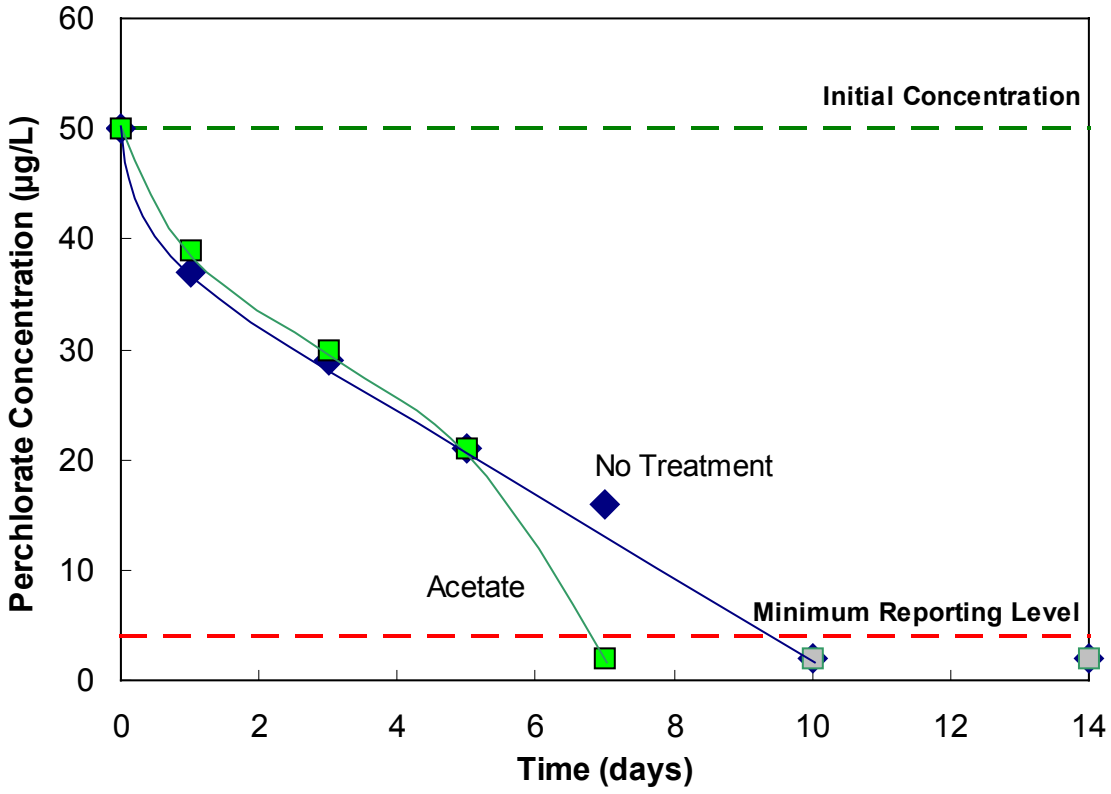


Figure 5-113
Impact of Acetate Addition on Perchlorate Reduction Rates for *Shallow 1* Sediment Cultures

Water Column Nutrients

A portion of the aqueous samples collected was processed as described in the "Water Column Variability" section of this document. Each of these samples (*Deep 1 Bottom, Thermocline, Surface, and Shallow 1 Bottom)* was amended with the acetate, metal and/or sediment combinations identified in the **Table 5-16**. Once amended, triplicate samples were agitated and allowed to react for a 14-day period. At the end of the contact time, filter-sterilized (0.45 µm) samples were collected and submitted to the USACE laboratory for perchlorate analysis.

Table 5-16
Nutrient Amendments for Water Column Cultures

Series	Nutrient Amendment
1	50 mg/L acetate
2	50 mg/L acetate and 50 mg/L autoclaved and dried "Deep 1" sediment
3	50 mg/L acetate and 1 mL/L of mineral solution†

† Mineral Solution Composition in Deionized Water (g/L)	
$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$	10
$ZnCl_2$	0.05
H_3BO_3	0.3
$FeCl_2\cdot 4H_2O$	1.5
$CoCl_2\cdot 6H_2O$	10
$MnCl_2\cdot 6H_2O$	0.03
$NiCl_2\cdot 6H_2O$	0.03

Results of the nutrient-amended aqueous cultures were compared to the corresponding unamended culture from the "Water Column Variability" tests. As shown in **Figure 5-114**, the addition of minerals to the *Deep 1 Bottom* culture caused the perchlorate-reducing ability of the indigenous microorganisms to completely cease. Results from the *Shallow 1 Bottom* culture where nearly identical. Interestingly, while each of the minerals has been identified as necessary micro-nutrients for many biological systems, some component(s) in the mineral solution appeared to be inhibitory. Additional detailed experiments would be required to identify the mineral(s) of concern and determine at what concentration the micro-nutrient becomes inhibitory.

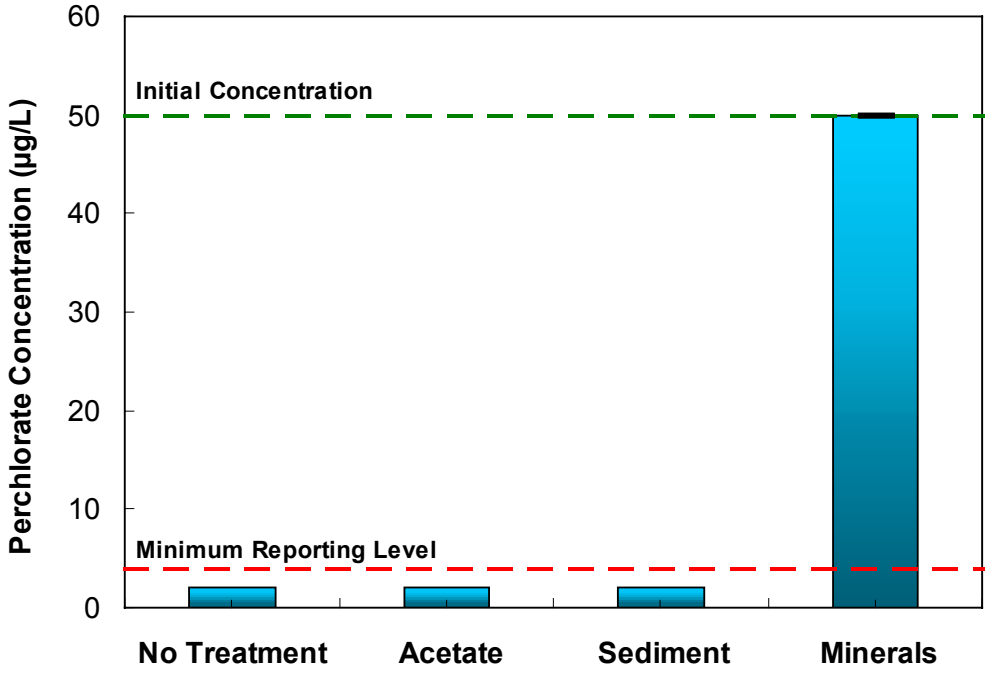


Figure 5-114
Impact of Nutrient Addition on *Deep 1 Bottom* Aqueous Cultures

In the "Water Column Variability" tests, the unamended *Deep 1 Thermocline* and *Surface* cultures were only able to reduce the initial 50-µg/L perchlorate by 50 and 16%, respectively. **Figure 5-115** shows the impact of adding acetate, sediment, or minerals to the *Deep 1 Surface* culture. Similar results were observed in the *Deep 1 Thermocline* culture. The addition of acetate did not improve perchlorate reduction and the addition of the mineral solution resulted in a complete inhibition of perchlorate reduction. However, sediment addition resulted in complete reduction of the perchlorate. Several possibilities may explain this improved reduction: 1) an increase in biomass; 2) scavenging of oxygen by reduced metals in the sediment; 3) increase in electron donor concentration; 4) perchlorate adsorption on/in to the sediment; or 5) addition of critical nutrients. However, the first four are not considered to be likely. Since the sediment was sterilized, any additional biomass added would be inactive, and through the sterilization and drying process, it is likely that any oxygen-scavenging metals would have been oxidized. As the addition of acetate did not result in a significant improvement in the reduction, it is unlikely that this alone could cause the observed response. An autoclaved control bottle of the entire system revealed that perchlorate adsorption on/in to the sediment is not an issue. This leaves the addition of some critical nutrient(s) not included in the mineral solution or the addition of a different concentration of the essential nutrient.

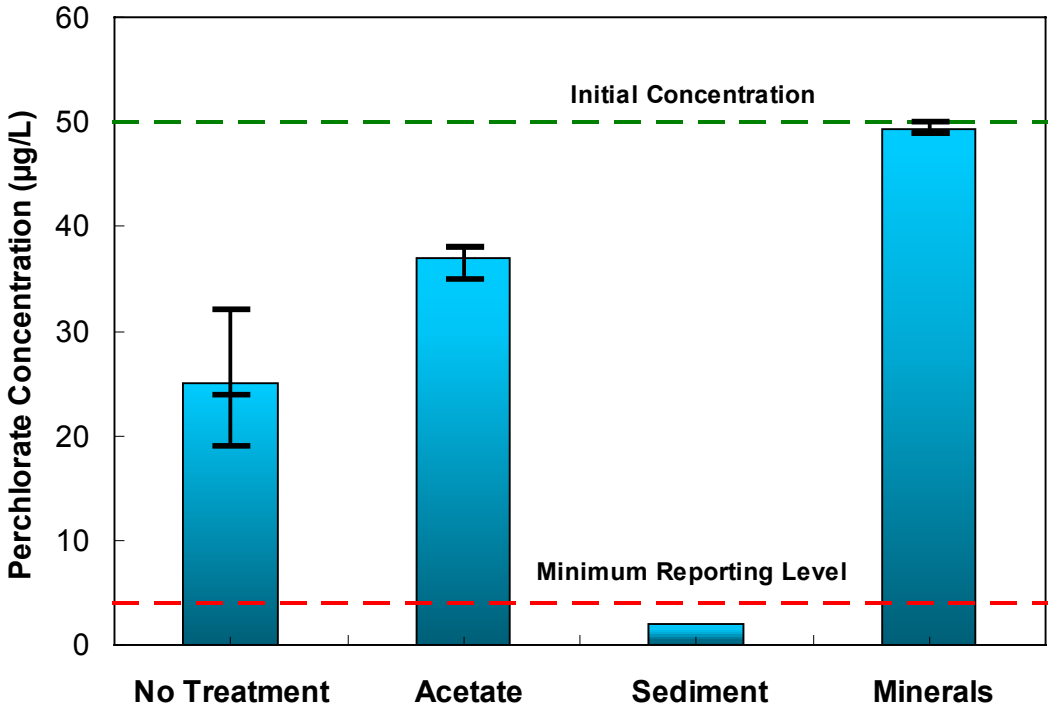


Figure 5-115
Impact of Nutrient Addition on *Deep 1 Surface* Aqueous Cultures[†]

This demonstrated that the perchlorate-reducing bacteria, that were likely to have been disrupted by the presence of dissolved oxygen at the thermocline or surface, could be induced to reduce the perchlorate.

[†] Bars represent average values of the three replicates. "Error bars" represent individual values.

Kinetic studies were performed for the *Deep 1 Bottom* sample location to characterize any potential impacts of various nutrient amendments on the measured rate of perchlorate degradation. Five additional cultures were prepared for each of the nutrient addition conditions (acetate, sediment, and minerals) and were individually sacrificed at 1, 3, 5, 7, 10 and 14 days. As previously observed, there was little to no difference in the rate of perchlorate degradation between the unamended and acetate-amended cultures. As seen in **Figure 5-116**, however, when nutrients were introduced through the addition of sterilized sediment, perchlorate reduction to below reporting limits (< 4 µg/L) improved from approximately 10 to 6 days.

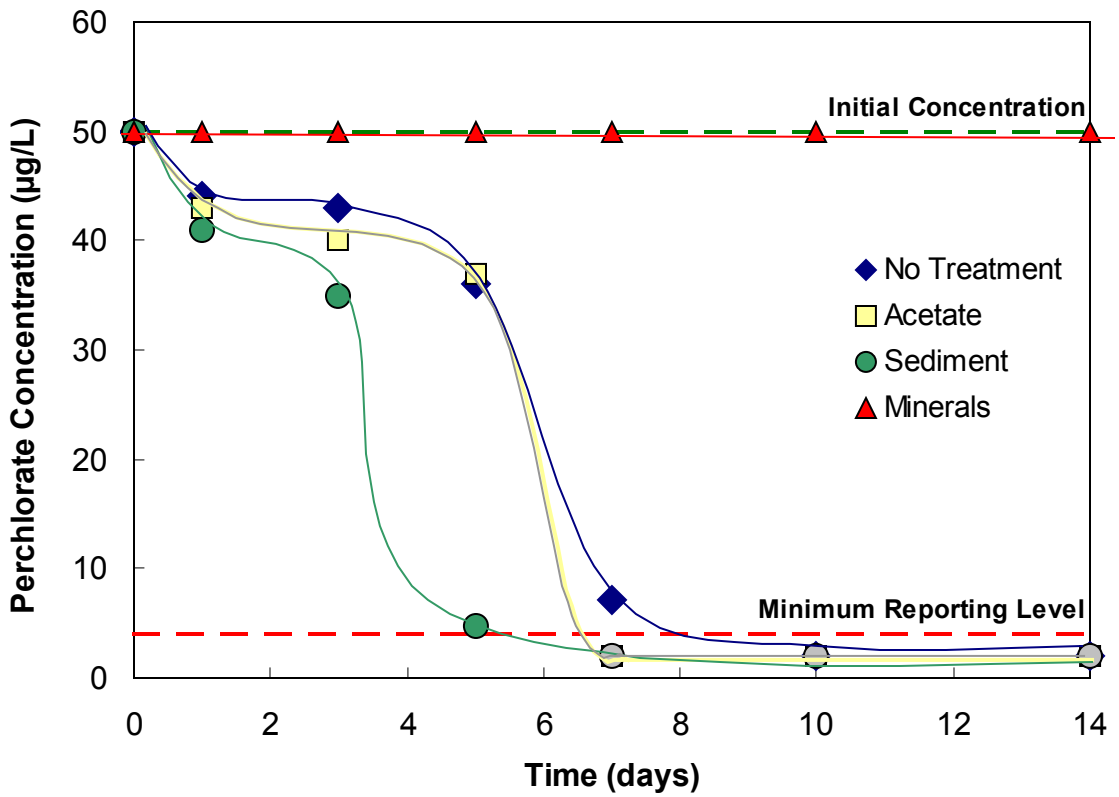


Figure 5-116
Impact of Nutrient Addition on Perchlorate Reduction Kinetics for *Deep 1 Bottom* Aqueous Cultures

These results confirmed the ability of the unknown nutrients in the sediment matrix to stimulate the activity of perchlorate-reducing bacteria present in the aqueous sample. However, additional ecology experiments would need to be performed to characterize the effect and activity of the many different microbial populations that contribute to perchlorate reduction and rate of biodegradation.

Oxygen

The inhibitory effect of oxygen was only evaluated in the water column. In addition to the effects of oxygen that can be observed in selected cultures from the "Spatial

Variability" tests, the oxygen tests were used to differentiate between the possible impacts of brief oxygen exposure to elevated dissolved oxygen levels for the *Deep 1 Bottom, Deep 1 Thermocline, Deep 1 Surface* and *Shallow 1 Bottom* samples.

Two aliquots were prepared from each water sample by either: 1) oxygenating the sample only or 2) oxygenation with a subsequent nitrogen purge and the addition of a small amount of sulfide to remove the available dissolved oxygen and lower the redox potential. Once prepared, all of the aliquots were then dosed with 50-µg/L perchlorate and 50-mg/L acetate, sealed, agitated, and returned to the inert environment. The cultures were allowed to react for a 14-day period before samples were filter-sterilized (0.45 µm) and submitted to the USACE laboratory for perchlorate analysis.

From **Figure 5-117** it can be seen that for the *Deep 1 Bottom* culture, the exposure of the organisms to high concentrations of oxygen had a significant negative impact on the ability of the indigenous microorganisms to effectively reduce perchlorate. Even when the oxygenated samples were sparged with nitrogen and dosed with sulfide to remove dissolved oxygen; the perchlorate-reducing capability of the culture was inhibited and could not recover within the 14-day incubation period. Similar results were observed for the additional samples prepared for the *Deep 1 Thermocline, Deep 1 Surface,* and *Shallow 1 Bottom* locations.

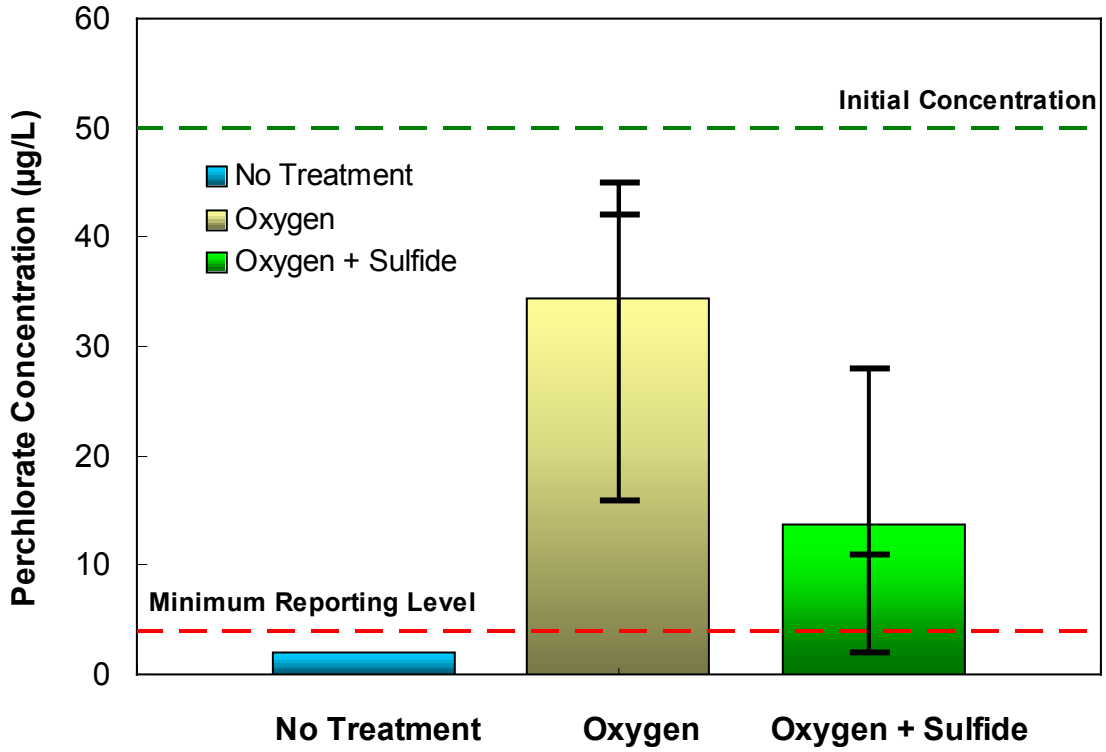


Figure 5-117
Impact of Oxygen on Perchlorate Reduction for *Deep 1 Bottom* Aqueous Cultures[†]

[†] Bars represent average values of the three replicates. "Error bars" represent individual values.

Temperature

It is expected that the lower temperatures at the bottom of the lake will slow the rate of perchlorate reduction. To measure this variability, a portion of the water and sediment samples collected was processed as described in the "Water Column Variability" and "Sediment Variability" sections of this document, respectively. The processed samples were agitated, nitrogen purged and stored in a low temperature incubator set at 6°C. After the respective incubation periods, filter-sterilized (0.45 µm) samples were collected and submitted to the USACE laboratory for perchlorate analysis.

Figure 5-118 compares the results of aqueous samples run at ambient and low temperatures. As expected, the rate of perchlorate reduction suffered for samples incubated at 6 ºC. This is best represented by the *Deep 1 Bottom* and *Shallow 1 Bottom* samples, in which the perchlorate-reducing bacteria (sampled from expected anoxic environments) performed the best at ambient temperature. When incubated at a low temperature, however, only 20% of the perchlorate was reduced indicating that the perchlorate-reducing bacteria located in the aqueous phase were inhibited by the low temperature environment. Since these bacteria were highly active at an ambient temperature (20 ºC), it is likely that the mixed culture in the aqueous matrix was comprised of primary mesophilic bacteria (capable of surviving between 20 – 40 ºC).

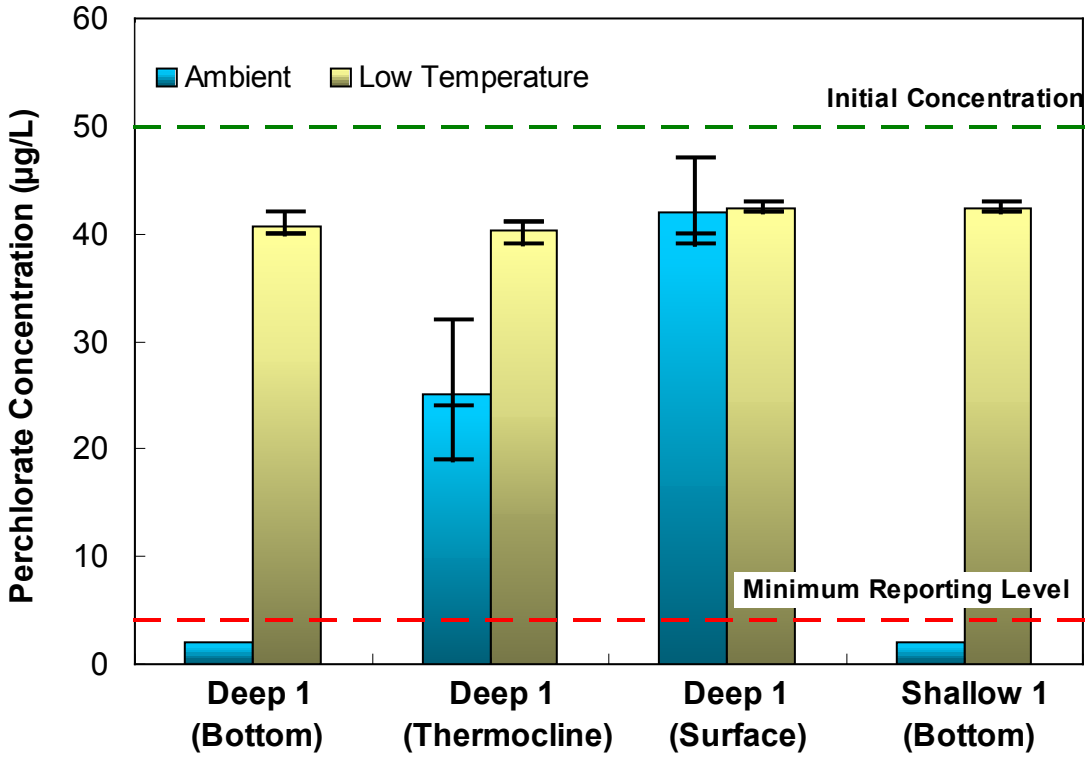


Figure 5-118
Effect of Temperature on Perchlorate Degradation for Aqueous Sample Cultures[†]

[†] Bars represent average values of the three replicates. "Error bars" represent individual values.

Figure 5-119 compares the results of sediment samples run at ambient and low temperatures. As expected, *Deep* sediment samples incubated at 6 ºC experienced similar diminished performance compared to the samples incubated at ambient temperature. Comparing the perchlorate reduction of aqueous and sediment samples from the *Deep 1* location incubated at 6 ºC, it is interesting to note that more perchlorate reduction was observed in sediment samples (60% compared to 20%). The perchlorate-reducing capability of the sediment-borne bacteria was possibly related to potentially larger numbers and/or different types of microorganisms in the sediment cultures.

Interestingly, results from the shallow sediment samples indicated that highly active populations of perchlorate-reducing bacteria existed in the *Shallow 2* and *Shallow 3* locations, as perchlorate reduction was observed below reporting limits (< 4 µg/L) for both ambient and low temperature conditions. The improved performance compared to *Shallow* aqueous samples is likely due to the fact that a larger population of perchlorate-reducing microorganisms and/or more diverse microorganisms are available in the sediment matrix (i.e., perchlorate-, nitrate-, perchlorate/nitrate- reducing bacteria, mesophiles, psychrophiles, etc.). Additional microecology experiments would be required to characterize the quantity and types of these microorganisms that are present in sediment compare to aqueous matrices.

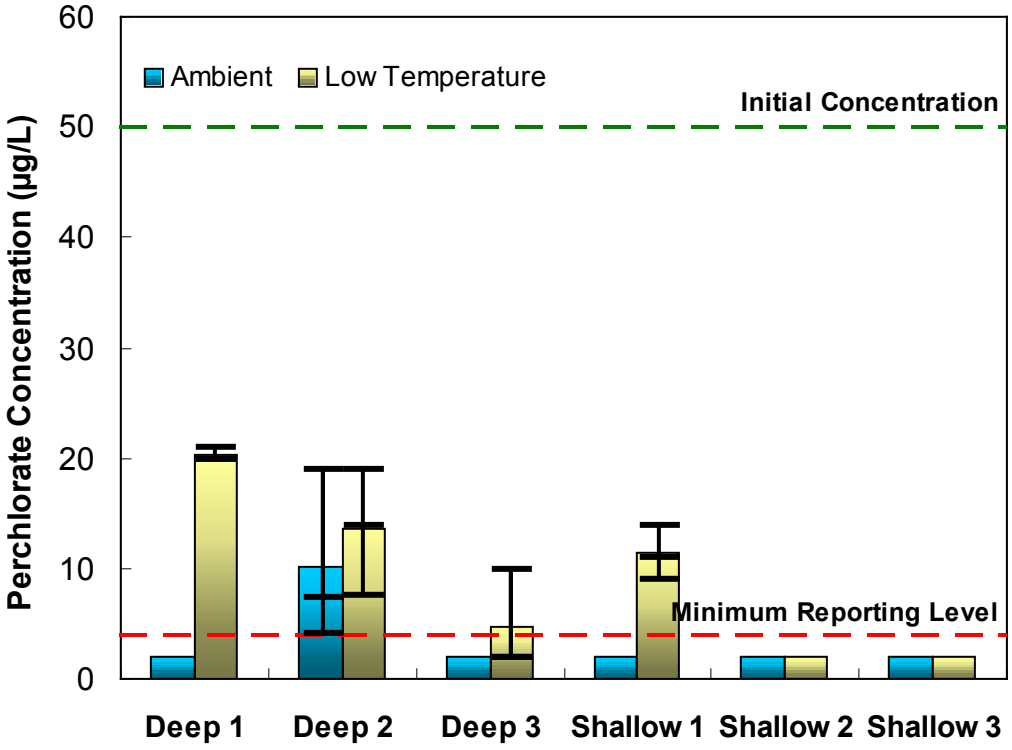


Figure 5-119
Effect of Temperature on Perchlorate Reduction for Sediment Sample Cultures[†]

[†] Bars represent average values of the three replicates. "Error bars" represent individual values.

5.2.2.4 Discussion

The overall results of this testing indicated that Lake Belton has the potential for the natural attenuation, or more specifically, intrinsic bioremediation of perchlorate. Based on the results of specific experiments performed, the following conclusions can be made:

- Indigenous perchlorate-reducing microorganisms are present throughout the water column and sediment of Lake Belton. All experiments performed with aqueous and sediments cultures demonstrated the capability to reduce 50 µg/L of perchlorate spiked to the samples. Several cultures, which were not amended with additional electron donor or nutrients, reduced perchlorate to non-detect levels (< 4 µg/L) in less than 14 days.
- The presence of oxygen impacts the rate of perchlorate biodegradation. Comparing results of samples collected at the bottom, thermocline, and near the surface of the lake, is apparent that the presence of dissolved oxygen has an inhibitory effect on the perchlorate-reducing ability of the microorganisms. Cultures present in the bottom or sediments of both deep and shallow portions of the lake were consistently able to reduce 50 µg/L perchlorate to below the 4 µg/L reporting limit within a 14-day incubation period. As a result, the majority of perchlorate bioreduction is expected to occur in the deeper areas of Lake Belton.
- Sufficient electron donor is available to the perchlorate-reducing bacteria for natural attenuation. Sufficient electron donor was present for complete reduction of perchlorate. Additional tests, performed by augmenting the sample with 50 mg/L acetate indicated that a further elevation of the electron donor did not consistently result in a significant improvement in the rate of perchlorate degradation.
- The amendment of sediment nutrients to water column cultures improved perchlorate reduction. Aqueous cultures, found to be impacted by the inhibitory effect of oxygen, were tested with the amendment of sterilized sediment. Results revealed that the perchlorate reduction was improved by approximately 90%. While several possibilities existed, it is likely that a critical nutrient(s) in the sediment was responsible for the observed increase in biological activity.
- Biological reduction of perchlorate is inhibited at lower temperatures. Results showed that mesophilic (moderate temperature tolerant) bacteria are dominant in the water column samples. In several samples, perchlorate reduction slowed from nearly 95 to 10% when incubated at 6 ºC. Compared to the aqueous cultures, sediment-borne cultures achieved improved perchlorate-reducing capability in a cold environment. Complete perchlorate reduction was observed in several sediment cultures at 6 ºC . These results indicated that the sediment contained more robust perchlorate-reducing bacteria (i.e.,mesophiles and psychrophiles) and larger populations of these microorganisms.

5.2.3 Perchlorate Transformation Study

5.2.3.1 Introduction

The fate of perchlorate in the environment can be influenced by any transformation (mainly microbial) that occurs. As plants senesce vegetative parts can be returned to the soil/sediment. The return of perchlorate to the soil/sediment may be a beneficial process due to the movement of perchlorate from stable environments (aerated stream water or low carbon deeper sediments) to areas where rapid transformation can occur (top organic rich soil layers). One of the most important aspects of the fate of perchlorate in these systems is the rate of transformation. Understanding the magnitude and environmental influences on these microbial degradation rates is imperative to understand the long term stability in the environment.

5.2.3.2 Methodology

5.2.3.2.1 Site Description and Sampling

Sediment and soil samples were obtained from two sites historically associated with ClO_4^- discharge in Texas. One site was the Naval Weapons Industrial Reserve Plant (NWIRP) in McGregor, Texas. The other site was the Longhorn Army Ammunition Plant (LHAAP) in Karnack, Texas, which was another primary manufacturer of solid propellant rocket motors. The locations of these sites are shown on **Figure 5-120**. The LHAAP, listed on the USEPA National Priorities List, is located in the Caddo Lake watershed. This site covers an area of about 8,500 acres. Perchlorate contamination in water, soil, sediment, vegetation, and animal tissue samples has been reported at this site (Smith et al., 2001).

Four sediment samples were collected from the top 10 cm of bottom sediments at four selected creek locations surrounding NWIRP, namely, Harris Creek at Highway 84 west of McGregor (T19), a branch of Harris Creek at Highway 84 (T18), Harris Creek at Highway 317 (T13), and S Creek at Highway 317 (T15). These multiple stream sites were hereafter referred as HW84 Mainstream, HW84 Sidestream, HW317, and HW317/MN, respectively (**Figure 5-120**). HW84 Sidestream is a groundwater discharge zone, with a spring identified at the head of the stream.

At the LHAAP site, several kilograms of relative dry soil were collected from the top 15 cm near Building 25C (a perchlorate grinding facility). The specific soil sampling location layout at LHAAP site has been described in detail elsewhere (Smith et al., 2001). The soil had prior exposure to ClO_4^-. Descriptions of sediments and soil are summarized in **Table 5-17**.

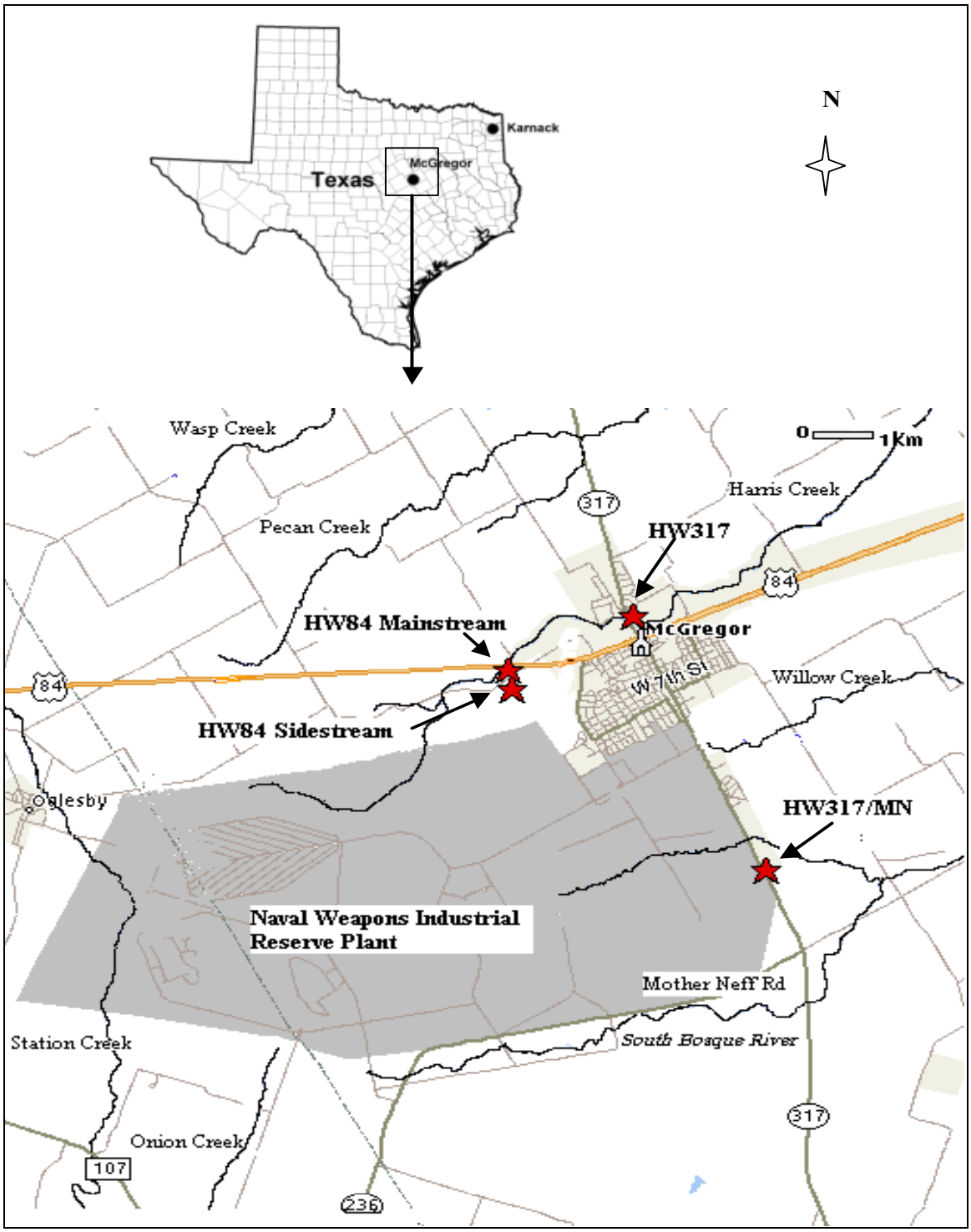


Figure 5-120
Locations of the NWIRP and LHAAP Sites

Table 5-17
Description of Sediments and Soils Used in Microcosm Studies on Perchlorate Transformation

Site	Location	Matrix	Description
HW84 Sidestream (T18)	NWIRP	Sediment	Continuously exposed to ClO_4^-
HW84 Mainstream (T19)	NWIRP	Sediment	Intermittently exposed to ClO_4^-
HW317 (T13)	NWIRP	Sediment	Intermittently exposed to ClO_4^-
HW317/MN (T15)	NWIRP	Sediment	Continuously exposed to ClO_4^-
Longhorn	LHAAP	Soil	Prior exposure to ClO_4^-

Water samples were taken simultaneously with sediments at each location. Water samples were not taken at the LHAAP site, because surface water was not available near Building 25C at the time of sampling. Sediment samples were maintained under anaerobic conditions by a layer of water over the surface. Water, sediment, and soil samples were stored on ice and then transported immediately to the lab for microcosm studies.

5.2.3.2.2 Laboratory Microcosm Studies

The sediment and water from individual locations were used to construct each microcosm. The microcosms consisted of 120 ml of a 1:3 sediment/water (by weight) slurry. Care was taken to avoid the introduction of oxygen. Bottles were sealed with septa and aluminum crimp caps, then incubated anaerobically at room temperature. Five ml of slurry were taken from each bottle with a clean bone marrow needle at a frequency of twice per week during the first few weeks, and once per week during the remaining period. All samples were analyzed for ClO_4^-, Cl^-, NO_3^-, NO_2^-, and SO_4^{2-}.

Generally, five treatments were conducted using HW84 Mainstream, HW84 Sidestream sediments, and Longhorn soil as representative samples to explore the influence of terminal electron acceptors. Treatments consisted of autoclaved (abiotic condition), control (no amendment), 5 ppm nitrate (corresponding to 1.1 ppm NO_3^--N) spiked, 50 ppm nitrate (corresponding to 11.3 ppm NO_3^--N) spiked, and 300 ppm sulfate spiked. Only the control treatment (no amendment) was conducted for HW317 and HW317/MN sediments. All microcosm treatments are summarized in **Table 5-18**. The autoclaved treatments were prepared by autoclaving the sediments and soils for 20 minutes at 15 psig and 121 ºC on 3 successive days, in order to assess the possible abiotic loss of perchlorate. Three replicates were run for each treatment. The initial ClO_4^- concentration for each treatment was 5 ppm (mg/L). Another treatment was conducted by using HW84 Sidestream sediment and DI water instead of site water to study the kinetics under lower levels of nitrate (i.e., 1 ppm nitrate-N spiked), because there were relatively high nitrate background levels in site water. One additional treatment was conducted for HW84 Sidestream to study the ClO_4^- degradation rate under a lower initial ClO_4^- concentration. The initial ClO_4^- concentration was spiked at 0.5 ppm for this treatment. The sampling interval was every two or three days.

Table 5-18
Summary of Treatment Conditions Used in Microcosm Studies on Perchlorate Transformation

				Site Water			DI Water
Site	**ClO_4^- ppm**	**Autoclaved**	**Control**	**NO_3^-- spiked 1.1 ppm**	**NO_3^-- spiked 11.3 ppm**	**SO_4^--spiked 300 ppm**	**NO_3^--spiked 1 ppm**
HW84 Sidestream (T18)	0.5	-	+	-	-	-	-
	5.0	+	+	+	+	+	+
HW84 Mainstream (T19)	5.0	-	+	+	+	+	-
HW317 (T13)	5.0	+	+	-	-	-	-
HW317/MN (T15)	5.0	-	+	-	-	-	-
Longhorn	5.0	+	+	+	+	+	+

"+" treatment conducted
"-" treatment not conducted

5.2.3.2.3 Analytical Methods

Perchlorate analysis procedures are described in **Appendix X**.

Total volatile solids (TVS) content offers a rough approximation of the amount of organic matter content in wetland sediments. The standard method 2540 G (APHA et al., 1998) was followed to determine volatile solids in semisolid samples.

5.2.3.2.4 Kinetic Data Analysis

Microbial degradation rates were calculated by assuming first-order kinetics. Microcosm degradation curves were fitted using non-linear regression by Sigmaplot software (SPSS Inc.) to the first-order kinetic equation:

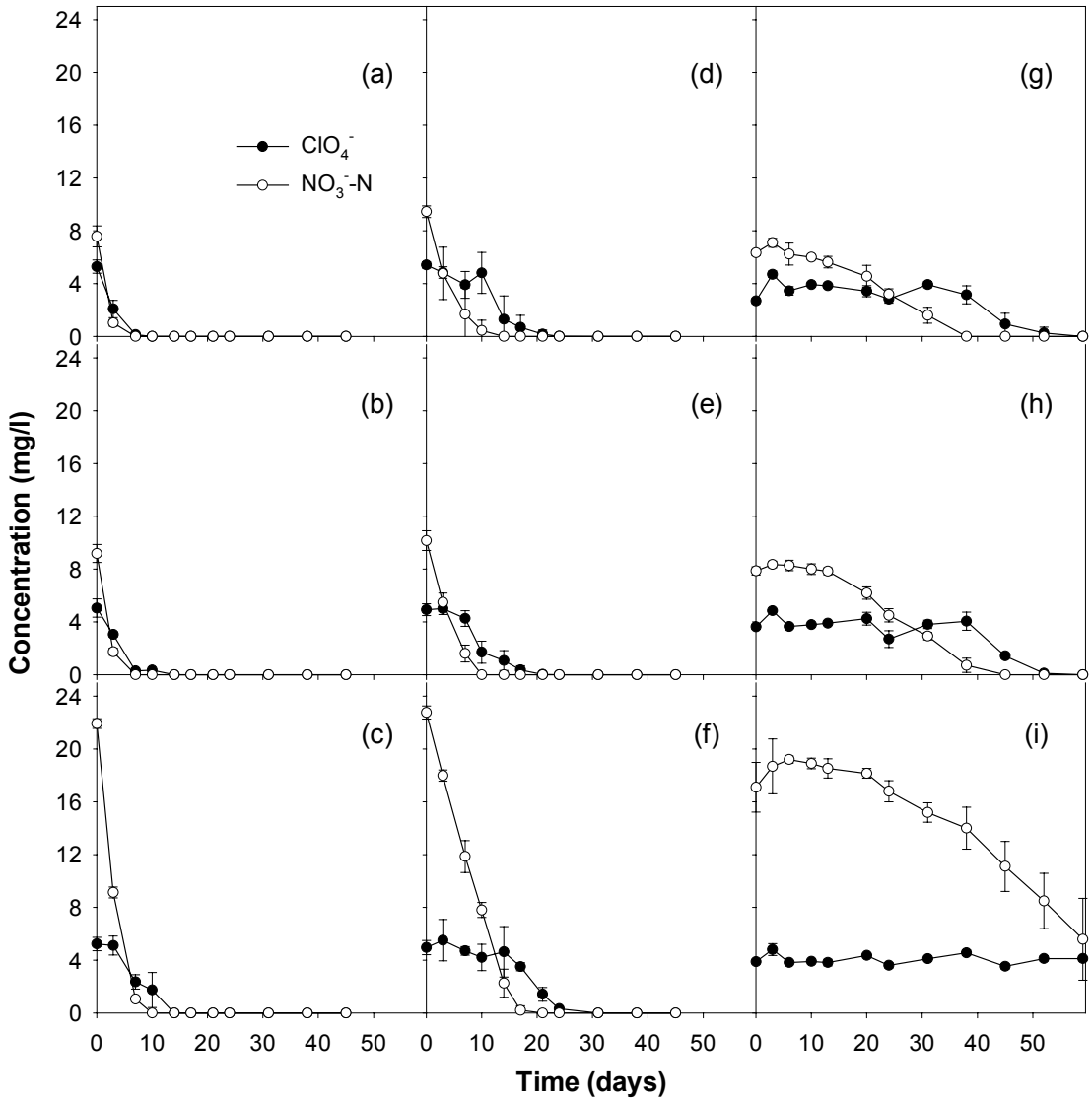
$$C = C_0 \exp(-kt)$$

where C = perchlorate concentration (ppm or ppb), t = time (day), C_0 = initial perchlorate concentration (ppm or ppb), and k = kinetic rate (day^{-1}).

5.2.3.3 Data

5.2.3.3.1 Degradation Curves of Perchlorate and Nitrate

The microcosm degradation curves of perchlorate and nitrate under different initial NO_3^--N for sediments and soil from three locations are presented in **Figure 5-121**. The results of three treatments are compared, including control (no amendment), 1.1 ppm NO_3^--N spiked, and 11.3 ppm NO_3^--N spiked. All treatments were at the same initial ClO_4^- concentration of 5 ppm. These treatments were chosen to cover NO_3^--N ranges from approximately 5 ppm to 25 ppm, corresponding to the nitrate concentration at a typical perchlorate-contaminated site.



HW84 Sidestream: (a) control, (b) 1.1 ppm NO_3^--N spiked, and (c) 11.3 ppm NO_3^--N spiked
HW84 Mainstream: (d) control, (e) 1.1 ppm NO_3^--N spiked, and (f) 11.3 ppm NO_3^--N spiked
Longhorn: (g) control, (h) 1.1 ppm NO_3^--N spiked, and (i) 11.3 ppm NO_3^--N spiked.
Error bars indicate the standard errors of the means for n = 3.

Figure 5-121
Degradation of Perchlorate and Nitrate Spiked at Different Nitrate Levels for Sediments and Soils from Three Locations

For all three treatments, perchlorate and nitrate were degraded to below the reporting limit (4 ppb) during the experimental period for HW84 Sidestream (**Figure 5-121** a, b, and c) and HW84 Mainstream (**Figure 5-121** d, e, and f). For Longhorn soil, perchlorate and nitrate were degraded to below the reporting limit within 60 days with up to 1.1 ppm NO_3^--N spiked (**Figure 5-121**). However, in the case of 10 ppm NO_3^--N spiked in Longhorn soil, ClO_4^- degradation did not occur during the 60 days of the experimental period and NO_3^--N (about 6 ppm) was still above the ClO_4^- concentration (approximately

4 ppm) on day 60 (**Figure 5-121**). There was less than 5% ClO_4^- degradation observed for all the autoclaved control treatments during the 60 days of the experimental period (data not shown).

No major difference was observed between the degradation curves with 1.1 ppm NO_3^--N spiked (**Figure 5-121** b, e, and h) and those of control treatments (**Figure 5-121** a, d, and g), which was probably due to the elevated initial nitrate background level in sediments and soil (6.3 mg/L to 9.4 mg/L as N), such that the small amount of NO_3^--N addition could not exert an obvious effect. Significant differences were observed for the degradation curves in treatments spiked with 11.3 ppm NO_3^--N (**Figure 5-121** c, f, and i).

For HW84 Sidestream (**Figure 5-121** a, b, and c), simultaneous perchlorate reduction and nitrate reduction were observed during the experimental period. For HW84 Mainstream and Longhorn soil, it was observed that rapid ClO_4^- degradation did not happen until NO_3^--N was degraded to a relatively low level (normally below the ClO_4^- concentration) (**Figure 5-121** d, e, f, g, h, and i). After the beginning of ClO_4^- degradation, simultaneous perchlorate reduction and nitrate reduction were observed. This suggested an interference effect of nitrate on perchlorate degradation. On the other hand, by examining all the control treatments (**Figure 5-121** a, d, and g), it was obvious that perchlorate and nitrate degradation curves followed different patterns for different sediments and/or soil. This significant difference of degradation behavior might be linked with the variation of organic substrate availability, bacterial population, and initial nitrate concentration in individual sediments and/or soils.

5.2.3.3.2 Degradation Kinetics and Lag Times

Reported half-saturation constants (K_s) for heterotrophic perchlorate reducers ranged from 45 mg/L to 470 mg/L (Logan et al., 2001), and K_s with respect to NO_3^--N was estimated to be 1.4 mg/L (Aesoy and Odegaard, 1994). With respect to the initial concentration of ClO_4^- (5 mg/L) and NO_3^--N (above 5 mg/L) in our study, ClO_4^- and NO_3^--N degradation would be expected to follow first-order and zero-order kinetics, respectively. The shape of degradation curves (**Figure 5-121**) supports this hypothesis. It appears that NO_3^--N degradation normally complied with zero-order kinetics during the initial rapid degradation period when the NO_3^--N concentration was still relatively high, then was followed by a transition to first-order kinetics when the NO_3^--N concentration was degraded to a lower level (below 2 mg/L). Both first-order and zero-order kinetics fit well for NO_3^--N degradation in terms of r^2 (> 0.86). However, first-order rates were selected to use for both ClO_4^- and NO_3^--N degradation for comparison.

Each microcosm treatment was run in triplicate. Data in the exponential decay period of each run were first fitted using nonlinear regression analysis to obtain individual first-order rate constants, which were then averaged to obtain kinetic rate constants of each treatment including standard deviation. Lag time (acclimation period without degradation or with less than 5% degradation loss) of each run was obtained from the degradation curves, and then averaged to obtain the lag time for each treatment. First-order rate constants and lag times determined for perchlorate and nitrate degradation from each degradation curve under different treatment conditions are summarized in **Table 5-19**. In

general, different sites showed variations in perchlorate and nitrate degradation rates and lag times, which were affected by numerous factors, such as organic substrate availability (represented by TVS), initial nitrate concentration, and prior exposure. The effects of these factors are discussed in detail individually in the following section.

Table 5-19
Perchlorate and Nitrate Degradation Kinetic Data for Sediments/Soils

Site	TVS[a] mg/g	Treatment	Initial NO_3^--N mg/L	Rate K_{ClO4-} d^{-1}	Rate K_{NO3-N} d^{-1}	ClO_4^- Lag d	NO_3^--N Lag d
HW84 Sidestream (T18)	115.9 ± 12.2	low nitrate	1	0.36 ± 0.01	0.66 ± 0.04	0.0 ± 0.0	0.0 ± 0.0
		control	7.6	0.37 ± 0.07	0.68 ± 0.05	0.0 ± 0.0	0.0 ± 0.0
		1.1 ppm NO_3^--N	9.2	0.25 ± 0.01	0.57 ± 0.03	0.0 ± 0.0	0.0 ± 0.0
		11.3 ppm NO_3^--N	21.9	0.21 ± 0.05	0.33 ± 0.01	3.0 ± 0.0	0.0 ± 0.0
HW84 Mainstream (T19)	84.5 ± 3.7	control	9.4	0.14 ± 0.02	0.30 ± 0.11	3.3 ± 3.5	0.0 ± 0.0
		1.1 ppm NO_3^--N	10.2	0.16 ± 0.05	0.26 ± 0.02	4.3 ± 2.3	0.0 ± 0.0
		11.3 ppm NO_3^--N	22.8	0.18 ± 0.03	0.13 ± 0.01	14.0 ± 0.0	0.0 ± 0.0
HW317 (T13)	160.5 ± 0.5	control	6.3	0.46 ± 0.04	1.42 ± 0.67	0.0 ± 0.0	0.0 ± 0.0
HW317/MN (T15)	70.6 ± 0.2	control	16.6	0.13 ± 0.05	0.11 ± 0.03	12.0 ± 1.7	0.0 ± 0.0
Longhorn	43.3 ± 5.7	control	6.3	0.16 ± 0.08	0.06 ± 0.01	35.7 ± 4.0	8.0 ± 5.6
		1.1 ppm NO_3^--N	7.9	0.14 ± 0.02	0.07 ± 0.00	35.7 ± 4.0	14.0 ± 0.0
		11.3 ppm NO_3^--N	17.1	NA	0.03 ± 0.01	60.0 ± 0.0	29.7 ± 9.1

Data are average ± standard deviation of triplicate run
[a]based on dry weight
[b]ClO4- degradation not observed during 60 days of study
NA – not available.

5.2.3.3.3 Effect of Organic Substrate Availability

The organic substrate availability, represented here by TVS, was found to be one of the crucial factors affecting microbial degradation rates and lag times. Control treatments for HW317 and HW84 Sidestream had a higher TVS content (160.5 mg/g and 115.9 mg/g, respectively), so both the ClO_4^- and NO_3^--N degradation rate (K_{ClO4^-} ranged from 0.37 d^{-1} to 0.46 d^{-1}; K_{NO3^--N} ranged from 0.68 d^{-1} to 1.42 d^{-1}) were significantly higher than those of other sediments and soil (K_{ClO4^-} ranged from 0.13 d^{-1} to 0.16 d^{-1} ; K_{NO3^--N} ranged from 0.06 d^{-1} to 0.30 d^{-1}) (**Table 5-19**). Additionally, lag times of ClO_4^- and NO_3^--N degradation generally increased with a decrease in TVS content of a similar magnitude as initial NO_3^--N concentration. No lag time was observed for both ClO_4^- and NO_3^--N degradation of control treatments for sediments of HW317 and HW84 Sidestream, but lag times of 35.7 days (ClO_4^- degradation) and 8.0 days (NO_3^--N degradation) were observed for Longhorn soil which had the lowest TVS content (43.3 mg/g) (**Table 5-19**). Compared to organic substrate availability, prior exposure does not appear to be a major factor affecting degradation rates and lag times. Sediment from HW317, which was only intermittently exposed to ClO_4^- (**Table 5-19**), had the highest perchlorate degradation rate (0.46 day^{-1}), while HW317/MN and HW84 Sidestream (which are continuously exposed to ClO_4^-) had relatively lower rates (0.13 and 0.36 day^{-1}). Likewise, lag times appear to

be more related to TVS than prior exposure as decreasing TVS is directly related to increasing lag times.

5.2.3.3.4 Effect of Initial Nitrate Concentration

Results indicated that nitrate was another important factor affecting ClO_4^- degradation. When comparisons were made among different treatments (control, 1.1 ppm NO_3^--N spiked, and 11.3 ppm NO_3^--N spiked) for a specific sediment (i.e. HW84 Sidestream, HW84 Mainstream, Longhorn, respectively), any variation in degradation rates and lag times would be due to the different nitrate concentration present since the organic availability (TVS) did not vary for different treatments of each sediment. Lag times of ClO_4^- degradation increased considerably with the increase in NO_3^--N concentration (HW84 Mainstream (3.3 ~14.0 days), HW84 Sidestream 0 ~3.0 days, and Longhorn soil (35.7 ~ 60.0 days)) (**Table 5-19**). In addition, with the increase in NO_3^--N concentration, ClO_4^- degradation rates for a specific sediment or soil under different treatment conditions were generally constant in terms of magnitude (e.g. HW84 Mainstream (0.14 ~ 0.18 day^{-1}), and Longhorn soil (0.14 ~ 0.16 day^{-1})), but in some cases the ClO_4^- rates declined slightly (HW84 Sidestream (0.37 ~ 0.21 day^{-1})). The slight decrease of K_{ClO4-} for HW84 Sidestream sediments with the increase in nitrate concentrations is probably caused by substrate availability due to simultaneous perchlorate and nitrate degradation throughout the experimental period. Only sediments from this site exhibited simultaneous perchlorate degradation when NO_3^--N concentration was greater than ClO_4^-. As the substrate supply would be relatively constant, it would be shared by populations using each electron acceptor and thus would be reduced in both cases. For all other sites, nitrate was essentially depleted before ClO_4^- degradation commenced and thus ClO_4^- degradation rates remained relatively constant. This implies that HW84 Sidestream sediments contained populations with as high an affinity for ClO_4^- as NO_3^-, or populations that were capable of using ClO_4^- but not NO_3^-. However, for most sediments examined, NO_3^- appears to prevent the use of ClO_4^- as an electron acceptor.

Generally, there was no observed lag time for NO_3^--N degradation in sediments except for the Longhorn soil. The lag phase was absent because acclimated denitrifying microorganisms existed in sediments. Nitrate degradation rates remained almost constant at lower NO_3^--N concentration (control treatment and treatment with 1.1 ppm NO_3^--N spiked), but decreased with 11.3 ppm NO_3^--N spiked (**Table 5-19**). Under the assumption of first-order kinetics, the rates should remain constant. However, the decline of NO_3^--N degradation rates under highly elevated NO_3^--N concentration may be linked with the limiting effect of organic substrate availability. The long lag time observed for Longhorn soil was probably associated with a low initial denitrifier population in the dry soil than that in sediments, and the lowest organic substrate content (TVS) compared to other sediments. Furthermore, NO_3^--N degradation rate constants were higher than ClO_4^- rate constant under the same treatment (i.e. K_{NO3^-N} = 0.68 d^{-1}, and K_{ClO4^-} =0.37 d^{-1} for control treatment of HW84 Sidestream), this might be caused by higher bacterial affinity for nitrate than that of perchlorate (because half saturation constant K_s for nitrate is much smaller than that for perchlorate).

5.2.3.3.5 Effect of Initial Perchlorate Concentration

Since the typical ClO_4^- concentration in the environment is at ppb levels, another microcosm control treatment (no amendment) with ClO_4^- of 0.5 ppm and the same initial NO_3^--N of 7.6 mg/L was conducted for sediment from HW84 Sidestream. Perchlorate degradation rate constants remained the same (K_{ClO4^-} = 0.37 d^{-1}) for both lower and higher initial ClO_4^- concentration. One interesting aspect was that there was a 1 day lag time for ClO_4^- degradation at 0.5 ppm, compared to no lag time for ClO_4^- degradation at 5 ppm. This supports the assumption that the lag time of ClO_4^- degradation was dependent on the relative ratio of a competitive electron acceptor (NO_3^-). A previous study by Attaway and Smith (1993) found that nitrate did not inhibit ClO_4^- reduction (no lag time), perhaps because the ClO_4^- concentration (9 mM, or 896 mg/L) used in the experiment was much higher than the nitrate concentration (9 mM, corresponding to NO_3^--N of 126 mg/L).

5.2.3.3.6 Effect of Sulfate Concentration

The effect of sulfate on ClO_4^- degradation was examined using microcosm treatment of 300 ppm SO_4^{2-}. It was found that sulfate did not exert an obvious effect on ClO_4^- degradation, which was consistent with previous findings (Attaway and Smith, 1993; Herman and Frankenberger, 1998; 1999). Compared to control treatments, ClO_4^- degradation rate constants at 300 ppm SO_4^{2-} remained almost constant, and no significant variation in lag time was observed. For example, K_{ClO4^-} was 0.14 d^{-1} and 0.15 d^{-1}; lag time of ClO_4^- degradation for sediment HW84 Mainstream was 3.3 day and 4.3 day, for the control treatment and treatment of 300 ppm SO_4^{2-}, respectively.

5.2.3.4 Discussion

Perchlorate reducing bacteria are believed to be distributed ubiquitously in the environment (Coates et al., 1998; 1999; Wu et al., 2001; Logan, 1998; 2001). Microcosm studies, using site sediment and water collected from four natural habitats near NWIRP, McGregor, have indicated that rapid intrinsic bio-remediation is possible in the stream sediments which are continuously or intermittently exposed to perchlorate. Microcosm treatments using soil from another perchlorate-contaminated site, LHAAP also were capable of ClO_4^- degradation although a long lag period (up to 60 days) may be necessary, depending on the environmental conditions. Intrinsic ClO_4^- degradation rates ranged from 0.13 day^{-1} to 0.37 day^{-1} for four sediments, corresponding to a half-life ($t_{1/2}$ = 0.693/k) range of 1.9 days to 5.0 days, with variation of rates depending mainly on the organic substrate availability.

Although it is clear that nitrate does interfere with ClO_4^- degradation, to date the pathway and mechanism involved is poorly understood. Most, but not all, perchlorate reducers could use nitrate as electron acceptor (Coates et al., 1999), and some denitrifying bacteria are capable of ClO_4^- degradation (Logan et al., 2001). Recently, Giblin and Frankenberger (2001) held the view that separate terminal reductases capable of reducing other electron acceptors were responsible for ClO_4^- and nitrate degradation in an isolated bacterium strain. However, the strain grew more rapidly with nitrate. Our results supported this hypothesis. Based on our results, we could extrapolate that the microbes in the sediments and soil from our study can use both ClO_4^- and nitrate as alternative

electron acceptor, depending on the relative electron equivalence ratio. In the presence of relatively high nitrate concentration, the bacteria will preferentially use nitrate as electron acceptor because the growth on nitrate is much faster. After nitrate has been depleted, the ClO_4^- reductase will function to use ClO_4^- as electron acceptor. Thus, the presence of nitrate only affects the lag time of ClO_4^- degradation under the assumption that organic substrate availability is not a limiting factor. The fact that ClO_4^- degradation rate (K_{ClO4-}) of HW84 Sidestream remained almost constant even if the NO_3^--N was lowered to 1.0 mg/L (**Table 5-19**), compared to 7.6 mg/L in the control treatment, also supported this assumption. If only one enzyme was involved, perchlorate and nitrate would become competitive inhibitors, and we would see a significant increase of ClO_4^- degradation rate because of the depletion of the competitive electron acceptor nitrate.

From the point view of thermodynamics in terms of energy yield of electron acceptors, perchlorate's energy yield (Gibb's free energy per electron equivalent $\Delta G_0' = -112.1$ kJ/e$^-$) as an electron acceptor is similar to that of nitrate ($\Delta G_0' = -112.2$ kJ/e$^-$), when hydrogen is used as the electron donor (Nerenberg et al., 2002). This implies that denitrification is not more energy-favorable than perchlorate degradation. The preference of ClO_4^- to NO_3^- as electron acceptor should be associated with different enzyme involved which lowered the activation energy. Further research should be conducted to fully understand the metabolism involved. The presence of nitrate may explain the persistence of ClO_4^- in the environment, especially when ClO_4^- concentration is considerably lower that that of nitrate concentration (i.e. in the most case of groundwater), which may require a relatively longer lag time for ClO_4^- degradation to happen.

Our studies also indicate that higher organic substrate availability can shorten the lag time of both ClO_4^- and nitrate degradation. Sediment HW317 was not continuously exposed to ClO_4^-, but it had the highest ClO_4^- and nitrate degradation rates (**Table 5-19**). This might imply one of the effective enhancement strategies of ClO_4^- natural attenuation by substrate amendment.

An attempt was made to correlate environmental conditions (i.e. organic substrate availability (represented by TVS), and/or nitrate concentration) with degradation rates and lag times. In general, no correlations were found, probably due to limited data as well as the complexity of the environmental system. Perchlorate degradation in the sediments and soil is affected by numerous environmental conditions (i.e. substrate, perchlorate concentration, population of perchlorate reducers, nitrate, physics of sediments and soil, temporal and spatial etc.) and other factors.

This study explores the intrinsic kinetics of ClO_4^- degradation in the natural sediments and soil, including the role of nitrate. More intensive *in situ* studies may be necessary to understand the effects of numerous other environmental conditions on the degradation of perchlorate in the natural system.

5.3 PLANTS

5.3.1 Laboratory Studies

5.3.1.1 Introduction

Evaluation of the potential movement of contaminants from water and/or soil to vegetation is critical for determining exposure routes to wildlife and humans. The conditions under which contaminants move from soil/sediment to plants are often initially characterized in the laboratory or greenhouse where the environment is more easily controlled and plant species can be selected for comparative purposes. Perchlorate is very water soluble and likely to move readily to vegetation. The uptake and distribution of perchlorate in plants was evaluated in short-term laboratory experiments. These tests provided the basis for determining uptake factors for perchlorate in the environment.

5.3.1.2 Methodology

5.3.1.2.1 Reagents and Standards

A 100 µg/mL certified sodium perchlorate solution (Accustandard, Inc) was purchased to prepare perchlorate calibration standards. Sodium hydroxide was purchased from Fisher Scientific and diluted to make the eluent required for the ion chromatography. Hydrosol is a diluted solution of Peters All Purpose Plant Food.

5.3.1.2.2 Uptake Experiments

Cucumber, lettuce, and soybean were selected for the uptake experiments. Seeds of plants were placed in polystyrene cups containing 50 g or 100 g Ottawa sand (Fisher Scientific) and grown in an incubator at 22 °C (15:9 light:dark photoperiod). The cups were covered with Petri dishes to avoid excessive loss of water. Our initial studies indicated that plant germination was not affected by up to 1000 ppb perchlorate (ng perchlorate/g sand). Since 100 ppb is a more environmentally relevant concentration, all uptake experiments were conducted at this concentration. The experimental design varied according to different plant species. For cucumber, three four-week experiments were conducted in the presence of varying ratios of Hydrosol: (1) 100% Hydrosol, (2) Hydrosol:Milli-Q water (50:50) and (3) Hydrosol: Milli-Q water (25:75). Due to our concern about the lack of nutrition for proper plant growth, another experiment in which cucumber was watered with 100% Milli-Q water lasted only two weeks. An eight-week cucumber uptake study was also conducted in which the sand was respiked with perchlorate after week 4. Lettuce was grown in the presence of 100% Hydrosol for six weeks. For soybean, two four-week experiments were conducted: (1) 100% Hydrosol, and (2) Hydrosol: Milli-Q water (50:50). There were four plants in each treatment group at every sampling point. Four control plants of the appropriate species (no perchlorate) were included for each of the respective uptake experiments. An additional control with perchlorate and no plant was sampled each week to assess possible microbial transformation of perchlorate in the sand.

Plants were removed each week and sectioned into two parts: portion of plant above sand level (primarily leaves) and portion of plant below sand level (primarily roots). Each portion of the plant sample was weighed, rinsed with water, and allowed to dry prior to extraction (described below). The amount of perchlorate remaining in sand was also determined weekly by adding a known volume of Milli-Q water (18MΩ) to the cups, and extracting the contents by mechanical agitation. Sand extracts were analyzed by ion chromatography (described below).

5.3.1.2.3 Tissue Extraction and Extract Cleanup

All plants were extracted in 11-mL cells using Milli-Q water (18MΩ) with a Dionex (Sunnyvale, CA) Accelerated Solvent Extractor (ASE 200) using the following procedure (Anderson and Wu, 2002). Cells were heated for 5 min at 100° C, filled with Milli-Q water, and pressurized to 1500 psi. Total extraction time was 15 min. At the completion of the extraction procedure, extract volume was recorded. For all plants, 1.0 mL of extract volume was cleaned with alumina solid phase extraction (SPE) cartridges and then diluted to 5 mL with Milli-Q water. Finally, the diluted extracts were filtered with a 0.45 µm Acrodisc® into 5-mL ion chromatography vials.

5.3.1.2.4 Analysis

All samples (plasma extracts, tissue extracts, and surface water) were analyzed by ion chromatography similar to EPA Method 314 (See **Appendix X** for specific procedures).

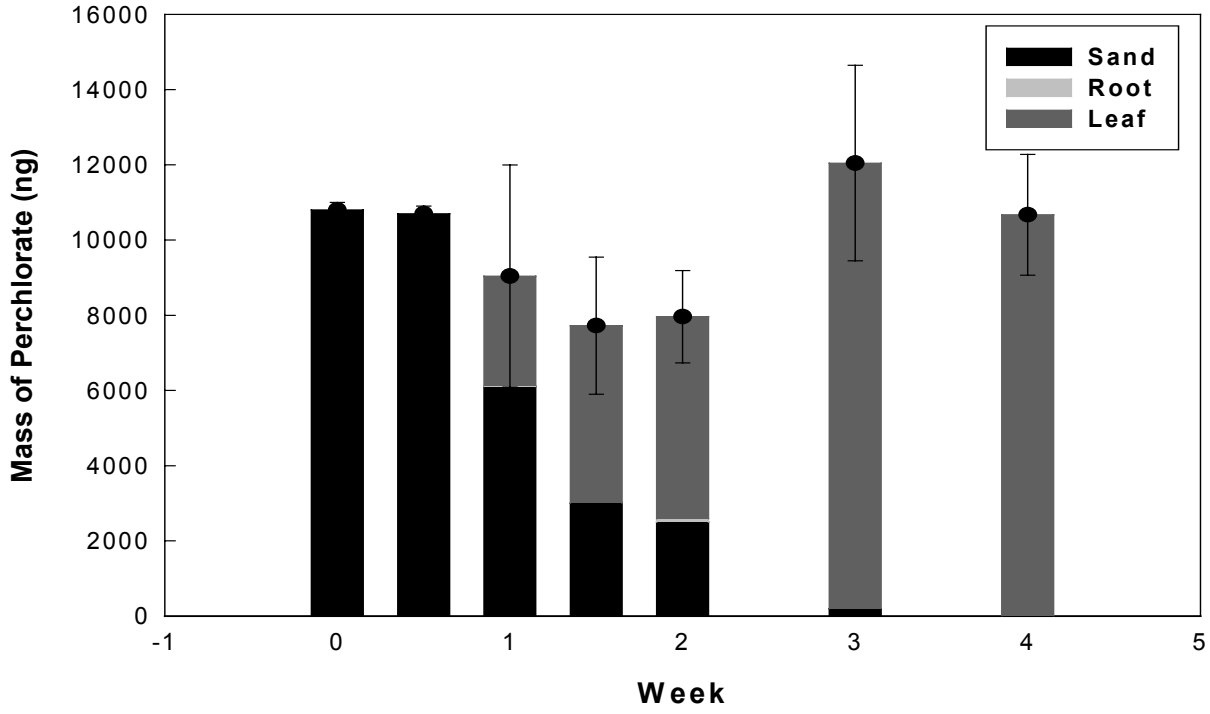
5.3.1.2.5 Data Analysis

All statistical tests were conducted using SAS software (Version 8). Comparisons of mean perchlorate concentrations between planted sand and unplanted sand were performed using Student's t-test. Two-way analysis of variance (ANOVA) was conducted to evaluate the effect of species on perchlorate uptake; Duncan's multiple range test was used after the ANOVA.

5.3.1.3 Data

Analysis of control samples (planted and unplanted cups without perchlorate) at the end of each experiment indicated that perchlorate was not detected in planted sand, roots, or leaves. In addition, loss of perchlorate from sand in the unplanted controls was negligible. For the uptake experiments, perchlorate was removed from sand by plants (**Figure 5-122**) and taken up in aboveground vegetation (**Table 5-20**) in all of the tests conducted.

In the four-week cucumber uptake experiment in the presence of Hydrosol, perchlorate concentration in the planted sand decreased to ND at Week 3. There was an 11% decrease in the unplanted sand during the same four weeks. Considerable uptake of perchlorate into cucumber leaves (**Table 5-20**) was observed. Similar results were obtained for the lettuce and soybean perchlorate uptake experiments. Comparisons of planted sand and unplanted sand in each of these experiments showed significant differences in perchlorate concentrations (P ranged from <0.0001 to 0.0401). There was only a slight decrease of perchlorate in unplanted sand over time, suggesting that microbial transformation and chemisorption/physisorption/ion exchange of perchlorate in the experimental system was negligible.



Error bars represent one standard deviation of total perchlorate

Figure 5-122
Perchlorate Distributions among Soil, Root and Leaves from a Four-week Cucumber (*Cucumis sativus* L.) Perchlorate Uptake Experiment in the Presence of Hydrosol:Milli-Q Water (25:75)

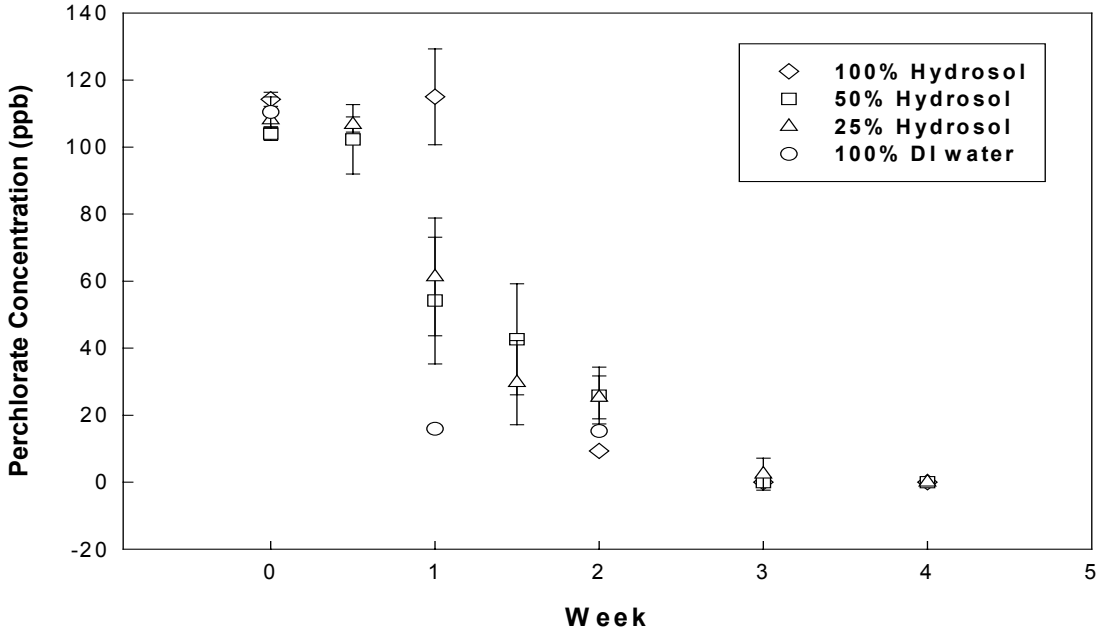
Table 5-20
Distribution of Perchlorate in the Test Systems

Plants	Sample Time	Perchlorate Concentration (ppb) ± SD				Mass of Perchlorate in Plant System (ng) ± SD
		Unplanted Sand	Planted Sand	Leaves	Root	
Cucumber						
Hydrosol(H)*	Week 0	111 ± 5 [c]	114 ± 2 [c]	NA	NA	11200 ± 250 [c]
	Week 1	NA	115 ± 14 [b]	35000 ± 9050 [b]	261200 ± 38900 [b]	13100 ± 1640 [b]
	Week 2	108 ± 0.68 [b]	9 [a]	20670 [a]	150800 [a]	2740 [a]
	Week 3	NA	ND [c]	22080 ± 17140 [c]	ND [c]	1200 ± 900 [c]
	Week 4	99 [a]	ND [c]	41060 ± 32010 [c]	ND [c]	2260 ± 1660 [c]
H*:Milli-Q (50:50)	Week 0	104 ± 2 [c]	104 ± 2 [c]	NA	NA	10400 ± 200 [c]
	Week 0.5	102 ± 10 [c]	102 ± 10	NA	NA	10200 ± 940 [c]
	Week 1	94 [a]	54 ± 19 [d]	98000 ± 44690 [d]	313900 ± 51400 [d]	8330 ± 3550 [d]
	Week 1.5	100 [a]	43 ± 17 [d]	159900 ± 33530 [d]	ND [d]	8350 ± 1780 [d]
	Week 2	106 [a]	26 ± 8 [d]	267700 ± 19190 [d]	ND [d]	12000 ± 1700 [d]
	Week 3	65 [a]	ND [d]	126500 ± 56960 [d]	ND [d]	6500 ± 3100 [d]
	Week 4	85 [a]	ND [d]	139000 ± 20550 [d]	ND [d]	9250 ± 1130 [d]

Plants	Sample Time	Perchlorate Concentration (ppb) ± SD				Mass of Perchlorate in Plant System (ng) ± SD
		Unplanted Sand	Planted Sand	Leaves	Root	
H*:Milli-Q (25:75)	Week 0	108 ± 2 [c]	108 ± 2 [c]	NA	NA	10800 ± 250 [c]
	Week 0.5	107 ± 2 [c]	107 ± 2 [c]	NA	NA	10670 ± 170[c]
	Week 1	99 ± 7 [c]	61 ± 18 [b]	127400 ± 31060 [b]	5500 ± 77770 [b]	9450 ± 3460 [b]
	Week 1.5	109 [a]	30 ± 13 [d]	201500 ± 22320 [d]	ND [d]	7550 ± 1600
	Week 2	108 [a]	25 ± 6 [d]	190600 ± 16030 [d]	9400 ± 18800 [d]	8000 ± 1460
	Week 3	72 [a]	2 ± 5 [d]	314400 ± 55700 [d]	ND [d]	12000 ± 2900
	Week 4	102 [a]	ND [d]	219100 ± 32950 [d]	ND [d]	10600 ± 1520
Milli-Q water	Week 0	110 ± 5 [b]	110 ± 5 [b]	NA	NA	5500 ± 230 [b]
	Week 1	85 [a]	16 ± 0.90 [b]	118100 ± 11280 [b]	ND [b]	4150 ± 520 [b]
	Week 2	98 ± 4 [b]	15.21 [a]	202400 [a]	ND [a]	1140[a]
Hydrosol*	Week 0	106 ± 4 [c]	106 ± 4 [c]	NA	NA	10600 ± 420[c]
	Week 0.5	96 ± 3 [d]	96 ± 3 [d]	NA	NA	9670 ± 1100 [d]
	Week 1	94 ± 5 [b]	55 ± 4 [c]	81600 ± 13550[c]	ND [c]	7840 ± 990 [c]
	Week 1.5	128 [a]	41 ± 9 [d]	114400 ± 7190 [d]	19500 ± 39000 [d]	8120 ± 1890 [d]
	Week 2	85 [a]	57 ± 19 [d]	99900 ± 13830 [d]	33700 ± 67300 [d]	10140 ± 3770 [d]
	Week 3	96 [a]	22 ± 16 [d]	142100 ± 10500 [d]	ND [d]	10870 ± 3160 [d]
	Week 4	62 ± 3 [b]	6 ± 5 [c]	101700 ± 45350 [c]	ND [c]	7650 ± 2930 [c]
	Week 5	184 [a]	36 ± 22 [b]	119400 ± 61930 [b]	ND [b]	19150 ± 6770 [b]
	Week 6	164 [a]	47 ± 22 [c]	146600 ± 900 [c]	ND [c]	20100 ± 5580 [c]
	Week 7	238 [a]	45 ± 20 [d]	102100 ± 9300 [d]	ND [d]	17120 ± 4870 [d]
	Week 8	149 [a]	20 ± 17 [d]	79800 ± 31300 [d]	ND [d]	14300 ± 3560 [d]
Lettuce						
Hydrosol*	Week 0	69 ± 2 [c]	69 ± 2 [c]	NA	NA	3440 ± 80 [c]
	Week 1	70 [a]	72 ± 2 [e]	115700 [a]	ND [a]	5170 ± 70 [e]
	Week 2	82 [a]	68 ± 8 [e]	241300 ± 16640 [d]	ND [a]	5100 ± 890 [d]
	Week 3	55 [a]	72 ± 19 [e]	70300 ± 23200 [e]	ND [e]	6110 ± 1560 [e]
	Week 4	43 [a]	8 ± 11 [e]	753800 ± 32600 [e]	ND [e]	5200 ± 1640 [e]
	Week 5	79 [a]	ND [e]	20760 ± 6240 [e]	19250 [a]	2480 ± 450 [e]
	Week 6	71 [a]	ND [c]	31900 ± 14370 [c]	ND [a]	1690 ± 170 [c]
Soybean						
Hydrosol*	Week 0	12 ± 3 [c]	128 ± 3 [c]	NA	NA	6420 ± 150 [c]
	Week 1	114 ± 9 [d]	83 ± 8 [d]	13000 ± 6300 [d]	9300 ± 18600 [d]	5940 ± 910 [d]
	Week 2	77 ± 3 [d]	11 ± 10 [d]	15300 ± 3600 [d]	28800 ± 22600 [d]	3640 ± 1770 [d]
	Week 3	62 ± 5 [d]	8 ± 2 [c]	17800 ± 3950 [c]	ND [c]	2720 ± 480 [c]
	Week 4	67 ± 4[d]	18 ± 16 [d]	14500 ± 3050 [d]	ND [d]	2800 ± 1550 [d]

NA = sample was not collected at this time point
ND = perchlorate was not detected
[a] = sample size (n = 1)
[b] = sample size (n = 2)
[c] = sample size (n = 3)
[d] = sample size (n = 4)
[e] = sample size (n = 5)
Week 0 indicates immediate extraction after perchlorate application.
*Diluted solution of Peters All Purpose Plant Food. Main components include nitrate, phosphate, magnesium, iron, copper, manganese, zinc, and molybdenum.

In this study, the potential effects of external nutrients were assessed by comparing perchlorate concentrations in planted sand, leaves, and roots grown in the presence of various ratios of Hydrosol to Milli-Q water. Evidence indicated that increased nutrient levels decreased the rate of perchlorate uptake into vegetation. For the cucumber experiments, sand concentrations of perchlorate in the presence of plants grown with 100% Hydrosol decreased the slowest, while those in 100% Milli-Q water decreased the fastest. Concentrations of perchlorate in the sand of experiments conducted with mixtures of Hydrosol and water decreased at a rate between those of pure Hydrosol and pure water (**Figure 5-123**). Consistent with the sand data, perchlorate uptake in leaves was the greatest when Hydrosol was not used to water the plants (**Table 5-20**). Although these perchlorate concentration differences in leaves may be due to decreased leaf mass because of a lack of nutrient(s), there is still an increased potential hazard for higher organisms due to the perchlorate concentration achieved in cucumber leaves. The differences in uptake could be due to certain nutrients in Hydrosol, especially nitrate, which may compete with perchlorate for uptake into plants; the presence of nitrate may essentially block perchlorate uptake. It is possible that perchlorate could only be taken up after most of the nitrate is removed. With fewer of these nutrients in sand, perchlorate was more available to accumulate in the plant. Due to the sampling protocol (weekly), perchlorate was rarely detected in roots. More frequent sampling of roots would be necessary to detect perchlorate before it translocates to leaves. A similar competition effect was observed in the soybean experiments, although in contrast to the cucumber experiments, the removal of perchlorate from sand occurred almost at the same rate for 100% Hydrosol and Hydrosol:Milli-Q water 50:50.



Error bars represent one standard deviation of the mean.

Figure 5-123
A Comparison among Sand Concentrations of Four Cucumber Perchlorate Uptake Experiments in the Presence of Varying Ratios of Hydrosol to Water

In the eight-week perchlorate uptake study, samples were respiked with 100 ppb perchlorate after Week 4. Concentrations in leaves peaked at Week 3 around 150 ppm and then peaked again at Week 6 (around 150 ppm) after the respike. It appears that the plants reached a maximum threshold of perchlorate, especially since the sand data indicated that there was still perchlorate available for uptake. This suggests that plants do not have the ability to hyperaccumulate perchlorate once a maximum burden in leaves is reached. At that point, the plant may begin to exude, transform, or transpire perchlorate. In the lettuce experiment, perchlorate concentrations in the leaves reached 750 ppm at Week 4 and then decreased to 20 ppm at Week 5, further supporting the idea of perchlorate exudation, transformation, or transpiration from leaves.

Although accumulation of perchlorate in leaves was observed in all plant species, there were significant differences in the ability of plants to take up perchlorate. Perchlorate concentrations in leaves were compared among cucumber, lettuce, and soybean experiments all treated identically (100% Hydrosol). Lettuce exhibited the highest accumulation of perchlorate at 750 ppm, followed by cucumber (41 ppm), and soybean (18 ppm). Lettuce showed a greater ability to accumulate perchlorate than the other two species. This difference is not completely explained by less plant mass for lettuce (the denominator in the concentration calculation) compared to the other two species. Significant differences ($P < 0.0001$) were observed for comparisons between perchlorate concentrations in lettuce vs. cucumber and soybean. There was no significant difference in perchlorate concentrations between cucumber and soybean.

Condensation was observed on Petri dishes covering the cups containing plants. This condensation was included in our analysis. Since water was only observed on Petri dishes covering the cups containing plants, the condensation is not due to evaporation from the sand, but rather transpiration from plant leaves. In the cucumber experiments, each water sample collected from the Petri dishes contained perchlorate, while in the soybean experiments, none of the water samples contained perchlorate. Further studies are needed to explain this process, but it appears that some plants can transpire perchlorate.

A perchlorate mass balance was calculated for each plant uptake experiment. In some experiments, there was an 80% loss of total mass by the end of the experiment. In other experiments, there was little perchlorate lost (< 10%) during the study period (**Figure 5-122**). The primary reason for differences in mass balance among the experiments appears to be due to excessive rinsing of dried samples. After drying, leaves and roots were rinsed with Milli-Q water before extraction to remove perchlorate attached to the external surface of plants. Rinse water was analyzed for perchlorate. If there was perchlorate present in the rinsate, the plant was rinsed again until no perchlorate was found in the rinse water. In some of the first experiments, plants were rinsed several times before extraction. Because of its high water solubility, perchlorate may migrate from plants to water during rinsing which would remove perchlorate from plants and contribute to loss of total perchlorate mass. Therefore, experimental uncertainty was increased in data from the cucumber experiments in pure Hydrosol and pure water. Other factors that may also contribute to the loss of perchlorate include: (1) plant-mediated

transformation of perchlorate to chloride, and (2) expiration of perchlorate with transpiration water from plant leaves.

5.3.1.4 Discussion

Cucumber, lettuce, and soybean demonstrated their potential to take up perchlorate from contaminated sand. There was a significant perchlorate concentration burden for cucumber and lettuce. Results also suggest perchlorate depletion from sand and subsequent uptake into leaves is strongly influenced by the presence of nutrients in the sand. Plant species also affected perchlorate accumulation; the highest perchlorate concentration was achieved in lettuce. Plants in perchlorate-contaminated areas or crops grown with perchlorate-contaminated water represent a significant route of perchlorate exposure to higher organisms, including humans. However, external nutrients in soil appear to reduce the levels of perchlorate in plants. There was some evidence that perchlorate may be transpired by plants through evapotranspiration.

5.3.2 Perchlorate Uptake by Algae

5.3.2.1 Delta Areas

5.3.2.1.1 Introduction

Algae sampling was originally intended to be performed in the Lake Belton delta area to assess the possibility of algae and associated algae blooms taking up perchlorate and becoming a transport mechanism for perchlorate in this lake. Algae sampling was not performed in Lake Waco based on the project team's assumption that Lake Waco is a relatively well mixed, homogeneous environment and algae would not move via preferential pathways in this lake. Lake Waco's shallow configuration, alignment with the prevailing wind direction, and the presence of a mechanical aeration system provided the basis for this assumption. Additionally, higher perchlorate concentrations historically had been detected in the streams tributary to Lake Belton. Therefore, the team hypothesized that if algae did uptake perchlorate, detections of perchlorate in algae in Lake Belton would be more likely than in Lake Waco. This portion of the study was conducted as part of the Delta Areas Study. All the methodologies and protocol followed are detailed in the *Final Lake Belton and Lake Waco Delta Area Field Sampling Plan* (MWH, 2002c). Any deviations from the Field Sampling Plan are discussed further below.

5.3.2.1.2 Methodology

The algae sample collection activities were scheduled during the warmest time of the year when algae blooms would be expected to be prolific (i.e., during the summer). Algae sample collection activities were attempted on June 11, 2003 at all of the delta grid points established in Lake Belton as shown in **Figure 5-18** (Section 5.1.2).

A plankton net (Student Net) was used to collect bulk algae samples as shown on **Figure 5-124**. At each selected grid location, the plankton net was lowered to approximately 3 feet above the lake bottom and raised to the surface to capture algae in the sampler. Upon

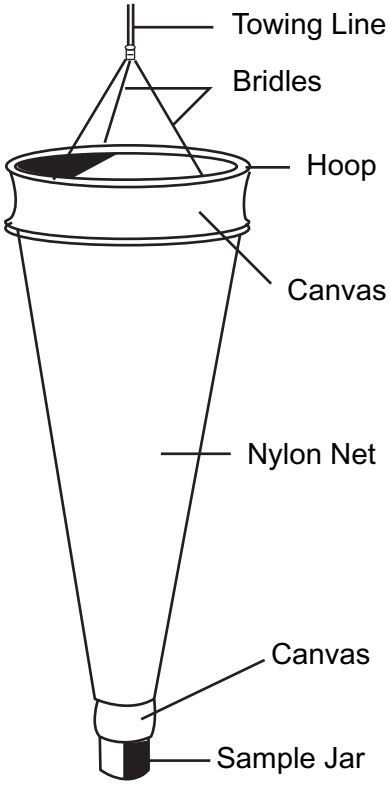
retrieval, the net was rinsed from the outside with distilled water to wash any algae that was adhering to the net into the cup at the bottom of the sampler. The water was allowed to drain from the cup and algae sample transferred to the appropriate sample container. The sampling procedure was attempted several times in order to obtain a sufficient volume of algae for the laboratory to perform the perchlorate analysis (i.e., 100 milliliters). However there were insufficient algae in the lake to collect any appreciable sample volume during this sampling event. Sampling activities were again attempted on August 21, 2003 both within and outside the delta areas. The locations within the delta area are shown in **Figure 5-18** (Section 5.1.2). The various locations outside the delta area where algae sampling was attempted are shown in **Figure 5-125**. The project team attempted to collect algae samples at a total of 80 locations within Lake Belton on two separate occasions and sufficient algae sample quantity was not available at any of the locations.

5.3.2.1.3 Data

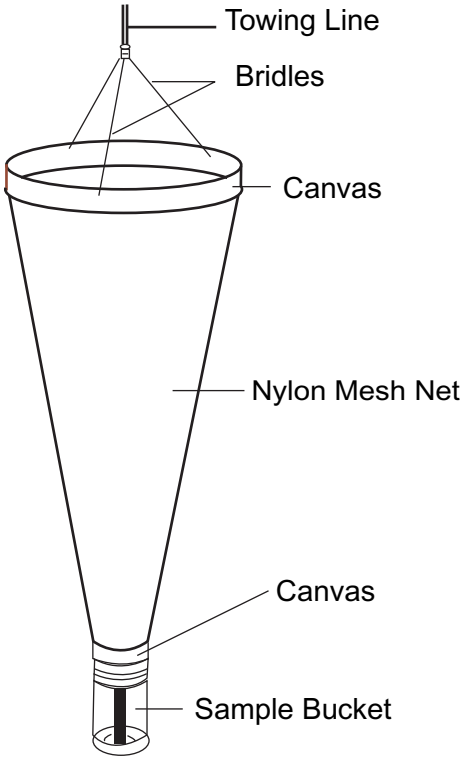
No data were generated as part of this study.

5.3.2.1.4 Discussion

Based on the two extensive attempts to collect algal samples in the summer of 2003, appreciable quantities of algae were not present in Lake Belton during this time. As a consequence, no data to evaluate the potential uptake of perchlorate by algae could be generated. It is not clear if the low occurrence of algae in Lake Belton was unique to these sampling events or is typically of the conditions in Lake Belton.



Student Net



Wisconsin Net

U.S. Army Corps of Engineers		
PROJECT: BOSQUE AND LEON RIVER WATERSHEDS STUDY		
DRAWING TITLE: PLANKTON NET SAMPLERS		
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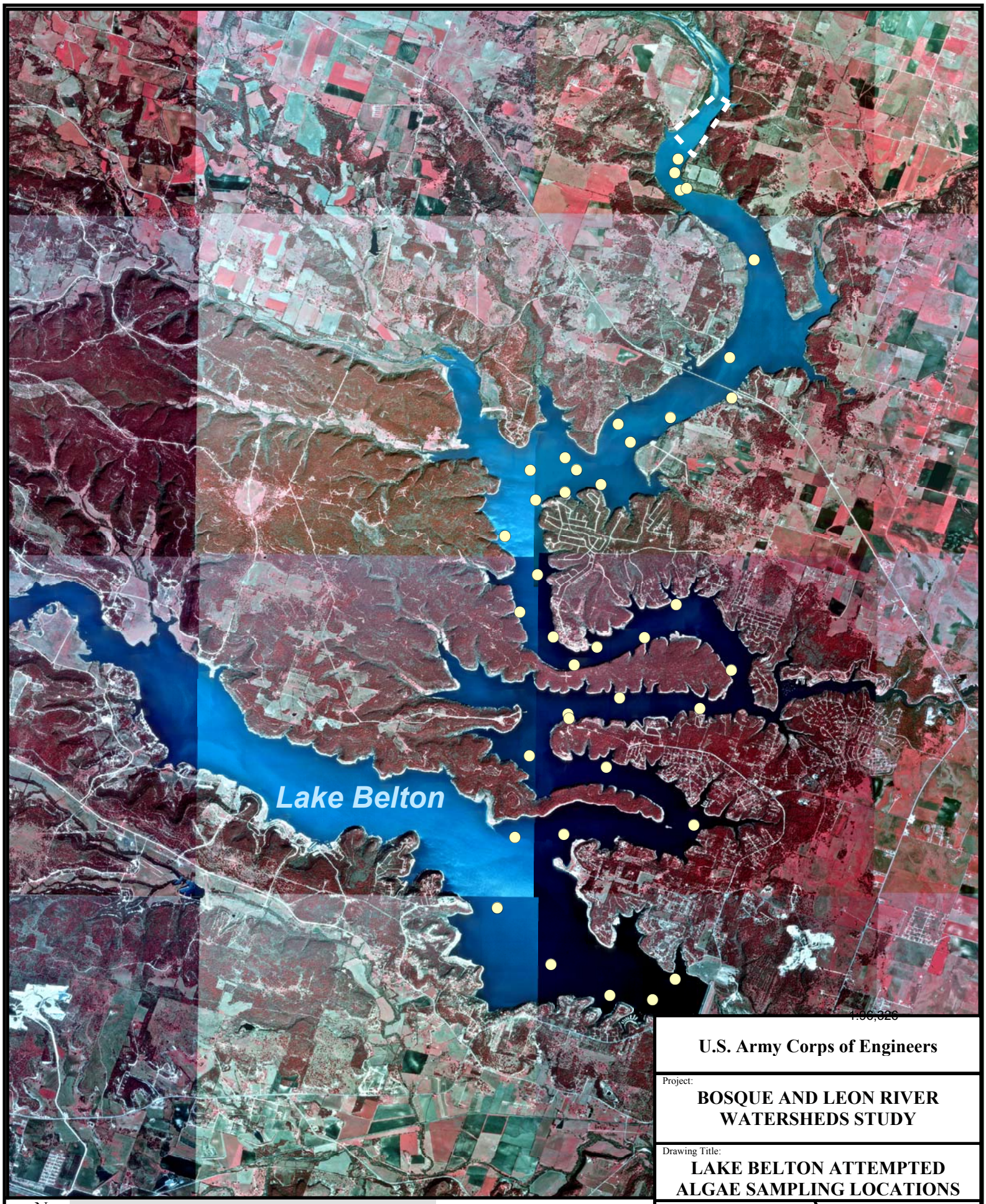
	SCALE No Scale	FIGURE 5-124



U.S. Army Corps of Engineers

Project:
BOSQUE AND LEON RIVER WATERSHEDS STUDY

Drawing Title:
LAKE BELTON ATTEMPTED ALGAE SAMPLING LOCATIONS

N

Legend

- Attempted Algae Sampling Locations
- Delta Area

0 0.5 1 Miles

Sheet 1 of 1 Sheets

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FIGURE: 5-125

5.3.2.2 Stream Periphyton

5.3.2.2.1 Introduction

Routes of perchlorate exposure in aquatic environments was identified as a data gap related to conditions within the study area. For example, comparative analysis of perchlorate accumulation in fish and water from field-collected specimens indicates that perchlorate levels may be higher in fish than in water, but laboratory analysis indicates that perchlorate does not bioconcentrate in fish. This suggests that fish in the field are being exposed by some route(s) other than direct absorption from the water. One possible route of uptake in fish is through the food chain. In most stream ecosystems that have been studied, there is little phytoplankton (floating algae), unlike lakes and larger rivers (Minns, 1995). Thus, primary productivity is due to periphyton (attached algae) growing on rocks and other solid objects. One possible route of exposure for fish is through consumption of periphyton that have taken up and accumulated perchlorate from the water. However, there is little information on uptake of perchlorate in periphyton.

5.3.2.2.2 Methodology

Samples of periphyton ("algae") were collected by grab sampling. Grab samples of algae were collected from at least 3 locations per sampling site, with each location at least 10 m apart. The sampling sites included Coryell Creek at Highway 84 (T36), Station Creek at Highway 107 (T23), the South Bosque at Highway 317 (T16), S Creek at Highway 317 (T15), Harris Creek at U.S. Highway 84 (T19) just west of McGregor, TX, and Wasp Creek at Highway 317 (T2). These sites are shown on **Figure 5-126**. At each location where algae were collected, three 60-ml samples of water were also taken for perchlorate analysis. Periphyton and water samples were kept on ice until transport back to the laboratory, then algae samples were frozen at -20 °C until analysis. Water samples were kept at 4 °C until analysis. Algae were thawed, desiccated under a fume hood, ground in a Warring blender, and extracted with water using accelerated solvent extraction. Algae extracts and water samples were analyzed for perchlorate using ion chromatography (**Appendix X**).

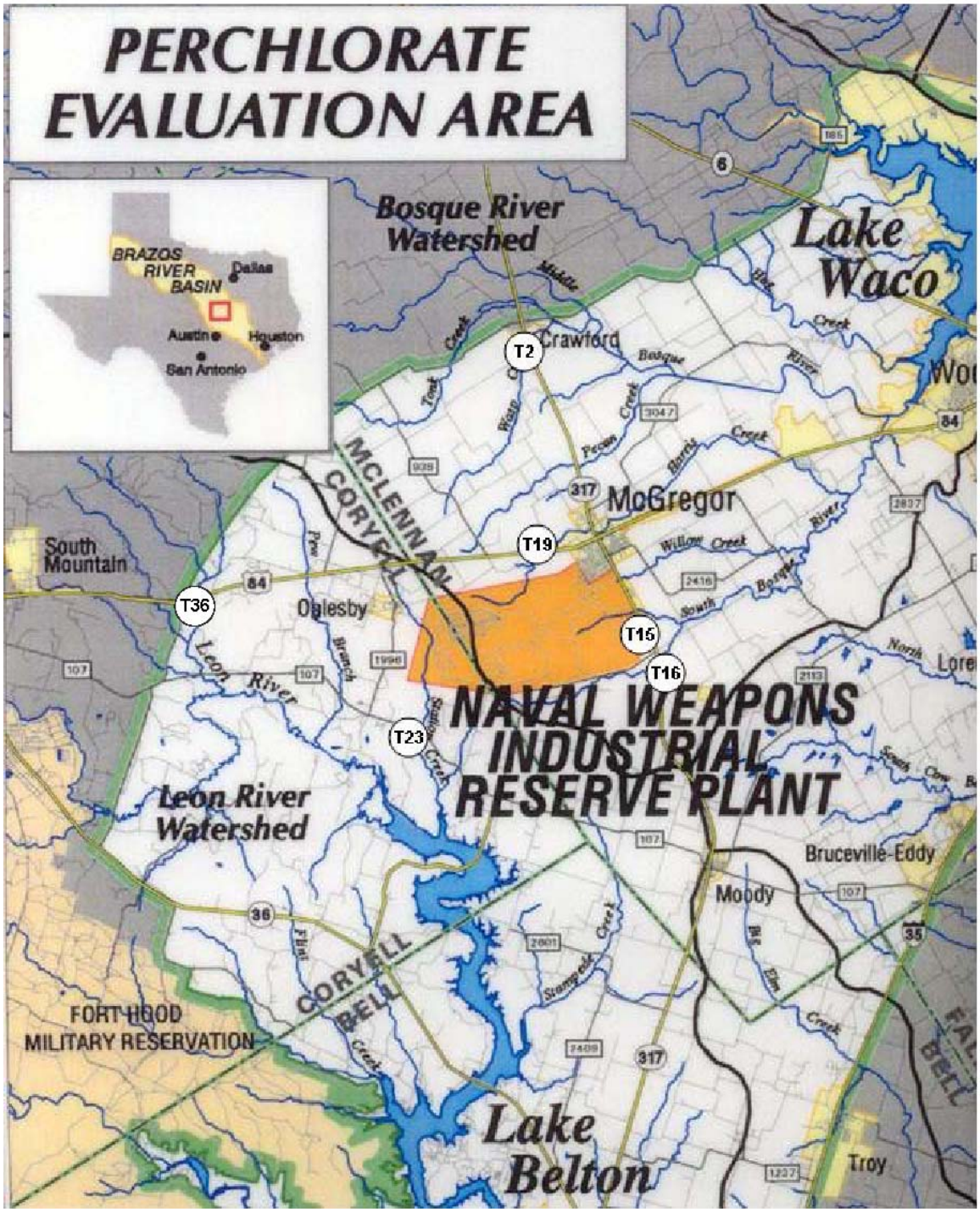

Figure 5-126
Map of the Study Area Illustrating the Approximate Locations where Stream Periphyton Samples Were Collected

5.3.2.2.3 Data

The concentrations of perchlorate in water and algae samples collected are presented in **Table 5-21** and **Table 5-22**, respectively. Concentrations in algae are reported on a dry weight basis. In general, these data indicate that the concentration of perchlorate in algae may be equal to or greater than that found in the water. Bioconcentration factors (concentration in the algae/concentration in the water) for Harris Creek (T19), Station Creek (T23), and S Creek at Highway 317 (T15) were 8.4, 14.7, and 1.0, respectively. A bioconcentration factor could not be calculated for South Bosque at Highway 317 (T16) due to the fact that perchlorate was not detected in the water. However, historical data are available (**Appendix C**) and would suggest a high bioconcentration factor (> 100) for this location.

Table 5-21
Mean ± SD Perchlorate Concentration in Water Samples Collected at Various Streams near NWIRP in March, 2002

Location	Perchlorate (ppb)
Coryell Creek (T36)	ND
Wasp Creek (T2)	ND
Harris Creek at Highway 84 (T19)	24.3 ± 0.3
Station Creek at Highway 107 (T23)	21.9 ± 0.9
S Creek at Highway 317 (T15)	323 ± 4.1
South Bosque at Highway 317 (T16)	ND

n = 3. ND = not detected

Table 5-22
Perchlorate Concentration in Algae within the Waco Watershed

Location	Perchlorate (ppb)
Coryell Creek (T36)	ND
Wasp Creek (T2)	ND
Harris Creek at Highway 84 (T19)	204
Station Creek at Highway 107 (T23)	323
S Creek at Highway 317 (T15)	322
South Bosque at Highway 317 (T16)	376

Perchlorate concentrations are in ng/g (ppb) expressed as tissue wet weight
Samples collected in March 2002
ND = not detected.

5.3.2.2.4 Discussion

Smith et al (2001) demonstrated that terrestrial plants may bioconcentrate perchlorate thousands of times the concentration in the soil. However, such large bioconcentration factors were not found when comparing levels of perchlorate in water to tissue concentrations in emergent vegetation. However, up to now there have been no studies examining the bioconcentration potential of fully submerged aquatic plants. This is

significant because such epiphytic algae species may serve as the primary producers in small streams. The results presented above indicate that epiphytic algae may indeed bioconcentrate perchlorate, hence providing a pathway for food chain transport of perchlorate to higher trophic levels. This may be a direct source of exposure, because many species of fish are specialized for feeding on periphyton, which forms much or all of their diet. Such fish include stonerollers, carp, suckermouth minnows, some suckers and certain darters (Smith, 1979). Other fish are ominivorous, and consume many items, both plant and animal, but still regularly consume periphyton. These fish include many species of minnows and shiners (*Pimephales*, *Notropis*, *Cyprinella*, and *Notemegonus* spp.), several species of suckers, some sunfish, killifish, and mosquitofish (Smith, 1979). Fish may indirectly be exposed to perchlorate accumulated in algae through food chain transfer, because numerous invertebrates also feed upon algae and other periphyton (Wallace and Webster, 1996). These organisms include crayfish, isopods, amphipods, Chrionomids (mayfly larvae), beetle larvae, and snails, all of which are fairly common in central Texas streams and are regularly consumed by at least some of the species of fish collected for analysis (Wallace and Webster, 1996).

The results reported above are notable for several reasons. First, they indicate that fish may be exposed to perchlorate levels much higher than that found in the water, so that monitoring water concentrations is insufficient to assess hazard, potential exposure or effects to fish. Second, these findings may help to explain results in Section 5.4.1, in which perchlorate was found in fish but not water; i.e., that food chain exposure via periphyton may contribute to fish body burdens. Third, it is notable that perchlorate was detected in the algae from South Bosque, but not in the water during this sampling period. However, low levels of perchlorate are routinely found in this river. This suggests that perchlorate concentrations in the South Bosque are highly variable, but that the concentrations in the algae is routinely higher, providing more opportunities for exposure than via uptake water. In fact if we use the highest concentration of perchlorate reported in this river in other sections of this report (i.e., 30 ppb reported in Section 5.4.2), then the minimum bioconcentration factor for algae in this stream is 12.5. If we take the average water concentration at this site reported in Section 5.4.2 (1.4 ppb), then the bioconcentration factor (BCF) for this site would be roughly 270 (assuming the concentration in algae remains relatively constant.). This would suggest that the BCF for algae in the South Bosque was higher (perhaps by even one order of magnitude) than at any other site. Differences in BCFs between sites may reflect differences in algal species composition, temporal dynamics of perchlorate levels in the streams, or physiochemical properties of the streams themselves.

5.3.3 Perchlorate Uptake by Riparian Plants

5.3.3.1 Introduction

Perchlorate uptake by plants can be viewed as both a possible sequestration and perturbation process of perchlorate from sediments and streams. Terrestrial plants are capable of removing perchlorate from sediments and stream water and translocating it to leaves/fruits/nuts. Aquatic vegetation is capable of uptake directly from bulk stream water. Exposure of organisms through ingestion of plant material may depend on the

availability of perchlorate to the plant (seasonal and spatial), distribution within the plant (leaves/fruits/nuts), as well as the length of exposure. Sampling of plant matter from both terrestrial and aquatic vegetation will help to evaluate the overall potential for perchlorate uptake in plants as well as characterize the importance of this exposure pathway.

The fate of perchlorate in the environment can also be influenced by the potential re-release of perchlorate from vegetation after uptake. As plants senesce, vegetative tissues are returned to the soil/sediment. The return of perchlorate to the soil/sediment may be a beneficial process due to the movement of perchlorate from stable environments (aerated stream water or low carbon deeper sediments) to areas where rapid transformation can occur (top organic rich soil layers). On the other hand, uptake of perchlorate can also be viewed as another mechanism of exposure, especially if bioconcentration occurs in plant material. Measuring the concentration in leaf litter facilitated a better understanding of the recycling of perchlorate.

5.3.3.2 Methodology

Six locations surrounding NWIRP were sampled: Harris Creek at Highway 84 (HW84 Mainstream, T19), the spring on Oglesby Road (HW84 Sidestream, T18), Harris Creek at Highway 317 (HW317, T13), S Creek at Highway 317 (HW317/MN, T15), Station Creek at Highway 107 (HW107, T23), and the South Bosque at Mother Neff Road (Mother Neff Rd, T16). These sampling locations were distributed along three major creeks flowing away from the site, including Harris Creek, South Bosque River, and Station Creek. Detailed descriptions of these sampling sites are summarized in **Table 5-23** and shown on **Figure 5-127**.

Table 5-23
Description of Riparian Plant Sampling Locations

Site Name	Location	Samples Taken	Remarks
HW84 Mainstream (T19)	Harris Creek at HW84	Water, Aquatic plants	Water depth fluctuation of about 0.3-1.5 m yearly (Long-term sampling)
HW84 Sidestream (T18)	Spring on Oglesby Road	Water, Aquatic plants, Terrestrial plants	About 250 m length; relatively constant annual flowrate of about 4 $X10^{-3}$ m^3/s (Long-term sampling for water and aquatic and terrestrial plants along the stream; Seasonal sampling of tree leaves during the growing season along a defined reach of about 75 m long; Deciduous tree leaves were collected)
HW317 (T13)	Harris Creek at HW317	Water, Aquatic plants	Water depth fluctuation of about 0.3- 1.5 m yearly (Long-term sampling)
HW317/MN (T15)	S Creek at HW317	Water, Aquatic plants Terrestrial plants	Water depth fluctuation of about 0-0.6 m yearly (Long-term sampling)
Mother Neff Rd. (T16)	South Bosque River at Mother Neff Road	Water, Aquatic plants	(Short-term sampling)
HW107 (T23)	Station Creek at HW107	Water, Aquatic plants	(Short-term sampling)

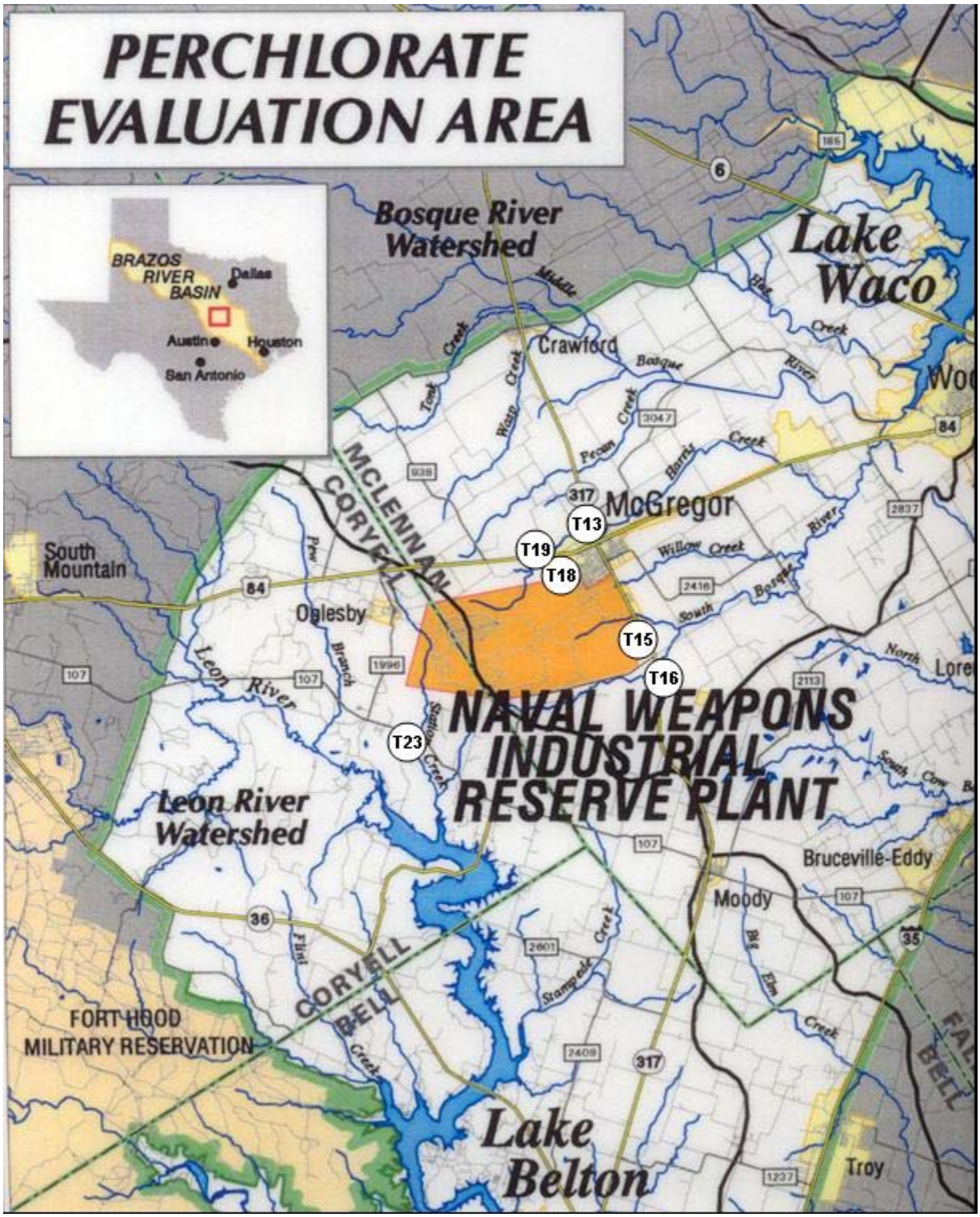

Figure 5-127
Map of Study Area Illustrating the Approximate Locations where Riparian Plant Samples Were Collected

Generally, water and vegetation sampling were conducted from June 2001 through November 2002, at an interval of every one to two months. Initially, short-term sampling was conducted for all the sites including Mother Neff Rd (T16) and HW107 (T23). Then, over a one-year period sampling was conducted to focus on the remaining sites. Two dominant aquatic plants, smartweed (*Polygonum* sp.) and watercress (*Rorippa* sp.), were collected whenever they were present in the streams.

In addition, starting from April, 2002, seasonal tree leaf sampling was conducted for more than sixty trees, including ash (*Fraxinus greggii*), china-berry (*Malia azedarach*), elm (*Ulmus parvifolia*), mulberry (*Broussonetia papyrifera*), and hackberry (*Celtis laevigata*), which grew on a forestland (approximately 75 m long and 25 m) wide) of HW84 Sidestream (T18). Trees along the reach were tagged and their locations were recorded. Tree leaves were sampled every two months during the growing period of macrophytes (from budding to leaf drop). At the end of October, 2002, four leaf litter traps (1.2 m length x 1.2 m width) were deployed at four locations on this forestland to collect the deciduous leaves. Deciduous leaves were collected after all the leaves had fallen from the trees. Distribution of trees sampled and the locations of litter traps are presented in **Figure 5-128**.

Bulk water samples were taken by using 30 ml plastic sample vials whenever there was water in the streams. Aquatic plants were rinsed with deionized water and then stored in plastic zip bags. Leaves of terrestrial plants were sampled from different locations and placed into pre-labeled bags. All samples were put on ice and transferred to the laboratory. Aquatic and leaf samples were mixed individually in the laboratory and then sub-samples were taken for further analysis.

Water samples were filtered (0.45 µm) and analyzed for perchlorate directly. Aquatic and terrestrial plants were extracted following the general method of Ellington and Evans (2000). Aquatic plants were rinsed once again with deionized water and blotted dry with paper towels. Aquatic and terrestrial plant tissues were cut into 0.5-1 cm pieces and dried in the refrigerator for several days. Collected plant material was subsampled and about 0.5 g of dried plant tissues (dry weight) were weighed and placed in 30 mL centrifuge tubes. Thirty mL of deionized water were added. In some cases duplicate or triplicate analysis were run on a given collection of plant material. However, in most cases only one sample from a given plant collection was evaluated. The centrifuge tubes were then tightly capped and placed in a boiling water bath for 1 hour, cooled to room temperature, and then placed in a 3 ºC refrigerator for one day. Samples were shaken regularly. Five ml of supernatant out of the total 30 ml was collected with a pipette and then added to 5 g of pre-cleaned aluminum oxide adsorbent (Al_2O_3, Aldrich, surface area, 155 m^2/g; 150 mesh) for 1 day at 3 ºC. Samples were filtered with prefilters (Millipore Corporation) and 0.45 µm IC syringe filters (Pall Corporation), diluted with 18 MΩ water (1:5), and analyzed for perchlorate (**Appendix X**).

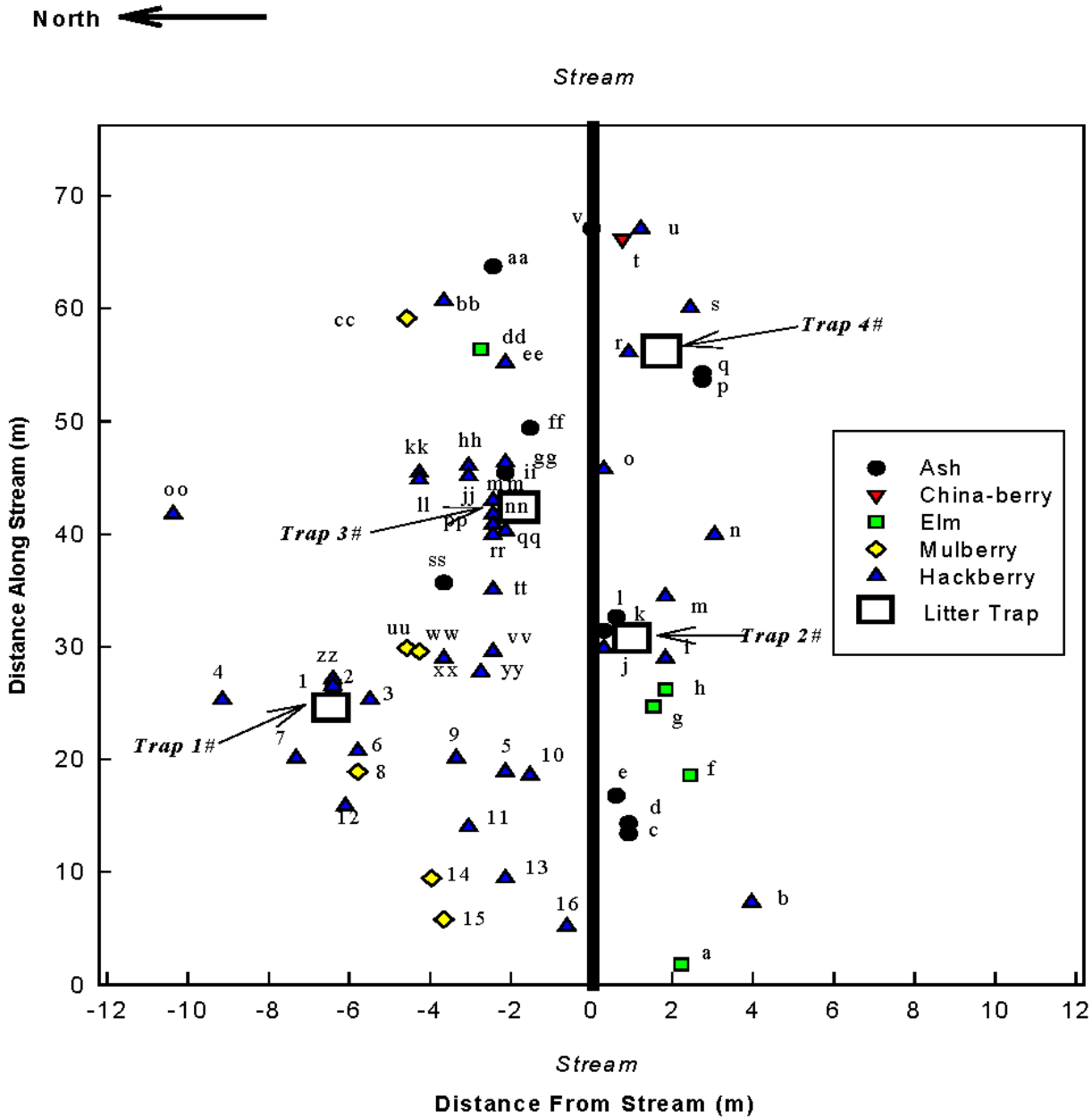

Figure 5-128
Distribution of Trees Sampled along a Defined Reach of HW84 Sidestream (T18) (Not to Scale). All trees Were Tagged with Different Letters or Numbers.

5.3.3.3 Data

5.3.3.3.1 Perchlorate Concentration in Surface Water

Perchlorate concentration in surface water was site-specific and temporally variable (**Figure 5-129**). The highest perchlorate concentration (up to 536 ppb) was observed at HW317/MN (T15). Compared to the other three sites, the HW317/MN location had the highest ClO_4^- concentration in the stream, probably because this location is close to an explosives disposal area within NWIRP (Hare, 2000). Perchlorate concentrations at HW84 Sidestream (T18) were relatively stable from January 2002 to October 2002 (20-40 ppb), but a peak of 123 ppb concentration was observed in October, 2001. HW84 sidestream's initial source is a spring. Both HW84 Sidestream and HW317/MN consistently contained ClO_4^-. Considerable fluctuation in perchlorate concentrations was observed at HW84 Mainstream (T19), but perchlorate was detected in most months (11 out of 16 samples). Perchlorate concentrations were below the detection limit (4 ppb) in August and September 2001, and August 2002, which was linked with rapid bacterial activity in natural wetland habitats at higher temperature. Significant variation in ClO_4^- concentrations was also observed at HW317. It was evident that both HW84 Mainstream and HW317 (T13) intermittently received influxes of ClO_4^-. The variation of ClO_4^- concentration may also have been caused by seasonal flowrate fluctuation in the streams due to different temporal evaporation and precipitation rates. On April 6th, 2002, surface water samples were collected at HW84 Mainstream and HW317 immediately after a heavy thunderstorm event and very low perchlorate concentrations were observed. HW317 was located approximately 3 km downstream from HW84 Mainstream and exhibited a similar temporal trend in ClO_4^- concentrations.

5.3.3.3.2 Perchlorate Uptake in Aquatic Plants

Overall, ClO_4^- uptake in smartweed was observed at all the selected sites where ClO_4^- was detected in surface water, except HW84 Mainstream (T19) (**Table 5-24**). Although the ClO_4^- concentration in water averaged 12 ppb at HW84 Mainstream (**Table 5-24**), ClO_4^- may have been depleted in sediment pore water due to microbial degradation in the sediments when smartweed samples were collected, so that ClO_4^- uptake was not observed. A previous kinetics study (5.2.3) demonstrated the rapid microbial degradation in sediment of HW84 Mainstream (intrinsic ClO_4^- degradation rate constant $k = 0.14\ d^{-1}$) (Tan et al., 2004). Sediment pore water concentrations monitored by in-situ dialysis samplers from another study indicated that ClO_4^- was not present in sediment at HW84 Mainstream (Tan, 2003), which supports this hypothesis. In addition, higher uptake in smartweed was generally observed at sites with higher average ClO_4^- surface water concentrations. Average ClO_4^- uptake in smartweed ranged from 9,140 to 40,600 ppb (µg/kg DW) for sites HW317/MN, HW84 Sidestream, and HW107, much higher than the range observed at HW84 Mainstream, HW317, and Mother Neff Rd. (< D.L. of 3,180 ppb). Watercress was collected from sites HW84 Sidestream, HW84 Mainstream, and HW107. Average perchlorate uptake in watercress ranged from 625 to 4,860 ppb (µg/kg DW) (**Table 5-24**).

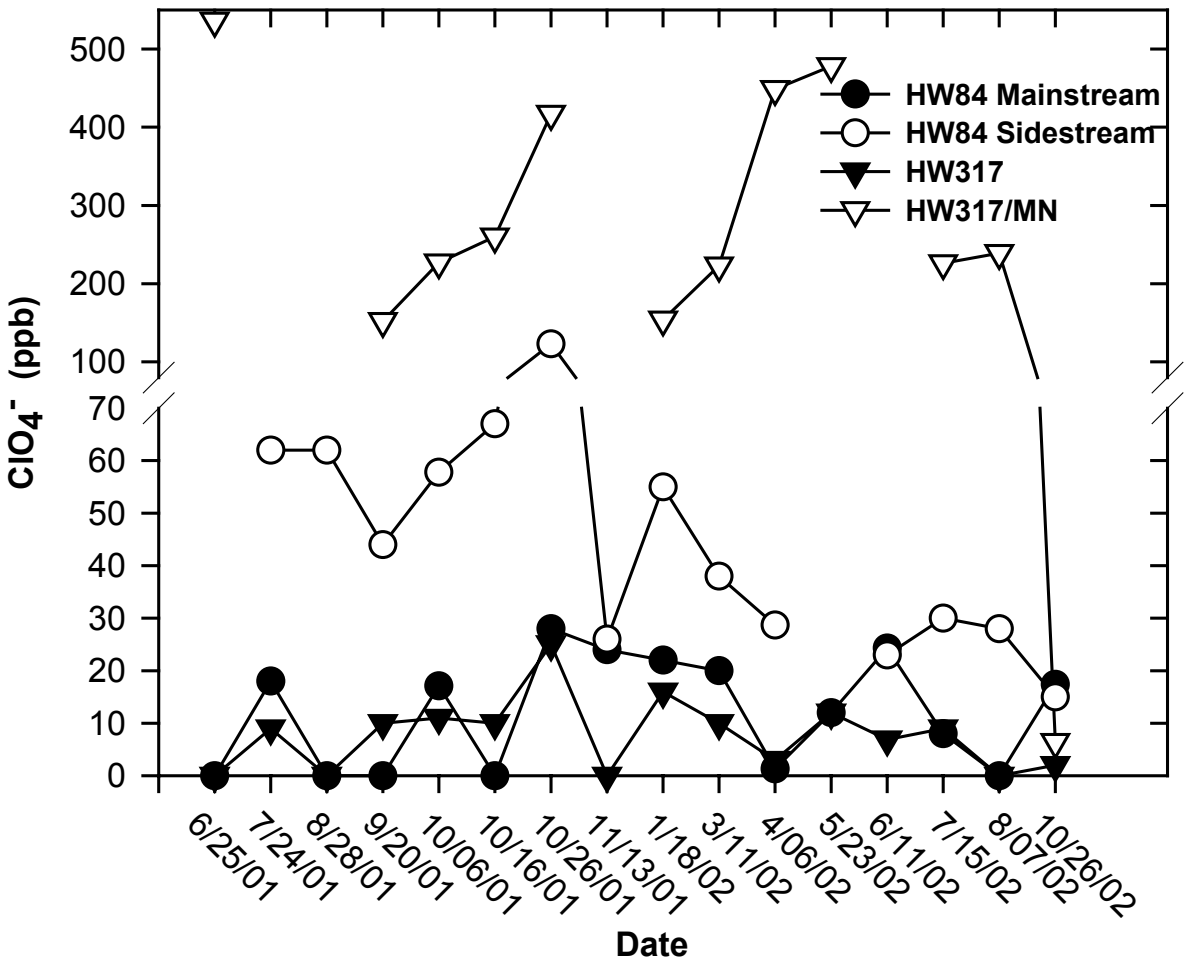

Figure 5-129
Perchlorate Concentrations in Surface Water at Different Stream Sites from June 2001 through October 2002

(Points not connected by solid lines means data missing because water samples were not taken.)

Table 5-24
Perchlorate Concentrations in Streams and Aquatic and Terrestrial Plants at Multiple Sites Surrounding NWIRP

Site	Matrix/Species	Concentration (ppb)				Sample Size (n)
		Min.	Average	Max.	SD	
HW84 Sidestream (T18)	Water	15	47	123	28	12
	Smartweed	5850	9240	12200	2650	4
	Watercress	< D.L. †	5043	13900	3750	22
	Ash (leaf)	< D.L.	2330	17000	3510	24
	China-berry (leaf)	631	5040	9440	6230	2
	China-berry (fruit)	< D.L.	< D.L.	< D.L.	-	1
	Elm (leaf)	< D.L.	2230	14200	3880	18
	Mulberry (leaf)	< D.L.	1310	6660	1720	21
	Mulberry (fruit)	< D.L.	467	934	661	2
	Hackberry (leaf)	< D.L.	4690	48200	10100	111
	Willow (leaf)	1580	1580	1580	0	1
HW84 Mainstream (T19)	Water	< D.L. ‡	12	28	11	15
	Smartweed	< D.L.	< D.L.	< D.L.	-	3
	Watercress	2630	4860	7090	3150	2
HW317 (T13)	Water	< D.L.	8	25	7	13
	Smartweed	< D.L.	3180	10500	4300	5
	Watercress	< D.L.	625	1560	705	6
HW317/MN (T15)	Water	6	281	536	157	12
	Smartweed	20700	40600	61600	20500	3
	Elm (leaf)	677	699	722	32	2
	Hackberry (leaf)	< D.L.	1300	2100	1140	3
	Willow (leaf)	< D.L.	6590	24300	8780	11
Mother Neff Road (T16)	Water	< D.L.	< D.L.	< D.L.	-	2
	Smartweed	< D.L.	< D.L.	< D.L.	-	2
HW107 (T23)	Water	15	17	20	3	2
	Smartweed	4280	9140	14000	6880	2

All samples were collected from June 2001 through October 2002
† D.L. (method detection limit) of ClO_4^- in water is 1 ppb.
‡ D.L. (method detection limit) of ClO_4^- in plants is 300 ppb (µg/kg DW).

Perchlorate uptake in aquatic plants was regressed against ClO_4^- bulk water concentration in streams. There was a significant linear relationship between bulk water ClO_4^- concentration and plant uptake in smartweed ($n = 19$; $R^2 = 0.7410$; $P < 0.0001$) and watercress ($n = 30$; $R^2 = 0.6022$; $P < 0.0001$) (**Figure 5-130** and **Figure 5-131**). Results indicated that plant uptake of ClO_4^- from streams was substantial with dry leaf concentrations up to 2 orders of magnitude greater than bulk water concentrations. From the slope, the plant bio-concentration factor (BCF) on the basis of plant dry weight was estimated to be 164 for smartweed and 267 for watercress, respectively. A laboratory study by Susarla et al. (2000) indicated that as high as 456 mg/kg ClO_4^- (based on plant fresh weight) uptake was taken up by smartweed exposed to 20 ppm ClO_4^- during a ten-day exposure experiment. Assuming 70% water content in smartweed, the bio-

concentration factor (BCF) of Susarla's study (2000) was estimated to be about 76 (on the basis of plant dry weight), which is slightly lower than our results. Ten days may not be sufficient to achieve equilibrium of perchlorate uptake and the concentration tested is unrealistic compared to most surface water concentrations of perchlorate.

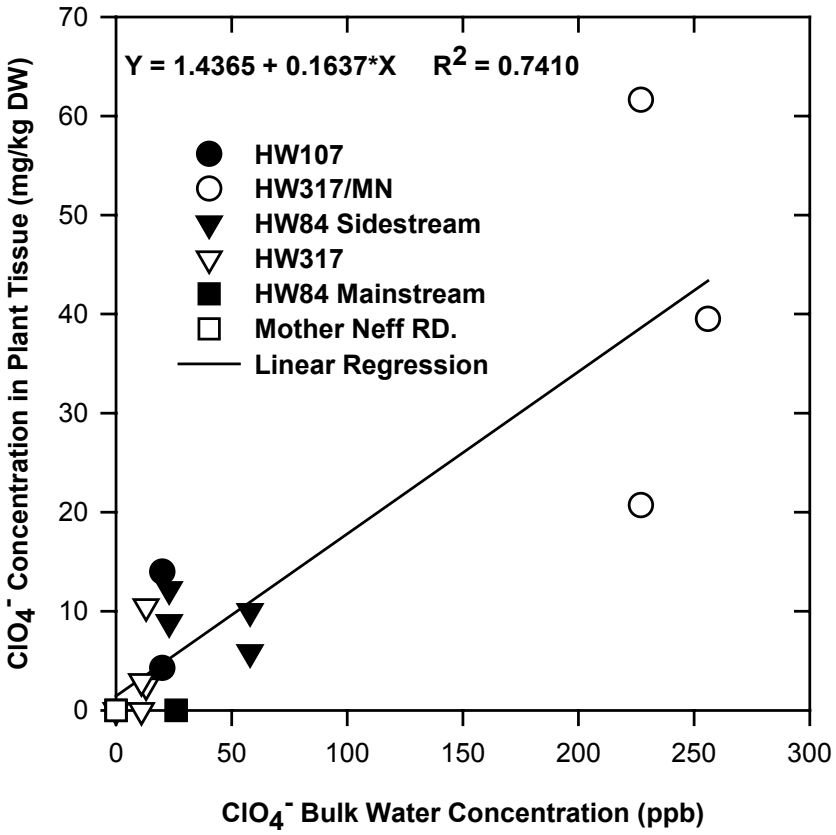

Figure 5-130
Relationship between ClO_4^- Bulk Water Concentration and Plant Tissue Concentration at Different Sampling Locations for Smartweed

(n = 19, P value of regression < 0.0001)

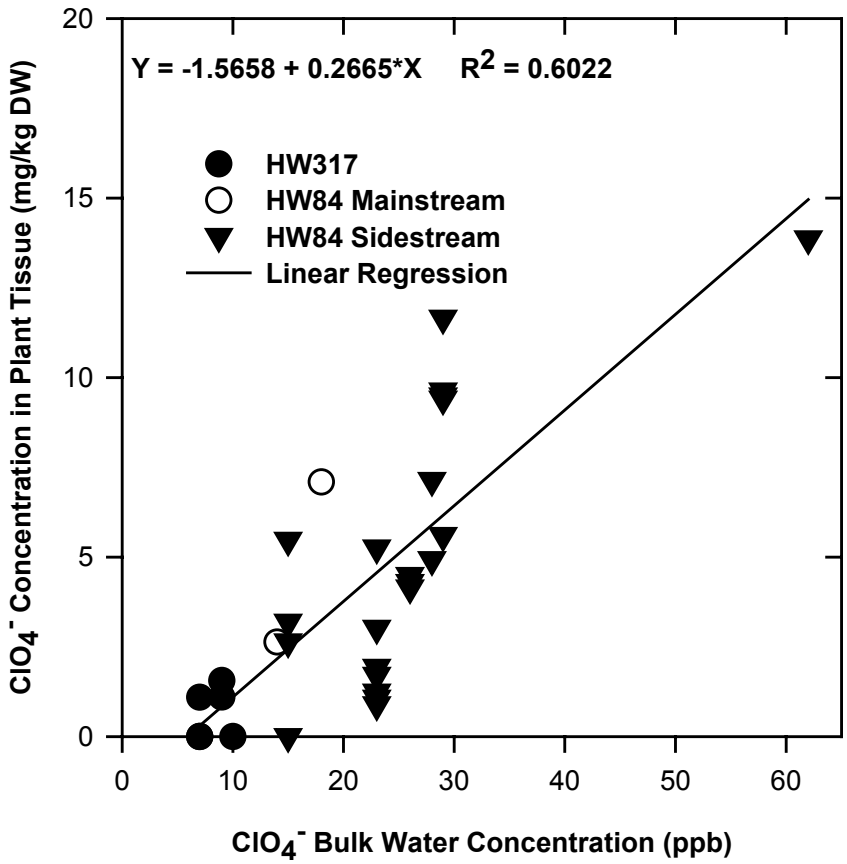

Figure 5-131
Relationship between ClO_4^- Bulk Water Concentration and Plant Tissue Concentration at Different Sampling Locations for Watercress

(n = 30, P value of regression < 0.0001)

Compared to watercress, smartweed has a more dense and longer root system that can extend deep into the sediment, so smartweed may take up perchlorate primarily from sediment pore water. Watercress is a floating aquatic plant with short roots that can take up perchlorate directly from surface water and shallower sediment pore water. Thus, the BCF estimated on the basis of the bulk surface water perchlorate concentration instead of the pore water concentration may underestimate the uptake potential of smartweed, because the pore water concentration is typically much lower than the bulk water concentration due to bacterial degradation in sediments. Because the actual sediment pore water concentrations at the locations where smartweed samples were collected were not available, bulk water concentrations were used to estimate the BCF in smartweed. Therefore, smartweed might have a higher perchlorate uptake potential than watercress even if the BCF of smartweed estimated on the basis of the bulk water concentration is lower than that of watercress. As a result, observed average perchlorate uptake in smartweed at HW84 Sidestream and HW317 was relatively higher than that of watercress (**Table 5-24**). At HW84 Mainstream, there was no perchlorate uptake in smartweed, due to the absence of perchlorate in the sediment pore water. In contrast, there was 4,860 ppb

in watercress because perchlorate was present in the bulk water for uptake stressing the importance of spatial exposure (**Table 5-24** and **Figure 5-131**).

5.3.3.3.3 Perchlorate Uptake Potential in Different Species of Terrestrial Plants

Terrestrial plant leaf samples were collected from two sites with consistent perchlorate concentrations, HW84 Sidestream and HW317/MN. Perchlorate uptake in all trees within 5 m from the stream was averaged to evaluate overall perchlorate uptake potential of different species (**Table 5-24**). All tree species investigated (ash, china-berry, elm, mulberry, hackberry, and willow) showed significant potential for perchlorate uptake, although perchlorate uptake was variable and species dependent. Highest perchlorate uptake was observed in hackberry (48,200 ppb), willow (24,300 ppb), and elm (14,200 ppb) (**Table 5-24**), implying these species may be good candidates for phytoremediation. In addition, perchlorate was preferentially accumulated in the leaf of china-berry and mulberry trees (average 5,040 ppb and 1,310 ppb, respectively) rather than the fruit (0 ppb and 467 ppb, respectively) (**Table 5-24**), implying that perchlorate was selectively partitioned in these plants.

5.3.3.3.4 Seasonal Uptake of Perchlorate in Terrestrial Plants

Sampling of tree leaves during the growing season at the HW84 Sidestream site indicated that perchlorate uptake in terrestrial plants was temporally and spatially variable and dependent on exposure duration. Generally, terrestrial plants up to 9 m from the stream accumulated significant concentrations of perchlorate (**Figure 5-132**). Trees located closer to the stream seemed to have higher perchlorate uptake, but in some cases trees located farther away from the stream also exhibited high perchlorate uptake. This may be caused by the heterogeneous distribution and penetration of root systems of trees. Some trees located further away from the stream may have widely-extended root systems to absorb water from the stream. For most trees, lower leaf ClO_4^- concentrations were observed in April and June, 2002, and the highest leaf tissue concentrations were observed late (August and October, 2002) in the growing cycle (**Figure 5-132**). Seasonal sampling of a willow (*Salix nigra*) tree from another site (HW317/MN) also indicated that perchlorate uptake in leaf tissue was seasonally variable (**Figure 5-133**). Perchlorate uptake in the willow tree increased with progression through the growth cycle. In April and May, 2002, there was no perchlorate uptake observed in new buds. In June and August, 2002, perchlorate uptake in leaves was 150 ± 210 ppb and 1,200 ± 250 ppb, respectively. The highest uptake in leaves was observed late in the growing cycle (15,800 ± 8,200 ppb and 14,600 ± 11,400 ppb for October, 2001 and October, 2002, respectively).

The reason that perchlorate concentrations in some trees showed declining trends in October, 2002 compared to that in August, 2002 is not clear (**Figure 5-132**). Phytodegradation may contribute to a partial decrease of perchlorate in plant tissues, but this process normally occurs relatively slowly (Nzengung et al., 1999; Aken and Schnoor, 2002). Nzengung et al. (1999) estimated phytodegradation accounted for approximately 11% ClO_4^- loss in a 26 day laboratory growing experiment using willow trees.

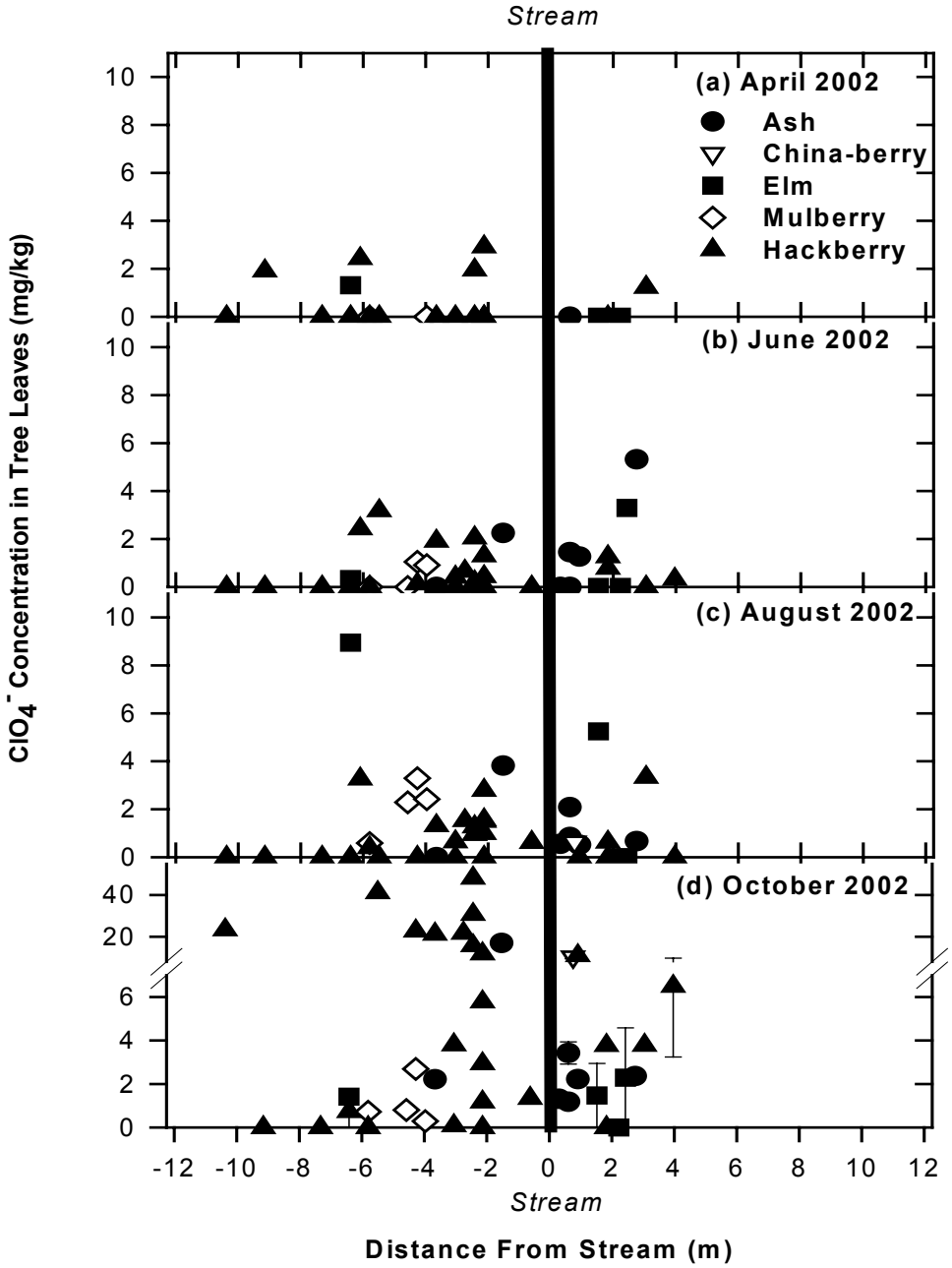

Figure 5-132
Perchlorate Seasonal Distribution in Tree Leaves along a Defined Reach of HW84 Sidestream (T18)

(Standard error bars represent the standard deviation of duplicate (n = 2) for some trees sampled in October 2002.)

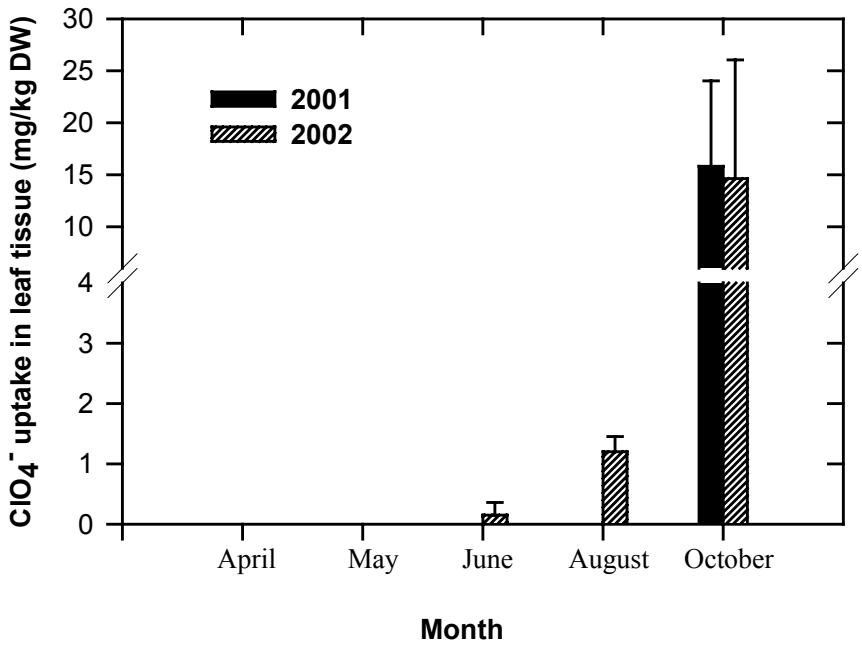

Figure 5-133
Seasonal Variation of ClO_4^- Uptake in a Willow Tree Located at HW317/MN (T15)

(Tree leaves were sampled from October, 2001 to October, 2002. Standard error bars represent the standard deviation of duplicate samples (n = 2).)

Another hypothesis is that the availability of other ClO_4^- sources, such as contaminated soil and groundwater, may also affect the uptake and distribution of ClO_4^- in terrestrial plants. However, information obtained from the HW84 Sidestream site suggests that terrestrial plants grown on this site absorb ClO_4^- mainly from the stream. In general, ClO_4^- was not detected in soil samples up to a depth of 60 cm beneath the surface. Only 1 out of 11 soil samples had a ClO_4^- concentration of 0.03 mg/kg DW. Results obtained suggested that soils near HW84 Sidestream were not contaminated by ClO_4^-. The boring log of a groundwater monitoring well OFFHC-14 located near HW84 Sidestream was composed of brown silty clay with limestone cobbles (0 - 1.2 m), slightly soft and moist clay (1.2 - 2.3 m), hard and silty argillaceous limestone/shale (2.3 - 3.0 m), and dry dark gray shale (3.0 - 5.2 m). Perchlorate concentrations were below the detection limit (4 ppb) in groundwater from OFFHC-14 from May 2000 to July 2001 (Personal communication with MWH Global in July 2003). This information suggested that trees at this site take up ClO_4^- mainly from the stream instead of from groundwater sources. An attempt was made to correlate tree size with ClO_4^- uptake, but no correlation was found. Tree size may affect ClO_4^- uptake in case of vertical contaminant source present, i.e. contaminated groundwater or soil, because bigger and taller trees tend to have a deeper root extension. However, in this case, ClO_4^- uptake in terrestrial plants was mainly influenced by the distance to the stream, since only the horizontal ClO_4^- source (stream) was available for uptake. On the other hand, large-trunk-size trees with a wide root

system could also have a relatively high ClO_4^- uptake even if they were located farther away from the contaminated stream.

5.3.3.3.5 Perchlorate in Leaf Litter Traps

Perchlorate concentrations in deciduous tree leaves collected from four litter traps (layout shown above in **Figure 5-128**) in January, 2003 were compared with concentrations in live tree leaves sampled prior to leaf drop in October, 2002. Average concentration of trees located in the proximity of litter traps (within a radius of 1.5 m away from individual litter traps) was considered to be typical ClO_4^- concentration in the late growth cycle. Generally, average ClO_4^- leaf tissue concentrations prior to leaf drop were higher than that in tree leaves after leaf drop for individual litter traps (**Table 5-25**). It was statistically significant (t-test, $P < 0.05$) that ClO_4^- concentrations in deciduous tree leaves in litter trap 3 and 4 were lower than those in live leaves prior to leaf drop (**Table 5-25**). However, in some cases (e.g., in litter trap 1 and 2), according to the statistical analysis (t-test), there was no significant difference in ClO_4^- concentration before and after leaf drop ($P > 0.05$).

Table 5-25
Perchlorate Concentration in Tree Leaves before and after Leaf Drop

Litter Trap	ClO_4^- Concentration in tree leaves (mg/kg DW)	
	Before leaf drop †	After leaf drop‡
1 #	0.71 ± 1.00 §	< D.L. (0.3)
2 #	2.16 ± 1.39	1.20 ± 0.28
3 #	4.79 ± 1.39	1.75 ± 0.26
4 #	10.93 ± 0.0	2.83 ± 0.21

† ClO_4^- uptake in live tree leaves located within a radius of 1.5 m away from individual litter traps (sampled in October, 2002)
‡ ClO_4^- concentration in deciduous tree leaves collected in individual litter traps (sampled in January, 2003)
§ Average ± standard error (n = 2).

The decrease of perchlorate concentration in deciduous leaves was most likely associated with leaching, rainfall events, and microbial degradation. Perchlorate from plants may be released into the environment, suggesting that plants may serve as source (re-release) and sink (uptake) of perchlorate. The selection of suitable plants may become a key factor in successful phytoremediation. The potential of evergreen plants to remediate perchlorate needs to be considered. If deciduous plants are to be used in phytoremediation of perchlorate, harvest and subsequent disposal after the plants hyperaccumulate perchlorate may be necessary.

5.3.3.4 Discussion

This research elucidated the fate of ClO_4^- in macrophytes in natural systems using the NWIRP site as a case study. Perchlorate concentrations in surface waters at multiple streams were temporally variable, depending on numerous factors such as contamination source, fluctuation of flowrate, and bacterial degradation. Significant ClO_4^- uptake was observed in smartweed and watercress (BCFs of 163 and 266, respectively) that dominated the natural wetland habitat. Perchlorate uptake in leaves of terrestrial plants was dependent on numerous factors, such as plant species, accessibility to the ClO_4^-

source, contamination levels in soils and groundwater, as well as exposure duration. Perchlorate taken up by trees may re-release to the environment after uptake. Results indicated that terrestrial plants at this site mainly take up perchlorate from streams rather than from groundwater. However, at some sites with a shallow groundwater table, if terrestrial plants absorb and accumulate ClO_4^- from groundwater and the BCF of a specific plant species is known, terrestrial plants may serve as an another alternative to monitor ClO_4^- contamination in groundwater by simply determining the ClO_4^- uptake in terrestrial plants overlying the groundwater table. Tree size may become an important factor to affect the uptake of ClO_4^-, since bigger and taller trees tend to have a deeper and more dense root system capable of reaching the groundwater. Thus, terrestrial plants could become a useful bio-monitoring tool as a supplement to costly groundwater monitoring wells. Information obtained will be helpful to tailor a site-specific design and management protocol of phytoremediation to remediate ClO_4^- contamination in other similar sites with non-point ClO_4^- contamination sources.

5.3.4 Market Basket Survey

5.3.4.1 Introduction

An important question in the study area is the potential for human exposure to perchlorate through the consumption of contaminated food. The use of irrigation water (surface or groundwater), which may be contaminated with perchlorate, to supply water for gardens is a relevant scenario in the study area. Under this scenario it is critical to establish the relationship between perchlorate concentrations in irrigation water and perchlorate concentrations in edible vegetation. In order to establish this relationship and determine the exposure potential for perchlorate to humans, edible plants or their surrogates were sampled from several locations within the study area. In addition, various vegetable samples from other locations were collected to complete the data set.

5.3.4.2 Methodology

Samples (vegetation, soil, and water) for this study were obtained by making personal contact with landowners and/or home owners along drainages within the study area. Descriptions of the sampled sites, along with the types of samples collected are provided below. The locations of these sites are indicated on **Figure 5-134**.

5.3.4.2.1 Harris Creek at Windsor Road (T30)

This location was near a longitudinal stream sampling station that was part of the overall project. The homeowner at this location allowed us to collect samples from the garden. The home owner indicated that water from Harris Creek was used to irrigate the garden. Water, soil, and potatoes were collected from this location.

5.3.4.2.2 Station Creek (T20-T23)

We were unable to identify a property owner or home owner along Station Creek (from the Texas A&M property near NWIRP to the Leon River) that had a garden. However, we collected samples from two locations along Station Creek: (1) at the Texas A&M property, and (2) near Highway 107. Samples collected included water, leafy vegetation (including sunflowers), berries and nuts.

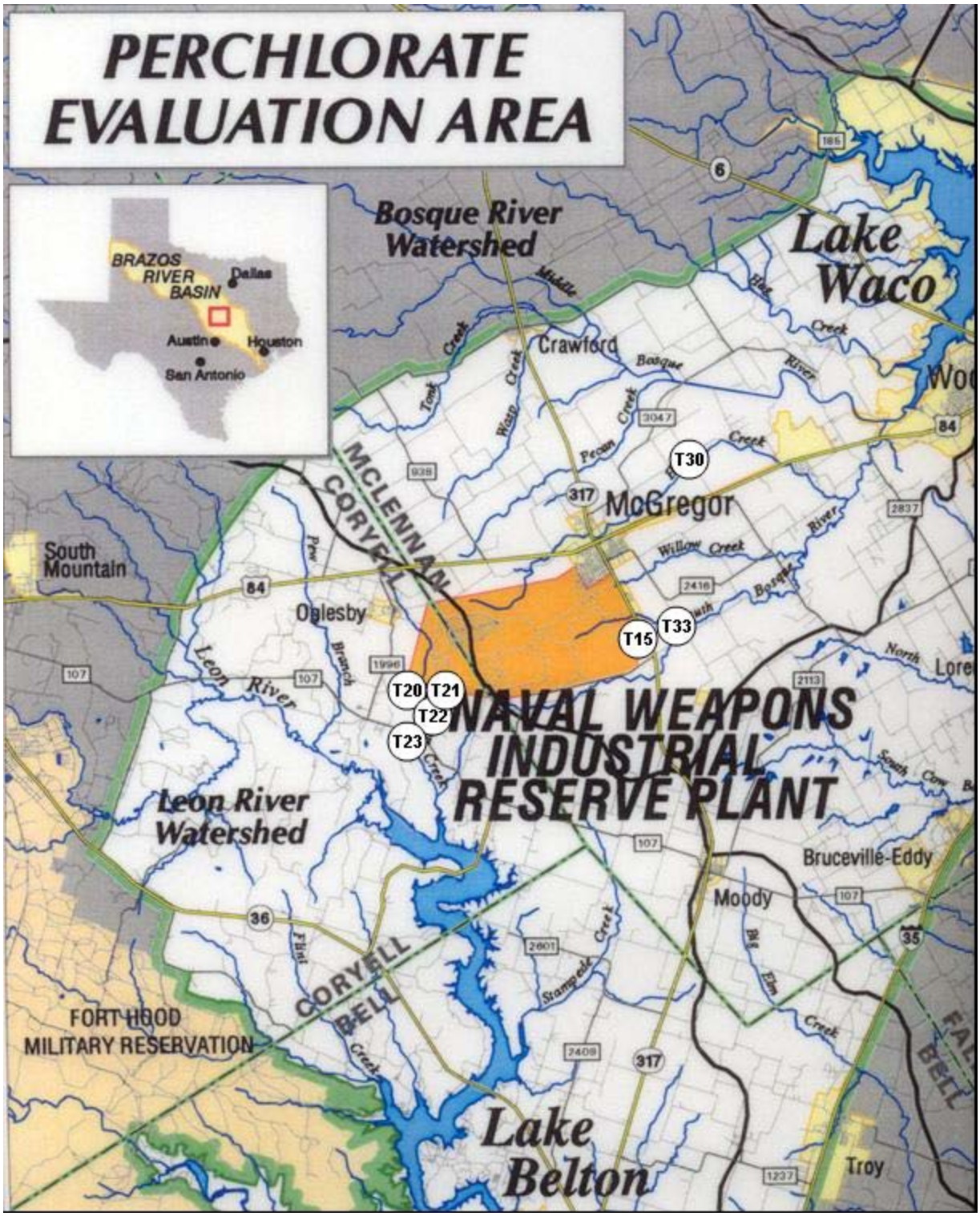

Figure 5-134
Map of Study Area Illustrating the Approximate Locations where Market Basket Samples Were Collected

5.3.4.2.3 South Bosque River at Indian Trail (T33)

This location was also near a longitudinal stream sampling station that was part of the overall project. The homeowner at this location allowed us to collect samples from the garden. The home owner indicated that water from the South Bosque was used to irrigate the garden. Water, soil, and okra were collected from this location.

5.3.4.2.4 S Creek at Highway 317 (T15)

This location represented the most contaminated stream in the study area based on monitoring data. The only resident near this stream before it flows into the South Bosque did not have a garden, however, we collected surrogate crop samples from this area. Samples collected included water, soil, leafy vegetation, wild squash, and field corn.

5.3.4.2.5 Additional Vegetable Samples

During the course of the study, vegetable samples from other locations were collected (along with irrigation water) in order to help complete the market basket survey dataset. These samples included: (1) vegetables (cucumber, tomato, lettuce, peppers) from 4 locations in New Mexico along with the corresponding irrigation water from each location, (2) vegetables/crops (tomato, wheat, etc.) from Gaines County in West Texas and the corresponding irrigation water, (3) vegetables (cucumber, tomato, and cantaloupe) from a garden in Kansas near a slurry explosives site and irrigation water from that location, and (4) organic-grown lettuce from Northern California (not irrigated with Colorado River water, but may have been fertilized with Chilean nitrate) and the corresponding irrigation water.

5.3.4.3 Data

Results from this study are shown in **Table 5-26**. This table includes results from within the study area as well as results from other perchlorate-impacted areas.

Table 5-26
Perchlorate Residues in Related Water, Soil, and Vegetation Samples

(Vegetation was collected to represent food items or surrogates for food items likely consumed by humans.)

Location	Perchlorate (ppb)			Accumulation Factor
	Water	Soil	Vegetation	
Harris Creek at Windsor Road	ND	ND	Potato - ND	
Station Creek at Highway 107 (T23)	NA	NA	Berries - ND	
			Nuts - ND	
Station Creek on A&M Property (T20-T22)				
"Spring"	3	NA	Leafy Greens - 18 ppb	≤ 6
"Water Crossing"	134	NA	Leafy Greens - 654 ppb	
"Ponded Water"	5	NA	Sunflower - 31 ppb	
South Bosque at Indian Trail (T33)	ND	ND	Okra - 2 ppb	
S Creek at Highway 317 (T15)	NA	ND	Smartweed - 1756 ppb	
			Milkweed - 3 ppb	
			Squash - 22ppb	
			Squash - 46 ppb	
			Field Corn - ND	

Location	Perchlorate (ppb)			Accumulation Factor
	Water	Soil	Vegetation	
Los Alamos County, New Mexico				
"PA"	ND	NA	Chile - 128 ppb	
			Squash - 99 ppb	
			Tomato - ND	
			Lavender - ND	
			Rhubarb - ND	
"LA"	ND	NA	Lettuce - J	
			"chanto" - 57 ppb	
			Pepper - ND	
			Lettuce - J	
			Tomato - ND	
"C"	ND	NA	Squash - ND	
			Cucumber - ND	
			Pumpkin - ND	
"SCP"	ND	NA	Chile - ND	
			Tomato - ND	
			Lettuce - ND	
			Egg Plant - ND	
			Chile - ND	
			Tomato - ND	
			Cucumber - ND	
Gaines County, Texas	2-20 ppb	NA	Corn - ND	≤ 20
			Pepper - ND	
			Tomato - ND	
			Cucumber - 40 ppb	
Morris County, Kansas	81 ppb	NA	Cucumber - 766 ppb	≤ 20
			Cantaloupe - 1645 ppb	
			Tomato - 221 ppb	
			Tomato - 42 ppb	
Northern California	ND	NA	Lettuce - 86 ppb	
			Lettuce - ND	
			Lettuce - ND	
			Lettuce - ND	
			Lettuce - ND	

NA = no sample collected (dry stream or no relevant soil sample)
ND = not detected
See **Appendix C** for historical data

5.3.4.4 Discussion

Laboratory studies and field sampling within the study area indicate that perchlorate is readily accumulated in plants. Vegetation in perchlorate-contaminated areas or crops grown with perchlorate-contaminated water could represent a significant route of perchlorate exposure to higher organisms, including humans. However, specific conditions within the study area lessen the probability of human exposure to perchlorate through vegetation. This conclusion is based on (1) where perchlorate occurs in surface water and the location of gardens irrigated by those streams and/or well water, (2) the concentrations of perchlorate observed in most streams and the respective accumulation

factors (≤ 20), and (3) the accumulation of perchlorate in plants (tree leaves) that are not typically consumed by humans.

Although the types of food items sampled within the study area were limited (primarily by the lack of gardens located along contaminated streams), additional food items collected from other areas in the U.S. suggest that the data collected in this study are consistent. In instances where contaminated water is used to irrigate gardens, perchlorate does accumulate in plants.

5.3.5 Mathematical Modeling of Perchlorate in Plants

5.3.5.1 Introduction

Models were developed to predict perchlorate uptake in terrestrial and aquatic plants. These models provide predicted perchlorate concentrations in vegetation that can be used in subsequent models of perchlorate uptake in terrestrial and aquatic animals. The aquatic vegetation models included an algae model and an aquatic macrophyte model.

5.3.5.2 Methodology

The models are based on the CERES model, originally developed by Dixon et al. (1978). CERES utilizes a combination of the compartmental and experimental systems in its modeling approach (**Figure 5-135**).The compartmental system approach focuses on tracing the quantities of substrates in the system, while the experimental component approach utilizes detailed analysis and mathematical representation to describe environmental and physiological processes. The modeled compartments in the model are roots, stems, leaves, and fruits. Each compartment is further sub-divided into sugar substrate, storage, water, and perchlorate amount.

Both models were programmed in Matlab; the algae model was programmed using differential equations and the aquatic macrophyte model was programmed using difference equations.

For the algae model, we used the model for growth and production of algae contained in The Enhanced Stream Water Quality Model QUAL2E (Brown and Barnwell, 1987). This model was used because of extensive experience using it in a risk assessment of atrazine (Solomon et al., 1996).

The second plant model simulates the uptake, transport, and distribution of the perchlorate anion in the vegetation of various terrestrial and aquatic plant species. The intrinsic processes that are incorporated in the model include plant growth, nutrient uptake and transport, nutrient and substrate storage, plant-water relations, photosynthesis, and respiration. The important extrinsic processes or factors include solar radiation, environmental temperature, and soil hydrology.

The models were tested with each of the structural tests described in Section 5.5.1.3.2.1 and passed each test. The parallel test, comparing the output from the Matlab model with that of the FORTRAN model, showed equivalent output.

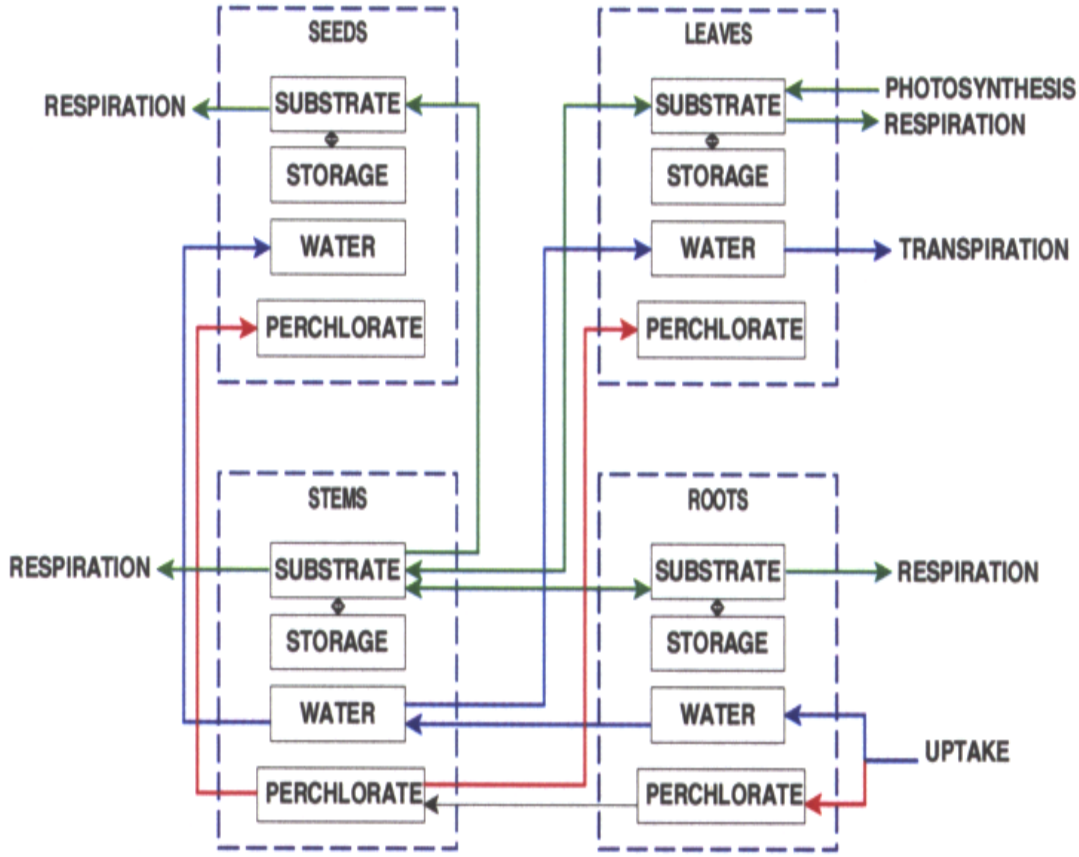

Figure 5-135
Flow Diagram of Macrophyte Model

5.3.5.2.1 Macrophyte Model Description

For the macrophyte model governing equations, we used those described by Dixon, et al. (1978). The plant's water uptake is the product of the plant's ability to take up water, its leaf area, and the volume of water available to the plant in its growing soil horizon:

$$U_t^i = f \cdot L'_{(t)} \cdot V_A$$

where :

U_t^i = incremental water uptake at time (t)
f = water flow constant
L' = Leaf Area Index
V_A = Volume of water in Soil Horizon A.

Distribution of water and perchlorate between compartments is defined by the difference in water and perchlorate between compartments:

$$F_{ab} = \begin{cases} (W_a - W_b)/r_{ab} & t_1 < t \le t_4 \\ 0 & otherwise \end{cases}$$

where:

F = flux from compartment A to compartment B (g/m^2 land area/hour)
W = amount of water in a given compartment (g/m^2 land area)
r_{ab} = water flux constant.

The amount of perchlorate in individual compartments is defined by:

$$M_a = F_{ab} \cdot C_{ClO4-}$$

where:

M_a = amount of perchlorate in the compartment at each time step (t)
F_{ab} = flux of water between the two involved compartments
C_{ClO4-} = ClO_4^- concentration in the incoming water.

The ratio of the amount of perchlorate in the compartment to the biomass of the compartment determines the perchlorate concentration:

$$Q_{a,t} = \frac{M_{a,t}}{B_{a,t} + W_{a,t}}$$

where:

$Q_{a,t}$ = concentration of perchlorate in compartment a at time t ($\mu g \bullet g^{-1}$)
$M_{a,t}$ = amount of perchlorate in compartment a at time t ($\mu g \bullet m^{-2}$)
$B_{a,t}$ = biomass of a given compartment ($g \bullet m^2$)
$W_{a,t}$ = mass of water in compartment a at time t ($g \bullet m^2$)

Plant biomass is calculated by summing the soluble and insoluble photosynthate fractions (Dixon, et al., 1978):

$$B_{a,t} = S_{a,t} + ST_{a,t}$$

where:

$B_{a,t}$ = biomass of compartment *a* at time *t* ($g \bullet m^2$)
$S_{a,t}$ = sugar substrate in compartment *a* at time *t* ($g \bullet m^2$)
$ST_{a,t}$ = plant storage tissue in compartment *a* at time *t* ($g \bullet m^2$)

Model Assumptions:

- transport between leaves and stems occurs from the time of bud formation to the time of abscission.

- transport between stems and fruits occurs from the time of net photosynthesis to the time of abscission.
- transport between the stems and roots is assumed to occur throughout the year.

5.3.5.2.2 Algae Model Description

We used the model for growth and production of algae contained in The Enhanced Stream Water Quality Model QUAL2E (Brown and Barnwell, 1987). The model is expressed as the differential equation:

$$\frac{dA}{dt} = \mu A - \rho A - \frac{\sigma_1}{d} A$$

where:

A	=	algal biomass concentration, mg•L^{-1}
t	=	time, hours
μ	=	the local specific growth rate, h^{-1}
ρ	=	the local respiration rate of algae, h^{-1}
σ_1	=	the local settling rate for algae, m•h^{-1}
d	=	average depth, m

We changed the original time step of a day to an hour. In the QUAL2E model, the algal specific growth rate is written as a function of light, and the nutrients nitrogen and phosphorus. For the purposes of these simulations, we assumed that nutrients are not limiting. Because no significant effect of perchlorate was observed, we did not include a growth limitation factor for perchlorate. In our growth experiments, we only measured net growth rate and not gross photosynthetic rate and respiration rate independently. Therefore, we used a revised model of algal dynamics in which growth rate, μ, and respiration rate, ρ, are both coupled to the expressions for the limitation factors for light, FL:

where:

$$\mu = \mu_{\max}(FL)$$

μ_{max}	=	maximum specific algal growth rate constant, h^{-1}
FL	=	algal growth limitation factor for light

$$X_T = X_{20}\theta^{(T-20^\circ)}$$

Both μ_{max} and μ are functions of temperature (Brown and Barnwell, 1987):

where:

X_T = the value of the coefficient at the local temperature (T)

X_{20} = the value of the coefficient at the standard temperature (20 C)

θ = an empirical constant for each reaction coefficient

Of the three options for the light functions in QUAL2E, we selected Smith's function. In this option, the algal growth limitation factor for light is formulated to include second order effects of light intensity:

$$FL_z = \frac{I_z}{\sqrt{K_L^2 + I_z^2}}$$

where:

FL	=	algal growth attenuation factor for light at intensity I_z
I_z	=	light intensity at a given depth (z), cal•cm^{-2}•min^{-1}
K_L	=	light intensity at 71% of the maximum growth rate, cal•cm^{-2}•min^{-1}
Z	=	depth variable, m

In QUAL2E, the light intensity, I_z, varies with depth according to Beer's law:

$$I_z = e^{-Kz}$$

where:

I	=	surface light intensity, cal•cm^{-2}•min^{-1}
K	=	light extinction coefficient, m^{-1}
Z	=	depth variable, m

Uptake of perchlorate from water into algal cells was assumed to follow from passive diffusion:

$$\frac{dC_{cellH2O}}{dt} = \mathrm{d}(C_{H2O} - C_{cellH2O})$$

where:

$C_{cellH2O}$	=	soluble perchlorate concentration in algal cells, ppb
C_{H2O}	=	water perchlorate concentration, ppb
d	=	diffusion coefficient, h^{-1}

Perchlorate concentration in algae water was calculated as the concentration in algal cells multiplied by the fraction of algal biomass in water. We assumed an adsorptive mechanism between cellular water concentration and insoluble algal biomass:

$$\frac{dC_{\text{algae}}}{dt} = k_1 \cdot C_{cellH2O} - k_2 C_{\text{algae}}$$

where:

C_{algae}	=	insoluble algal perchlorate concentration, ppb
A	=	algal biomass, ppm
k_1	=	adsorption rate coefficient, h^{-1}
k_2	=	dissociation/degradation coefficient, h^{-1}

Algal perchlorate concentration was calculated as both adsorbed and absorbed perchlorate divided by the algal biomass.

5.3.5.2.3 Algae Model Calibration

We used data from laboratory studies on uptake of perchlorate into duckweed (*Lemna minor*) to calibrate the model. Depth and settling rate in the lab experiments were assumed to be zero. Initial algal biomass was set to 0.55 g. We allowed the simulated algal biomass to increase over the 10 day simulation experiment (**Figure 5-136**). Water concentration was set to 100 ppb. Predicted perchlorate concentration in duckweed showed dynamics similar to observed values (**Figure 5-137**). We concluded that the model was calibrated sufficiently to conduct additional simulation experiments.

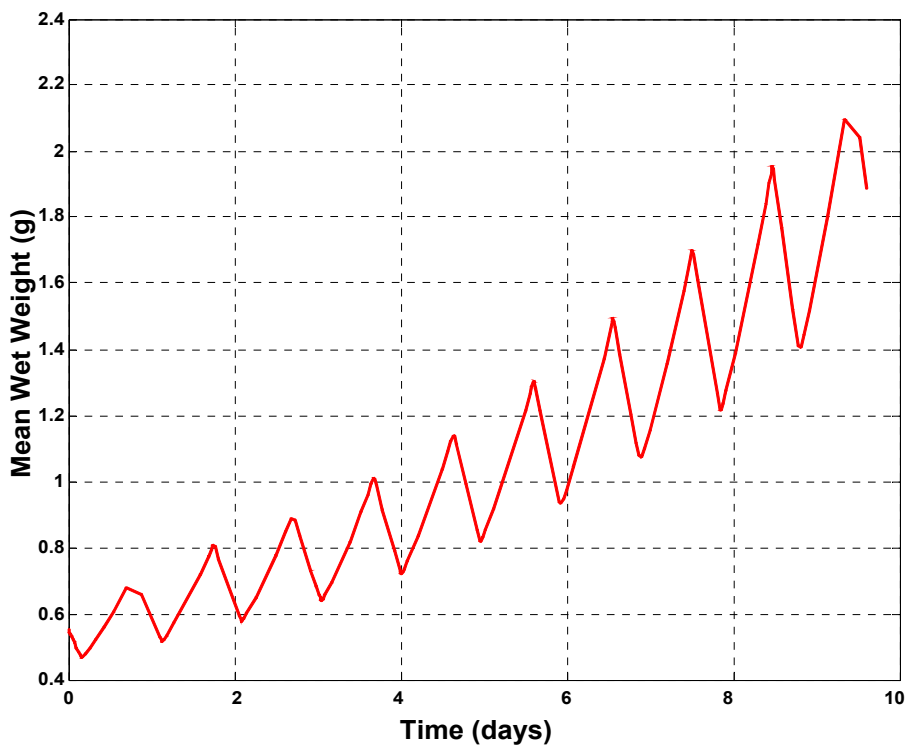

Figure 5-136
Simulated Algal Growth in Ten Day Experiment

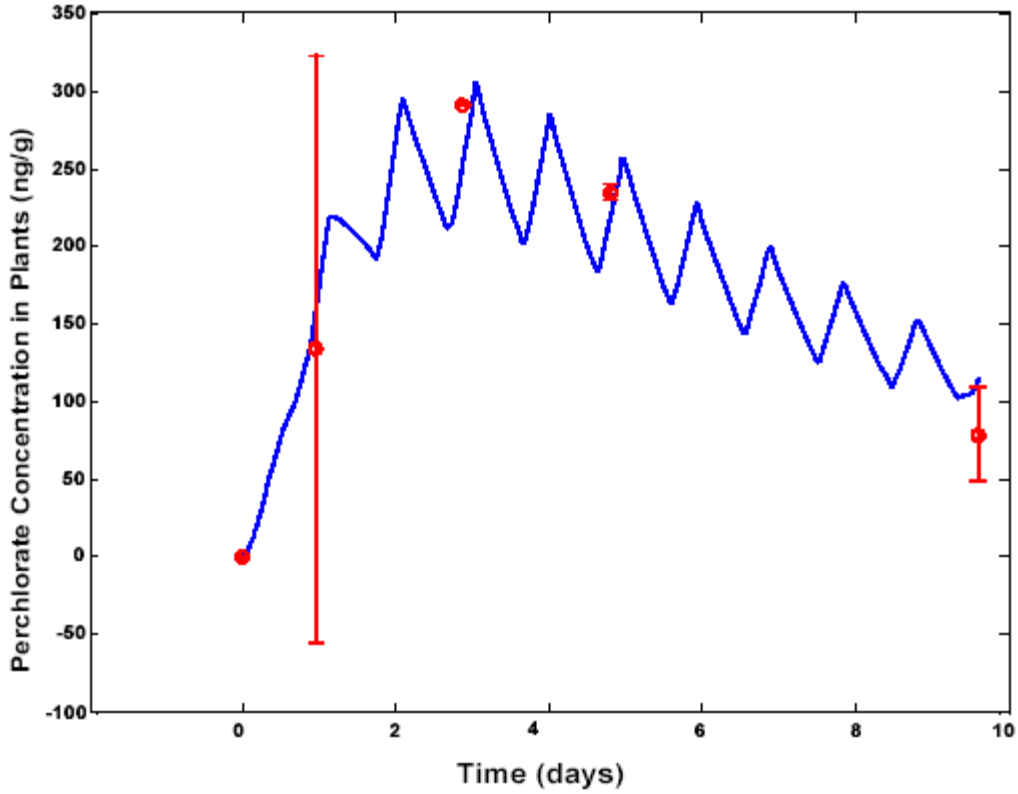

Figure 5-137
Predicted and Observed Perchlorate Concentration in Duckweed in Ten Day Experiment

(Predicted values are in blue. Observed values, in red, are mean ± standard deviation.)

5.3.5.3 Data

5.3.5.3.1 Exposure Scenario

Simulations representing "worst-case" scenarios were run for terrestrial plants (a generic tree species), aquatic macrophytes (*Polygonum* spp), and algae. For the terrestrial plants and aquatic macrophytes, we used data collected from S Creek at Highway 317 (T15). The perchlorate concentrations used 270.48 ± 157.56 ppb. We assumed that both terrestrial and aquatic plants were exposed to the same concentrations. Simulations were run for algal populations in Harris Creek at Highway 317 (T13), the only sampling location that had a detectable perchlorate concentration (7.17 ppm) in algae.

5.3.5.3.2 Aquatic Macrophyte Simulations

The simulation results for *Polygonum* spp. (**Figure 5-138**) show an initial increase in perchlorate concentration at the start of the growing season. As plant biomass increases, relative to perchlorate uptake, there is a small decrease in concentration. When the plants reach maximum growth, biomass remains relatively constant, uptake of perchlorate continues, and concentration also increases. At the end of the growing season, both

biomass and perchlorate decrease as a result of senescence and mortality, resulting in a decrease in concentration.

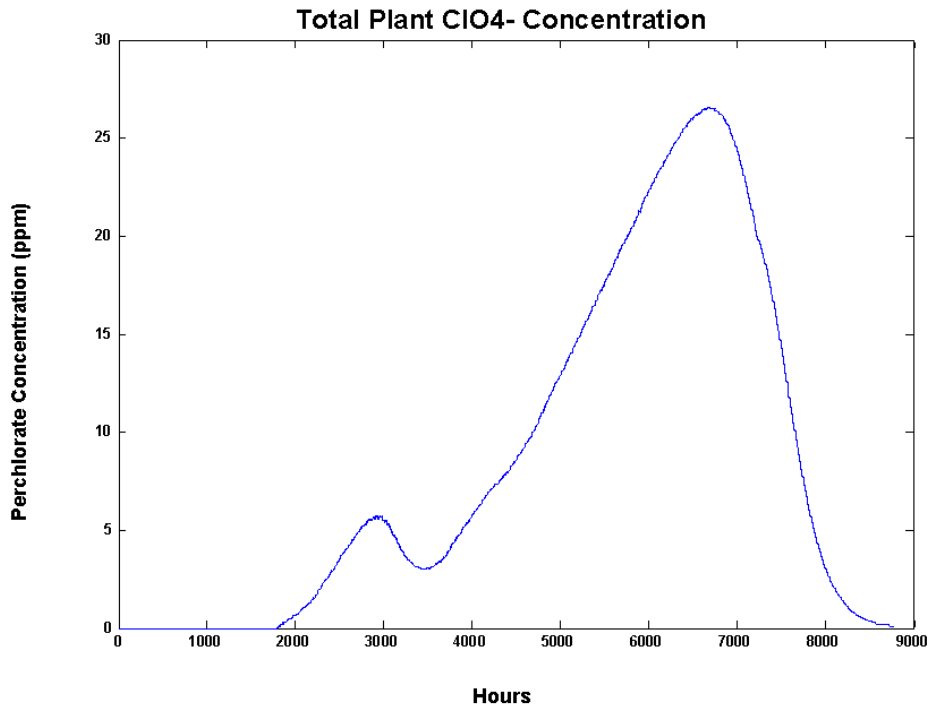

Figure 5-138
Predicted Perchlorate Concentration (ppm) in *Polygonum* spp. from S Creek at Highway 317 (T15)

The simulation results for each plant compartment show predicted perchlorate concentrations in each compartment (**Figure 5-139**).

The concentration pattern at the beginning of the growing season is similar in all compartments. There is an initial increase in concentration resulting from a relatively rapid uptake of perchlorate compared with plant growth. As plant biomass increases, there is a decrease in concentration, as reflected in the total plant concentration. There are significant differences, however, in the seasonal concentration dynamics. In the root compartment, the concentration remains at a relatively low level as the root biomass increases relative to concentration. In the stem compartment, the levels of biomass and concentration stay relatively the same, resulting in a concentration that remains at the same level. The concentrations in both the leaves and fruits increase significantly more than in the roots and stems. This is a result of the water being transpired from those compartments and the sequestration of perchlorate. The model predicts that the concentrations in both leaves and fruits continue to increase after the end of the growing season. Although there is no perchlorate accumulating in the leaves during this time of year, the amount of perchlorate decreases slightly less than the leaf biomass, resulting in a high concentration.

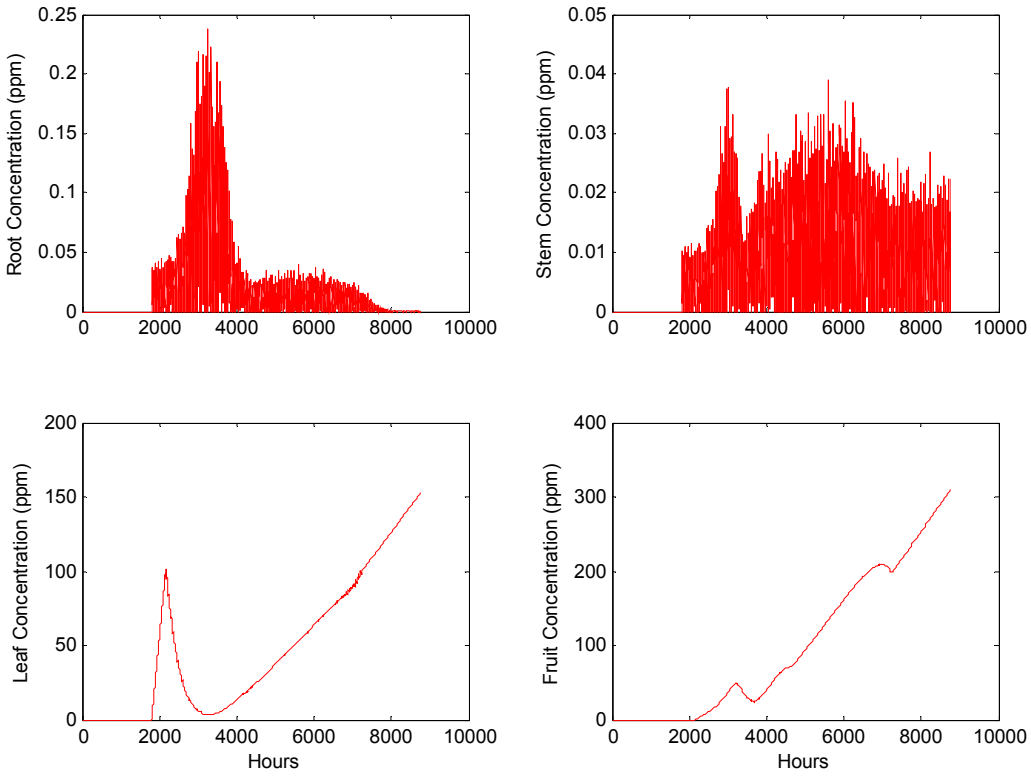

Figure 5-139
Predicted Perchlorate Concentrations in Roots, Stems, Leaves, and Fruits in *Polygonum* spp. from S Creek at Highway 317 (T15)

5.3.5.3.3 Terrestrial Plant Simulations

The simulation of terrestrial plants, representing perennial tree species, differed from the simulation of *Polygonum* spp. in several respects (**Figure 5-140**). The most obvious difference is that perennial plants begin with significant stem and root biomass. This tends to reduce the concentration in all compartments. Also, there is less water available in the soil compared with wetland sites. A third factor is that the height of trees increases the resistance to water movement throughout the plants. The dynamic patterns of perchlorate concentration, however, are quite similar to those of *Polygonum* spp. The predicted perchlorate concentration in tree leaves on October 29 is 15.75 ppm compared to the mean sampled concentration on that date of 21.90 ppm.

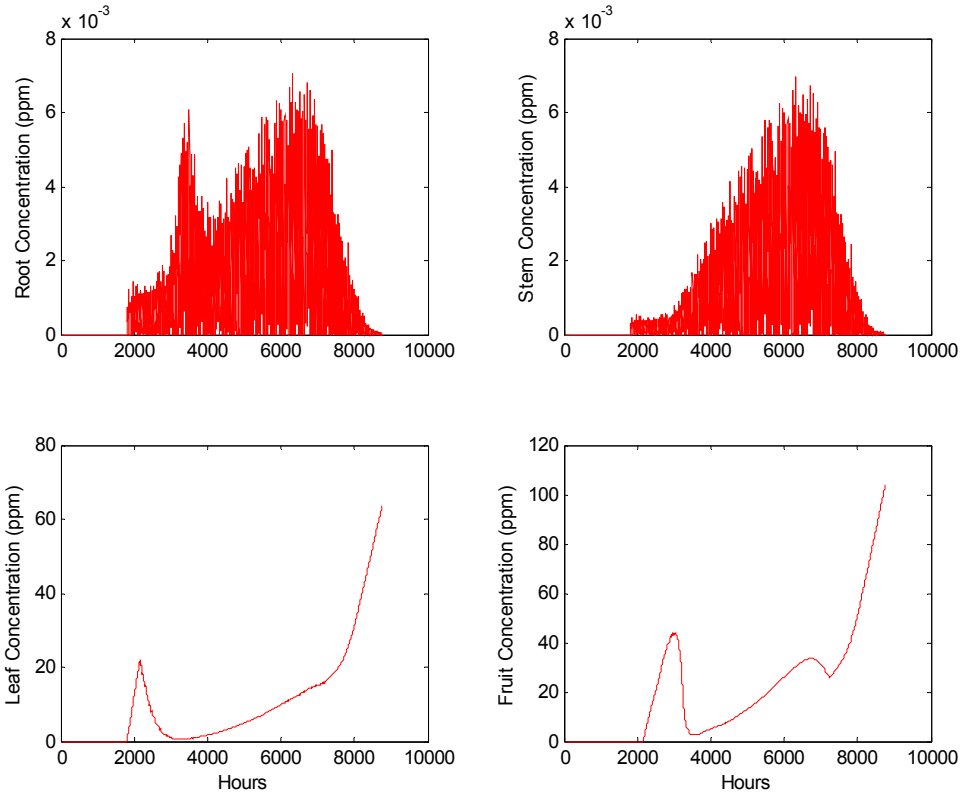

Figure 5-140
Predicted Perchlorate Concentrations in Roots, Steams, Leaves, and Fruits in Tree leaves from S Creek at Highway 317 (T15)

5.3.5.3.4 Algae Simulations

The water concentration at the Harris Creek sampling site averaged 11.28 ppb after September 6, 2001. That value was used as the exposure concentration. These simulation results show predicted algal biomass increases seasonally (**Figure 5-141**). Perchlorate concentration increases to a peak after about 20 days into the growing season but then decreases as biomass increases (**Figure 5-142**). There is an increase in perchlorate concentration at the end of the growing season as biomass decreases but because of the low algal biomass density, the perchlorate exposure through the aquatic food chain is low. The predicted perchlorate concentration on October 6 is 4.65 ppm dry weight compared with the observed value of 7.17 ppm.

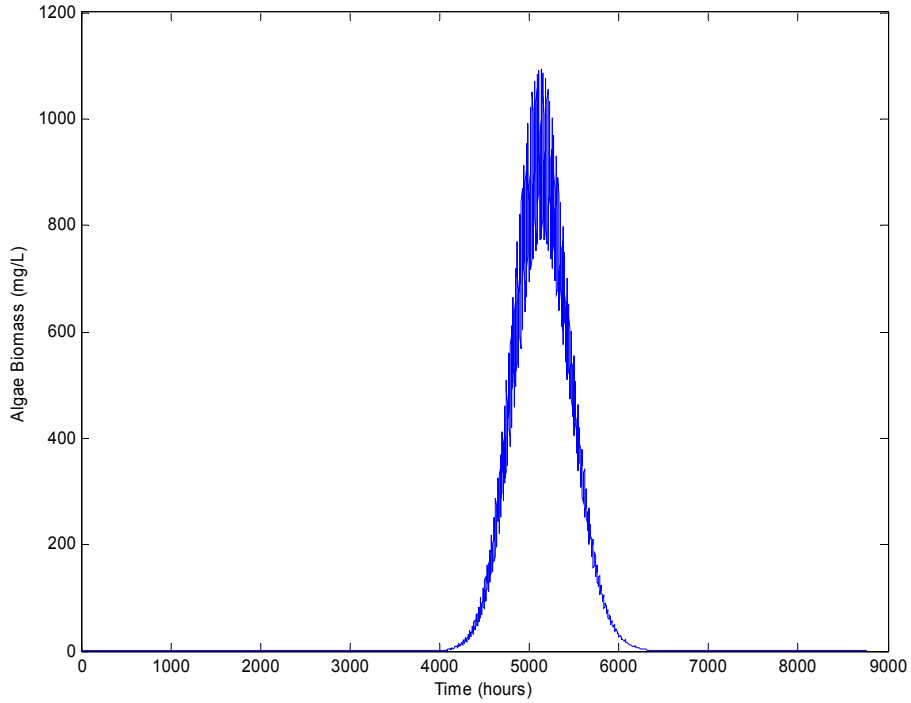

Figure 5-141
Predicted Algal Biomass in Simulated Exposure to Perchlorate Concentration from Harris Creek Site (T13)

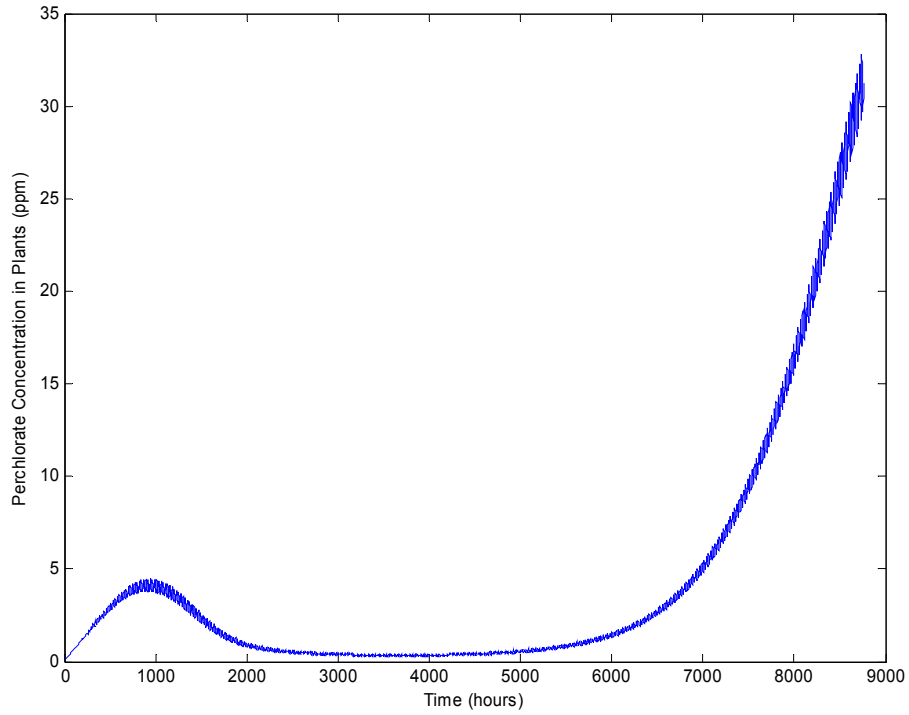

Figure 5-142
Predicted Algal Perchlorate Concentration for Harris Creek (T13) Simulation

5.3.5.4 Discussion

The vascular plant model was developed under the assumption that water is the driving force behind the uptake and distribution of perchlorate in plants. Because the model predicts tissue concentrations that are in line with laboratory and field values, it is reasonable to assume that water movement in plants is an important driving force in the uptake and distribution of perchlorate.

The model also indicates that perchlorate is capable of bioaccumulation in the leaves and fruits of exposed plants. If this result is true, there is significant potential for trophic transfer of perchlorate if wildlife and humans consume exposed plants. Although parameter estimates were based on calibration with lab experimental data, direct parameter estimation may improve the accuracy of the model predictions.

5.4 AQUATIC ANIMALS

5.4.1 Fish

5.4.1.1 Perchlorate Residues in Native Fish

5.4.1.1.1 Introduction

In order to confirm perchlorate exposure, fish were collected from aquatic environments within the study area and analyzed for the presence of the perchlorate anion. The goals of this effort were to assess the exposure (and potential effects) of perchlorate in fish as well as to assess the exposure potential for perchlorate in humans through the consumption of contaminated fish.

5.4.1.1.2 Methodology

5.4.1.1.2.1 Preliminary Assessments

The first assessment of perchlorate residues in fish was conducted in May, 2001. At that time, fish were collected from various streams within the Lake Waco and Lake Belton Watersheds. Based on the results of the May, 2001 sampling event, an immediate follow-up sampling was requested in August/September, 2001. This fall sampling was on fish fillets and also included sediment collections.

5.4.1.1.2.2 Routine Monitoring

At each location where fish were captured, at least two water samples were collected in precleaned glass vials (Wheaton) from just under the water surface. Water samples were then analyzed for perchlorate according to **Appendix X**. In the streams, fish were sampled with a backpack shocking apparatus set at a current of 2-4 amps and a frequency of 30-60 cps, or were collected by seining. All fish collected from the streams were identified to species, frozen whole in liquid nitrogen on site, and kept at -20° C for perchlorate analysis. The fish were then dried under a fume hood, ground in a Waring blender, and extracted on an Accelerated Solvent Extraction apparatus, using water as the solvent. Extracts were then analyzed for perchlorate according to **Appendix X**. The sampling sites included Station Creek (T20-T23), South Bosque River at Highway 317 (T16), S Creek at Highway 317 (T15), and a tributary of Harris Creek off US Highway 84 (T17) just west of McGregor, TX. These sites are shown on **Figure 5-143**. In Lake Waco and Lake Belton, fish were collected with an aluminum boat equipped with an electroshocking apparatus. All boat electroshocking was carried out within 50 m of the shoreline. Fish were collected from two to four stations per lake, with each station comprising an approximately 100-150 m stretch of shoreline. At least three water samples (as above) were collected at each station where fish were collected. Fish collected from the lakes were identified to species, anesthetized on ice, and sacrificed by cervical scission. Fillets were then collected, frozen in liquid nitrogen on site, and kept at -20 °C until perchlorate analysis according to **Appendix X**. In some cases, heads were also collected because laboratory data (see Section 5.4.1.3) indicated that perchlorate may

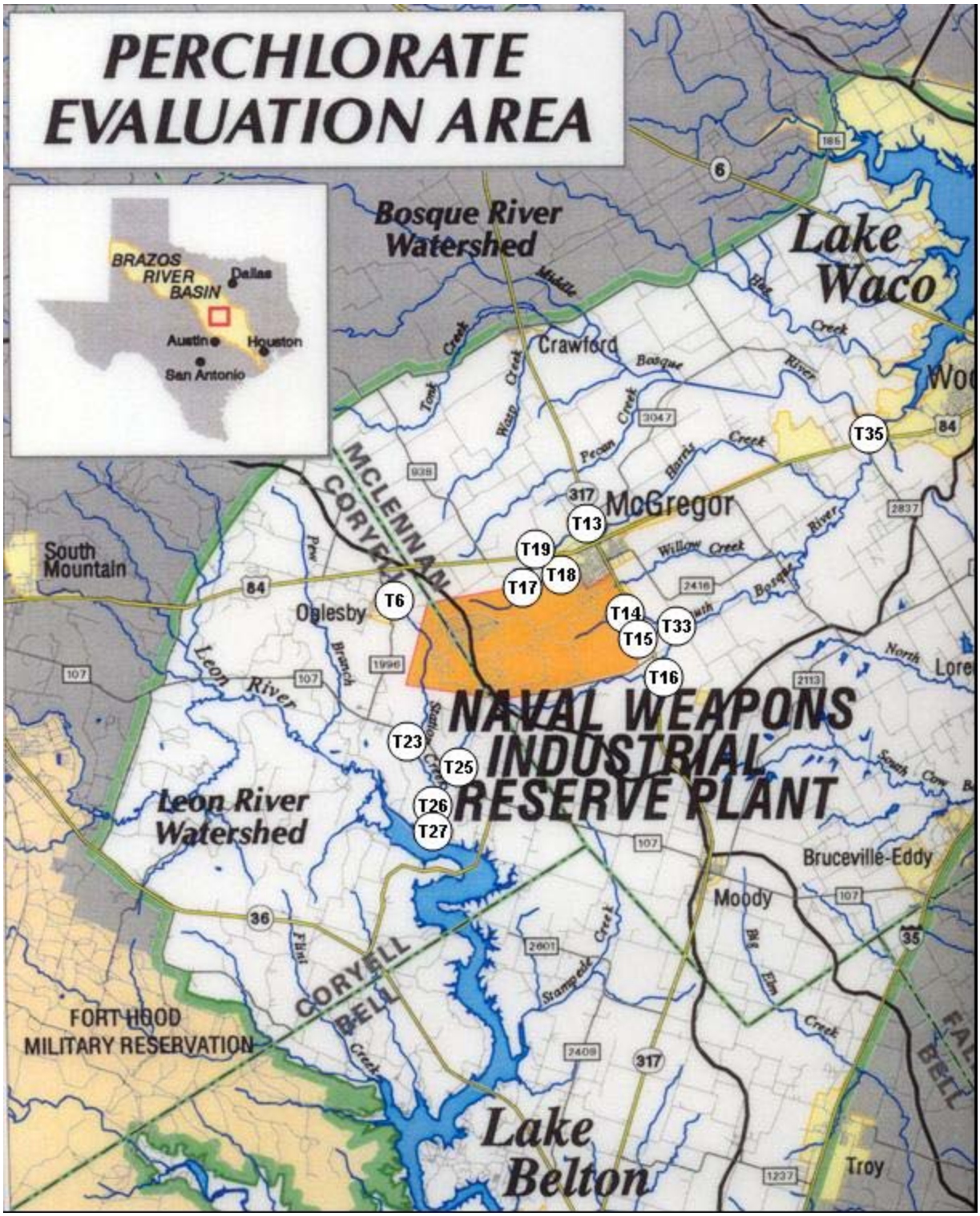

Figure 5-143
Map of Study Area Illustrating the Approximate Locations where Fish, Water, and Sediment Pore Water Samples Were Collected

accumulate in the head at concentrations that are greater than in the fillet. Because perchlorate concentrations in the fillets may have been near the detection limit, heads were analyzed because it was hypothesized that the concentrations in the head may be a more sensitive indicator than perchlorate accumulation in the edible portions of the fish.

5.4.1.1.3 Data

5.4.1.1.3.1 Preliminary Assessments

Results of the initial assessment of perchlorate residues in fish are presented in **Table 5-27**. Because of the location of thyroid follicles in fish and the size of the fish captured, this initial assessment focused on residues analyses of fish heads.

Table 5-27
Preliminary Data on Perchlorate Concentrations in Fish Heads within the Lake Waco and Lake Belton Watershed

Location	Species	Perchlorate (ng/g)
South Bosque at Indian Trail (T33)	Suckers (4)	2030 [930]
		1940 [890]
		2110 [970]
		2290 [1050]
	Catfish (3)	1360 [630]
		1010 [470]
		1850 [850]
	Largemouth Bass (1)	600 [280]
Harris Creek at Highway 317 (T13)	Sunfish (4)	740 [340]
		890 [410]
		2-ND
	Catfish (4)	730 [350]
		190 [90]
		290 [140]
		270 [130]
	Largemouth Bass (1)	ND
Harris Creek at Highway 84 (T19)	Sunfish (4)	710 [340]
		360 [170]
		1060 [500]
		690 [330]
Station Creek at Highway 107 (T23)	Sunfish (4)	ND
		540 [240]
		290 [130]
		2730 [1230]

ND = not detected by the analytical procedure [detection limit in dry tissue = 100 ppb]
Perchlorate concentrations are in ng/g (ppb) expressed based on tissue dry weight. Data represent the results of analyses of individual tissues (heads) with the number of analyses indicated parenthetically. Respective wet weight concentrations are presented in brackets. Samples were collected in May, 2001 from areas previously identified through water quality analyses as having received perchlorate.

As part of the preliminary assessment, fillets from catchable-size fish were also collected within the Leon and South Bosque Rivers and their tributaries during August/September, 2001. At the time of fish collection, water samples (**Table 5-28**) and sediment pore water samples (**Table 5-29**) were also collected. All samples (59 fillet samples from 6 locations, 18 sediment samples from 7 locations, and 41 water samples from 16 locations) were analyzed for perchlorate contamination using ion chromatography.

Only four (4) fillets tested positive for quantifiable perchlorate residues (**Table 5-30**). Two additional fish had trace quantities of perchlorate (perchlorate levels detectable, but below the limit of quantitation). Perchlorate was not detected in sediment samples from locations where fish were collected or from additional areas within the Lake Waco and Lake Belton Watersheds. Perchlorate was detected in several water samples from locations within the two Watersheds, consistent with our previous data indicating various levels of perchlorate contamination in flowing water.

Table 5-28
Perchlorate Concentrations in Surface Water within the Lake Waco and Lake Belton Watersheds Corresponding to the Dates of Fish Sampling during a Preliminary Assessment

Location	Sample ID	Perchlorate (ng/mL)	Collection Date
Harris Creek at Highway 317 (T13)	00490	ND	08/28/01
	00253	ND	09/06/01*
	00247	ND	09/06/01*
Harris Creek at Oglesby Road (T17)	00457	ND	08/28/01
	00264	ND	09/06/01*
	00265	ND	09/06/01*
Harris Creek at Highway 84 (T19)	00483	ND	08/28/01
Spring on Oglesby Road (T18)	00462	62	08/28/01
	00263	50	09/06/01
	00261	48	09/06/01
Unnamed Tributary near WWTP at Highway 317 (T14)	00453	ND	08/28/01
	00271	36	09/06/01
	00270	34	09/06/01
S Creek at Highway 317 (T15)	02303	388	09/07/01
	02245	402	09/07/01
South Bosque at Highway 317 (T16)	00452	ND	08/28/01
	02301	ND	09/06/01*
	02302	ND	09/06/01*
South Bosque at Indian Trail (T33)	00469	36	08/29/01*
	00477	34	08/29/01*
South Bosque at Highway 84 (T35)	00492	ND	08/28/01*
	00459	ND	08/28/01*
	00520	ND	08/28/01*
	00466	ND	08/28/01*
	00481	ND	08/28/01*
Station Creek at Highway 107 (T23)	00451	ND	08/28/01
	02277	32	09/06/01
	02276	29	09/06/01
	02314	30	09/07/01
	02319	32	09/07/01
Station Creek at Leon River (T27)	00267	ND	09/06/01
	00266	ND	09/06/01
	02298	ND	09/07/01*
	02294	ND	09/07/01*
Station Creek at Old River Road (T26)	00464	ND	08/29/01
	00275	ND	09/06/01
	00246	ND	09/06/01
Station Creek at Oglesby Road (T6)	00455	ND	08/28/01
Onion Creek at Highway 107 (T25)	00450	33	08/28/01

*Date of fish collection
ND = not detected (detection limit = 1 ppb)

Table 5-29
Perchlorate Concentrations in Sediment Pore Water within the Lake Waco and Lake Belton Watersheds Corresponding to the Dates of Fish Sampling during a Preliminary Assessment

Location	Sample ID	Perchlorate (ng/mL)	Collection Date
Harris Creek at Highway 317 (T13)	00234	ND	09/06/01*
	00235	ND	09/06/01*
Harris Creek at Oglesby Road (T17)	02244	ND	09/06/01
Unnamed Tributary near WWTP at Highway 317 (T14)	02304	ND	09/06/01
	02321	ND	09/06/01
S Creek at Highway 317 (T15)	02300	ND	09/07/01
	02320	ND	09/07/01
South Bosque at Indian Trail (T33)	00478	ND	08/29/01*
	00479	ND	08/29/01*
South Bosque at Highway 84 (T35)	00468	ND	08/28/01*
	00448	ND	08/28/01*
	00519	ND	08/28/01*
	00420	ND	08/28/01*
	00518	ND	08/28/01*
Station Creek at Leon River (T27)	00269	ND	09/06/01
	00268	ND	09/06/01
	02243	ND	09/07/01*
	02272	ND	09/07/01*

*Date of fish collection.
ND = not detected (detection limit = 4 ppb)

Table 5-30
Perchlorate Concentrations in Fish Fillets Within the Lake Waco and Lake Belton Watersheds

Location	Species	Perchlorate (ng/g)
Harris Creek at Highway 317 (T13)	Green sunfish (2)	2-ND
	Largemouth Bass (4)	1-TRACE 3-ND
	Yellow bullhead (1)	1-ND
Harris Creek at Oglesby Road (T17)	Green sunfish (2)	2-ND
	Yellow bullhead (1)	1-TRACE
South Bosque at Highway 84 (T35)	Carp (2)	2-ND
	Green sunfish (1)	1-ND
	Sucker (1)	1-ND
	Channel catfish (6)	6-ND
	Large mouth bass (6)	1-690 5-ND
	White bass (1)	1-ND
	Flat head catfish (1)	1-ND
	Sunfish (2)	2-ND
South Bosque at Indian Trail (T33)	Largemouth bass (16)	1-590 15-ND
	Sunfish (6)	6-ND
	Sucker (1)	1-ND
South Bosque at Highway 317 (T16)	Green sunfish (2)	1-260 1-ND
Station Creek at Leon River (T27)	Channel catfish (4)	1-260 3-ND
	Yellow bullhead (1)	1-ND

ND = not detected by the analytical procedure [detection limit in wet tissue = 170 ppb].
TRACE = perchlorate was detected, but the perchlorate concentration in the extract was below the limit of quantitation (2.5 ppb).

(Perchlorate concentrations are in ng/g (ppb) expressed based on tissue wet weight. Data represent the results of analyses of individual tissues (fillets), except where indicated, with the number of analyses indicated parenthetically. Samples were collected in August-September, 2001 from areas previously identified through water quality analyses as having received perchlorate.)

5.4.1.1.3.2 Routine Monitoring

Water and tissue concentrations for perchlorate are presented for samples collected in October, 2001 (**Table 5-31** and **Table 5-32**, respectively), March, 2002 (**Table 5-33**), and August, 2002 (**Table 5-34** and **Table 5-35**). Sites sampled are shown in **Figure 5-144**. Perchlorate water concentrations for March are indicated in **Appendix C**. All body burden data are expressed on a per dry weight basis, and were based on whole body analysis. Most of the fish collected from streams in the study area were not of legal size (>8 inches).

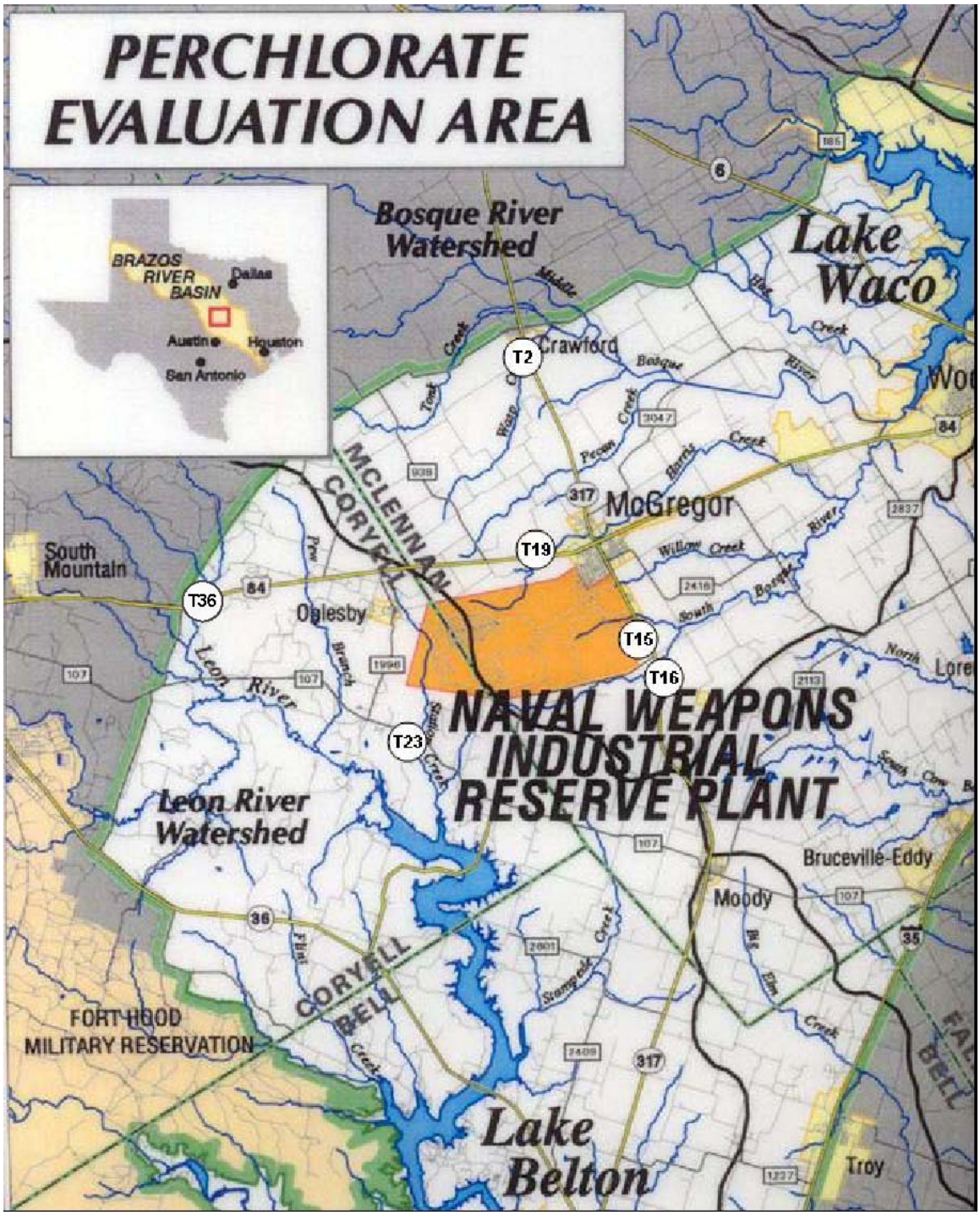

Figure 5-144
Map of Study Area Illustrating the Approximate Locations where Fish Samples Were Collected

Table 5-31
Average (± Standard Deviation) Perchlorate Concentration in Water Samples Collected at Various Streams Near NWIRP in October, 2001

Sampling Site	Conc. (ppb)
Coryell Creek (T36)	ND
Wasp Creek (T2)	ND
Harris Creek at Highway 84 (T19)	28.8 ± 4.4
Station Creek at Highway 107 (T23)	23.6 ± 0.9
S Creek at Highway 317 (T15)	202.6 ± 23.5
South Bosque at Highway 317 (T16)	10.8 ± 9.3

n = 3
ND = not detected (method detection limit = 1ppb)

Table 5-32
Whole Body Concentrations of Perchlorate (ppb) in Fish Collected Near NWIRP in October, 2001

Species	Collection Sites				
	Coryell Creek (T36)	Wasp Creek (T2)	Harris Creek (T19)	South Bosque (T16)	Station Creek (T23)
Green sunfish	ND (n=2)	ND (n=3)	170 ± 159 (n=5)	81 ± 181 (n=5)	ND (n=2)
Longear sunfish	ND (n=4)	ND (n=2)	-	88 ± 95 (n=8)	ND (n=1)
Stoneroller minnow	ND (n=1)	ND (n=3)	-	-	79 ± 136 (n=7)
Yellow bullhead	ND (n=7)	-	-	ND (n=1)	ND (n=3)
Redfin shiner	-	-	-	-	ND (n=1)
Blackspotted topminnow	-	-	-	ND (n=2)	-
Blacktail shiner	-	ND (n=1)	-	ND (n=1)	220 (n=1)
Black bullhead	-	-	ND (n=1)	-	ND (n=2)
Mosquitofish	-	ND (n=1)	-	ND (n=1) 1198 (n=1)	-
Largemouth bass	-	-	-	-	ND (n=2)

ND = not detected
- = fish not collected

Table 5-33
Whole Body Concentrations of Perchlorate (ppb) in Fish Collected Near NWIRP in March, 2002

Species	Collection Sites			
	Coryell Creek (T36)	Harris Creek (T19)	South Bosque (T16)	Station Creek (T23)
Green sunfish	ND (n=6)	ND (n=9)	ND (n=8)	ND (n=11)
Longear sunfish	ND (n=5)	-	ND (n=11)	ND (n=5)
Stoneroller fish	ND (n=12)	-	ND (n=6)	ND (n=2)
Yellow bullhead	ND (n=6)	-	ND (n=2)	ND (n=4) 250, 125
Redfin shiner	ND (n=2)	-	-	ND (n=8)
Blacktail shiner	ND (n=2)	-	ND (n=5)	-
Black bullhead	-	ND (n=4)	-	-
Darter	-	-	ND (n=1)	-
Mosquitofish	-	-	ND (n=1)	-
Largemouth bass	-	-	ND (n=1) 373, 246	-
Bluegill sunfish	-	-	-	ND (n=1)

ND = not detected
- = fish not collected

Table 5-34
Average (± Standard Deviation) Perchlorate Concentration in Water Samples Collected at Various Streams Near NWIRP in August, 2002

Site	Conc. (ppb)
Coryell Creek (T36)	ND
Wasp Creek (T2)	ND
Harris Creek at Highway 84 (T19)	24.2 ± 0.1
Station Creek at Highway 107 (T23)	14.2 ± 0.9
S Creek at Highway 317 (T15)	343.1 ± 0.1
South Bosque at Highway 317 (T16)	ND

ND = not detected (method detection limit = 1ppb)
n = 3

Table 5-35
Whole Body Concentrations of Perchlorate (ppb) in Fish Collected Near NWIRP in August, 2002

Species	Collection Sites			
	Coryell Creek (T36)	Wasp Creek (T2)	South Bosque (T16)	Station Creek (T23)
Green sunfish	ND (n=5)	569 ± 205 (n=3) ND (n=2)	ND (n=5)	ND (n=4) 541 (n=1)
Stoneroller minnows	ND (n=5)	ND (n=3) 1593 ± 335 (n=2)	ND (n=4) 3153 (n=1)	ND (n=5)

ND = not detected

In general, detection of perchlorate in fish tissues was sporadic, but, when it was found, tissue concentrations were higher than that found in the water.

Results from analysis of edible portions (fillets) of fish collected from Lakes Belton and Waco are presented in **Table 5-36** (February, 2002) and **Table 5-37** (March, 2002). Perchlorate was not detected in any water samples. All fish were of legal size (>8 inches for most fish), although some of the species collected (drum, spotted gar) are not typically consumed by humans. Refer to **Plate 6** for sampling locations in Lake Belton and Lake Waco.

Generally, the trends in the lakes are similar to that in the streams: sporadic detection of perchlorate at concentrations in the fillets greater than in the water. For black crappie, perchlorate was more likely to be detected in the head than in the fillet. Perchlorate was more likely to be detected in the fillets than in the heads for largemouth bass, spotted gar, and drum.

Table 5-36
Summary of Perchlorate Analysis for Fish Collected from Lakes Waco and Belton in February, 2002

Lake	Location	Species	Number Collected[a]	Number Detects[b]	Concentration[c]
Lake Waco	Site 1[d]	Largemouth Bass	10	1	990
		Carp	3	0	
		Channel Catfish	4	0	
		White Bass	3	0	
		Drum	2	0	
		Smallmouth Buffalo	2	0	
		Shad	4	0	
		White Crappie	1	0	
		Bluegill	3	0	
		Warmouth Sunfish	2	0	
	Site 2[d]	Largemouth Bass	1	1	550
		Channel Catfish	1	0	
		River Carpsucker	1	0	
Lake Belton	Site 1[d]	Largemouth Bass	4	0	
		Black Crappie	3	0	
		Shad	1	0	
		Smallmouth Buffalo	1	0	
		Spotted Gar	1	0	
	Site 2[d]	Largemouth Bass	2	1	720
		Channel Catfish	5	1	970
		White Bass	3	0	
		Black Crappie	1	0	
		Spotted Gar	1	0	
		Drum	2	0	

[a]Total number of fish collected at that site
[b]Number of fish in which perchlorate was above the detection limit (330 ppb)
[c]Concentration (ppb) of perchlorate in fish for which perchlorate was above the detection limit
[d]GPS coordinates for the sampling sites are as follows: Lake Waco Site 1 – N31°30.752' W 097° 15.130'; Lake Waco Site 2 – N 31° 31.702' W 097° 14.739'; Lake Belton Site 1 - N31° 16.090' W 097° 28.789'; Lake Belton Site 2 - N31° 15.986 W 097° 28.484'.

Table 5-37
Summary of Perchlorate Analysis for Fish Fillets and Heads Collected from Lakes Waco and Belton in May, 2002

Location[a]	Species	# detected / # analyzed		Concentration[b] (ppb)	
		fillet	head	fillet	head
Lake Waco					
Site 1	White bass	0/3	0/3		
	Largemouth bass	0/1	1/1		4560
	Drum	0/3	0/3		
	Flathead catfish	0/1	0/1		
	Channel catfish	0/3	1/3		626
	Black crappie	0/3	1/3		1960
	Bluegill sunfish	0/1	0/1		
Site 2	Carp	0/2	-		
	Largemouth bass	1/3	0/3	1400	
	Drum	0/3	0/1		
	Shad	0/1	0/1		
	Redear sunfish	0/1	0/1		
	Black crappie	0/3	2/3		1770; 1510
Lake Belton					
Site 1	Spotted gar	2/2	0/1	2040; 3960	
	Carp	0/2	-		
	Smallmouth buffalo	0/1	-		
	Largemouth bass	0/2	0/1		
	White bass	0/2	0/2		
	Drum	0/3	0/2		
	Shad	0/3	0/3		
	Bluegill sunfish	0/2	0/2		
	Flathead catfish	0/1	-		
Site 2	Spotted gar	1/3	-	1910	
	Drum	1/3	0/3	2740	
	White crappie	0/1	0/1		
	Shad	0/3	0/3		
	Channel catfish	0/2	-		

[a]Sites are the same as described in **Table 5-36**
[b]Concentration (ppb) of perchlorate in fish for which perchlorate was above the detection limit.
Perchlorate concentrations are in ng/g (ppb) tissue dry weight

5.4.1.1.4 Discussion

The results presented here are similar to those found in Smith et al. (2001), who also sporadically detected perchlorate in fish tissues at concentrations that were greater than in the water. Possible explanations for this may be that a) perchlorate uptake occurs via other routes than from the water (e.g., via algae, see Section 5.3.2), or b) there is a high temporal variation in perchlorate levels in the water over time, and elimination from fish tissues may lag behind decreases in ambient water concentrations (illustrated in laboratory studies presented in this report). In general, the frequency of detection of perchlorate in fish tissues seems to be associated with proximity to the NWIRP. It is important to note that most (> 95%) of the fish caught in streams in the study area were not of legal size (> 8 inches), thus they would not likely be consumed by humans.

Curiously, perchlorate was found in 3 of 5 green sunfish and 2 of 5 stonerollers collected from Wasp Creek in August, 2002. There is no logical explanation for this occurrence. Based on findings during this study, including groundwater and surface water interaction, there is no known migration pathway for perchlorate from NWIRP to result in exposure of fish in this stream. Wasp Creek does not originate on NWIRP grounds, as do Station and Harris Creeks and the South Bosque River. Wasp Creek is located in a separate sub-watershed, the boundary of which is approximately 1.5 miles from NWIRP, and the distance from known groundwater plumes of perchlorate is substantial. Nonetheless, the analytical quality assurance for these fish samples was satisfactory, so there was no analytical reason to qualify the data. Therefore, these data create a source of uncertainty for the potential exposure of fish to perchlorate in Wasp Creek.

In the lakes, perchlorate was occasionally detected in the fillets of several sport fish, most notably largemouth bass and channel catfish. However, because the U.S. EPA has not yet instituted an acceptable consumption limit or benchmark dose for food-borne exposure to perchlorate, it is not possible to determine if this is within acceptable limits. In addition, for black crappie, perchlorate was detected in the head but not the fillet on several instances. Although the head is not usually considered an edible tissue, the lack of perchlorate detection in the fillets of this species may be due to detection limits rather than absence of perchlorate in edible tissues.

Both laboratory and field data indicate that human exposure to perchlorate through consumption of fish caught in the study watersheds is possible, but highly unlikely. This conclusion is based on (1) where perchlorate occurs in surface water and the size of fish supported by those streams, (2) the preferential accumulation of perchlorate in rarely consumed tissue (head) rather than the fillet, (3) fillet residue data on larger fish caught near the exposure points and the lakes, and (4) laboratory studies on perchlorate tissue distributions in exposed fish.

5.4.1.2 Thyroid Histology in Native Fish

5.4.1.2.1 Introduction

Perchlorate is known to affect thyroid function, causing subsequent hormone disruption and potential perturbations of metabolic activities. In teleost fish, thyroid hormones promote growth (Donaldson et al., 1979), stimulate early gonadal development (Cyr and Eales, 1996), stimulate steroidogeneis (Sullivan et al., 1989; Cyr and Eales, 1996), and initiate metamorphosis in many fish (Inui and Miwa, 1985; Inui et al., 1995). Because of the involvement of thyroid hormone in fitness components such as reproduction, growth, and development, thyroid endocrine disruption could ultimately be manifested at the population, community or ecosystem level of biological organization. In most vertebrates, thyroid hormone also interacts with retinoid signaling pathways (retinoids are a class compounds that include vitamin A and derivative, and regulate limb development in embryonic and larval vertebrates [Chatterjee and Tata, 1992]).

Thus, the focus of this research is to explore spatial and temporal patterns of perchlorate concentrations in water and fish, as well as examining effects of perchlorate on thyroid function in fish.

5.4.1.2.2 Methodology

Central stonerollers (*Campostoma anomalum*) and green sunfish (*Lepomis cyanellus*) were collected from the streams listed in Section 5.4.1.1, as well as from Wasp Creek at Highway 317 (just south of Crawford, TX) and Coryell Creek (chosen as reference sites). Fish were collected with a backpack electroshocking device, as described above. They were anesthetized on site, and the heads were preserved in Bouin's fixative for histological analysis. Fish heads were left in Bouin's fixative for 2 days to decalcify and fix tissues. After 2 days, the Bouin's was removed and cassette storage containers were placed under gently running water overnight to continuously rinse the tissue. Storage containers were then filled with 70% ethanol, and the ethanol was replaced daily until the yellow color in the tissues disappeared. Tissues were processed with the Tissue-Tek V.I.P. 2000 Processor (Miles Laboratories, Elkhart, IN) and then embedded in paraffin. Sections were cut with a microtome (5 um) and mounted on microscope slides. The slides were then stained with hematoxylin and Eosin Y for observation following Humason (1967).

Slides were observed with a light microscope and the number of follicles per section and the height of the follicular epithelium were measured in 10 sections randomly selected from each fish in each exposure group. In each fish, a total of 10 follicles were selected, and the height of 10 epithelial cells per follicle were measured. Five follicles from the rostral end (the first section to have at least 5 follicles) and from the caudal end (the section with at least 5 follicles not included in the first group) of the thyroid follicular region were measured. Individual sections were randomly chosen based on the overall quality (whole follicles, no folds or tears) of the section. For each follicle, epithelial cell height was measured using a micrometer. For each fish, the percent hyperplastic follicles, and percent of follicles with depleted colloid, were also noted.

5.4.1.2.3 Data

The results from histological analysis of thyroid tissues for fish collected from NWIRP streams in October 2001 and August 2002 are presented in **Figure 5-145** and **Figure 5-146**, respectively. The hypothesis of differences among sites was tested using a nonparametric (Kruskall-Wallis) test, because the data did not meet the criteria for using parametric statistics (normality and homogeneity of variances). The data from the October 2001 sampling indicate that, for thyroid follicle epithelial heights, the level of effect is in the order of Harris Creek > Wasp Creek > Station Creek > Coryell Creek.

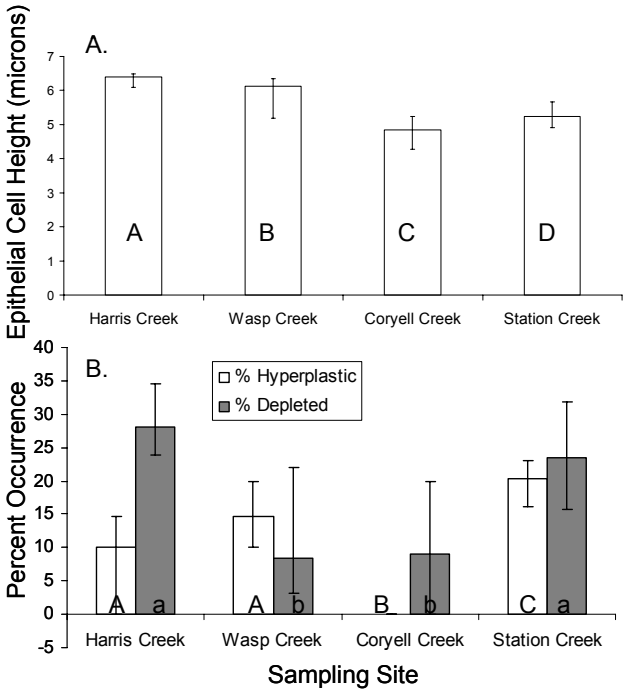

Figure 5-145
Thyroid Follicular Epithelial Cell Heights (A) and Percent Occurrence of Follicles with Hyperplasic Epithelia or Depleted Colloid (B) from Stoneroller Minnows Collected from Various NWIRP Streams in October, 2001

Bars and error bars represent medians with first and third quartiles. Bars labeled with different letters are statically significantly different ($P < 0.05$, Kruskall-Wallis test).

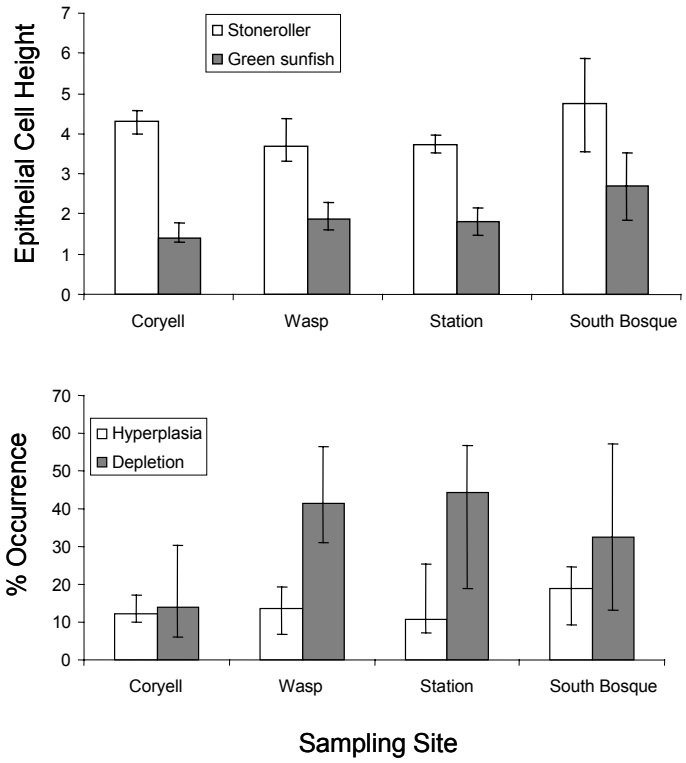

Figure 5-146
Thyroid Follicular Epithelial Cell Heights for Stonerollers and Green Sunfish (A) and Percent Occurrence of Follicles with Hyperplasic Eptihelia or Depleted Colloid in Stonerollers (B) Collected from Various NWIRP Streams in August, 2002

Bars and error bars represent medians with first and third quartiles. Bars labeled with different letters are statically significantly different ($P < 0.05$, Kruskall-Wallis test).

For percent occurrence of hyperplasic follicles, the relative levels of effect were Station Creek > Wasp Creek = Harris Creek > Coryell Creek, and for percent occurrence of follicles with depleted colloid the levels of effect were Harris Creek = Station Creek > Wasp Creek = Coryell Creek. In addition, all three metrics were used to classify the sites as to magnitude of effect by calculating an average rank for each individual metric (**Table 5-38**). For example, for the follicle epithelial cell heights, Harris Creek was given a rank of "1", Wasp Creek was ranked a "2", Station Creek a "3" and Coryell Creek a "4". In the case of ties (no statistically significant differences) the tied sites were given average ranks. For instance, for depleted colloid, Harris Creek and Station Creek were each given a rank of 1.5 (average of 1 and 2) and Wasp Creek and Coryell Creek were each given a rank of 3.5 (average of 3 and 4). For each site, the ranks were averaged over all three metrics (epithelial cell height, percent hyperplasic follicles, percent depleted colloid) in order to determine relative overall level of impact. According to this method, the order of overall level of impact was Harris Creek > Station Creek > Wasp Creek > Coryell Creek (**Table 5-38**).

Table 5-38
Ranking of NWIRP Sites According to Magnitude of Response for Several Thyroid Histopathological Metrics

Sampling Site	Rank				Average Rank
	Epithelial height		Hyperplasic follicles	Depleted colloid	
	Stoneroller	Green sunfish			
A. October 2001					
Station Creek (T23)	3	-	1	1.5	1.8
Harris Creek (T19)	1	-	2.5	1.5	2.3
Wasp Creek (T2)	2	-	2.5	3.5	2.7
Coryell Creek (T36)	4	-	4	3.5	3.8
B. August 2002					
Station Creek (T23)	3.5	2.5	2	2.5	2.625
South Bosque (T16)	1.5	1	2	2.5	1.75
Wasp Creek (T2)	3.5	2.5	2	2.5	2.625
Coryell Creek (T36)	1.5	3	4	2.5	2.75

* See **Figure 5-144** for sampling locations.

For the data from the August 2002 sampling, the epithelial cell heights were calculated for green sunfish and stonerollers, while the percent occurrence for hyperplasia and colloid depletion were calculated for stonerollers alone. The data for stoneroller and green sunfish epithelial cell heights indicate relative levels of effect in the order of Coryell Creek = South Bosque > Wasp Creek = Station Creek, and South Bosque > Station Creek = Wasp Creek > Coryell Creek, respectively. For percent depleted colloid, the relative levels of effect was in the order of Wasp Creek = Station Creek = South Bosque > Coryell Creek (**Table 5-38**). There were no statistically significant differences between sites for percent hyperplasic follicles, although Station Creek and South Bosque had the highest values (**Figure 5-146**). The average ranks among sites (averaged over all metrics) were in the order of South Bosque > Station Creek = Wasp Creek > Coryell Creek.

5.4.1.2.4 Discussion

Overall, the level of effect was associated with proximity to the NWIRP. Fish collected from streams that originated on or passed through the NWIRP grounds, especially Harris Creek and the South Bosque River, showed more thyroid histology impacts than fish from other areas.

Although both Wasp Creek and Coryell Creek were intended to be reference sites, results for fish collected from Wasp Creek were not entirely consistent with fish collected from Coryell Creek. The Wasp Creek fish seemed to be intermediate between the NWIRP streams and Coryell Creek. Although perchlorate and thyroid histology impacts were found in some fish collected from Wasp Creek (discussed in Section 5.4.1.1), there was no logical explanation for the perchlorate residue data. Additionally, thyroid histological impacts were identified in some fish from Wasp Creek that had no detectable concentrations of perchlorate in tissue.

In comparing the epithelial cell heights between green sunfish and stonerollers, the patterns seen in the sunfish more closely match the perchlorate contamination histories of the streams. Wasp Creek does not originate on NWIRP grounds, as do Station and Harris Creeks and the South Bosque River. Wasp Creek is located in a separate sub-watershed from NWIRP, and the distance from known groundwater plumes of perchlorate is substantial. There is no known migration pathway for perchlorate from NWIRP to result in exposure of fish in this stream. Therefore, data indicating thyroid histopathological impacts in fish from Wasp Creek create a source of uncertainty, as some other component in the water (or the lack of some nutrient) could be impacting thyroid histology.

In conclusion, the thyroid histopathology data presented suggest that these fish were impacted by perchlorate, although perchlorate is not the only environmental contaminant that could influence changes in thyroid histology (a lack of iodine, for example, could also influence thyroid structure and function). The conclusion that fish were impacted by perchlorate is supported by an integrated approach using multiple vitiates ("weight of evidence" Suter, 1993). This approach is more robust than looking at just one biomarker of effect. Although thyroid histology is adversely impacted in fish from streams near NWIRP, laboratory studies indicate that fish can recover from these effects as perchlorate is removed. In addition, it is not clear whether the negative thyroid impacts observed are sufficient to cause changes in fish populations in the study streams. In general, fish were abundant in most of the streams sampled, suggesting that any impact of perchlorate was not being manifested at the community or population level.

5.4.1.3 Kinetics of Perchlorate Uptake and Depuration in Laboratory Fish

5.4.1.3.1 Introduction

Although the mechanisms by which perchlorate acts on the thyroid have been well established, there is little information on the dose-response of perchlorate in ecologically relevant species. Uptake and depuration kinetics of perchlorate may provide a better understanding of perchlorate fate and effects in fishes, as well as providing information on the dose-response of perchlorate in environment. Also, there is little information on tissue distribution of perchlorate in fish. Such information would be useful in determining which tissue to use for biomonitoring purposes, and would be vital for developing physiologically-based toxicokinetic models in fish. The rates of perchlorate uptake and depuration in channel catfish (*Ictalurus punctatus*) and eastern mosquitofish (*Gambusia holbrooki*) were examined following short-term exposure. Because perchlorate release is often characterized by the short-term release of relatively high concentrations of toxicant

(Smith et al., 2001), and because depuration of highly water-soluble compounds is expected to occur relatively rapidly, the experimental design employed a limited-time exposure and high perchlorate concentration.

5.4.1.3.2 Methodology

5.4.1.3.2.1 Uptake

Female eastern mosquitofish (*Gambusia affinis*) and channel catfish (*Ictalurus punctatus*) were exposed to 0, 0.1, 1, 10, 100, and 1000 mg/L sodium perchlorate for 2, 10, and 30 days with 5 replicate aquaria of each exposure group. After these exposure times, perchlorate was determined in the whole bodies. Channel catfish were exposed to 100 mg/L sodium perchlorate for 5 days with 20 replicates to determine the uptake of perchlorate into specific tissues. Sex was not determined in the catfish. Mosquitofish and catfish were obtained from commercial hatcheries and treated with antibiotics prior to exposure. All fish were allowed to acclimate to the laboratory conditions for at least 5 days prior to exposure.

Fifteen mosquitofish were randomly assigned to each 15 L aquaria water consisting of 60 mg/L Instant Ocean sea salts in deionized water with the appropriate amount of sodium perchlorate stock solution added to each test aquarium. Fish were fed commercial fish flake food daily. Water samples were also taken on the first day and last day of each exposure and once a week for the longer exposures and analyzed to determine actual perchlorate concentration in each aquarium. Following each exposure, the mosquitofish were euthanized in MS-222 (3-aminobenzoic acid ethyl ester and $NaHCO_3$, each mixed in distilled water at 1.5 g/L) and the length and weight of each fish was recorded. Approximately 8 fish from each aquaria were frozen in liquid nitrogen for perchlorate analysis, with the remainder used for other analyses.

One catfish was assigned to each 15 L aquarium. Aquaria water was composed of reconstituted fresh water as above with the appropriate amount of sodium perchlorate stock solution added to each test aquarium. Fish were fed commercial shrimp pellet food each day, and feeding time, daily observations of behavior, fish health and condition, and number of dead fish were recorded. Water changes and water quality analyses were as discussed above. Following exposure, the catfish were euthanized in 1.5 g/L MS-222 and The kidney (KY), liver (LV), gill (GL), gonad (GD), gastrointestinal tract (GI), head (HD), and fillet (FL) were dissected out and preserved in liquid nitrogen for determination of perchlorate concentrations into each tissue.

For perchlorate analysis, fish were desiccated under a fume hood, ground in a Warring blender, and extracted in an Accelerated Solvent Extractor, using water as the solvent. Extracts were cleaned using silica solid phase extraction (SPE) cartridges. The diluted extracts were analyzed as described in **Appendix X**.

5.4.1.3.2.2 Elimination

This experiment was carried out in a static renewal design system. In this system, fish were exposed to 100 mg/L sodium perchlorate solution for 2 days, then transferred to

pre-cleaned aquaria containing clean water. Fifty-two mosquitofish and 20 catfish were initially exposed to 100 mg/L sodium perchlorate for 2 days, and then transferred to aquaria containing clean water. At 0, 1, 2, 5, and 10 days of elimination, 4 catfish and 5 mosquitofish were removed, rinsed in 3 successive tanks filled with DI water to remove sodium perchlorate on their skin and gills, and anesthetized with MS-222 (1.5 g/L). Anesthetized fish were dissected quickly to collect tissues (muscle, liver, gastrointestinal (GI) tract (stomach + intestine), gills and head). All tissue samples were immediately frozen in liquid nitrogen, and stored at -70 °C until perchlorate analysis. In a subsequent experiment, catfish were again exposed for 2 days and allowed to depurate for 20 days. At 0 and 20 days, we dissected 2-day exposed catfish following above-mentioned procedure. For mosquitofish, we followed above methods, except that we used whole body to determine the concentration of perchlorate. After exposing mosquitofish to 100 mg/L sodium perchlorate for 2 days, they were transferred to clean water. Seven fish in each replicate were collected at 0, 1, 2, 5, 10, and 20 days. All samples collected from catfish and mosquitofish were also rinsed with DI water 3 times to remove potential perchlorate on surface of samples, weighed, air dried and extracted with distilled, deionized water and analyzed using ion chromatography, as described in **Appendix X**. Because the size of liver was too small to extract perchlorate from samples, we combined two catfish livers.

For perchlorate analysis, fish were thawed and desiccated under a fume hood, ground in a Warring blender. They were then extracted in an Accelerated Solvent Extractor, using water as the solvent. Extracts were analyzed for perchlorate as described in **Appendix X**.

5.4.1.3.2.3 Rate Constant Determination

The uptake rate constants (K_1) were calculated from $K_1=K_2 \cdot Cm/Cw$; Cm is the concentration in tissues and Cw is the concentration in water. When the fish are transferred to clean water, the elimination rate constant (K_2) was determined using a non-linear regression model: $Cm_T = C_{mo}e^{-K2T}$ where C_{mo} is the concentration of perchlorate in the selected tissue at the beginning of the experiment, C_{mT} the concentration of perchlorate in the selected tissue at the end of the experiment, and T the time of elimination (day). The half-life ($T_{1/2}$) of perchlorate in tissues was estimated according to the equation $T_{1/2} = -\ln 0.5/K_2$.

5.4.1.3.3 Data

5.4.1.3.3.1 Uptake of Perchlorate in Mosquitofish

Perchlorate was not detected in mosquitofish exposed to the lowest concentrations of perchlorate (0, 0.1, and 1 mg/L sodium perchlorate), regardless of the exposure time. Perchlorate was detected in mosquitofish exposed to 10, 100, and 1000 mg/L sodium perchlorate, but the whole body tissue concentrations were approximately 10 times less than the exposure concentration (**Figure 5-147**).

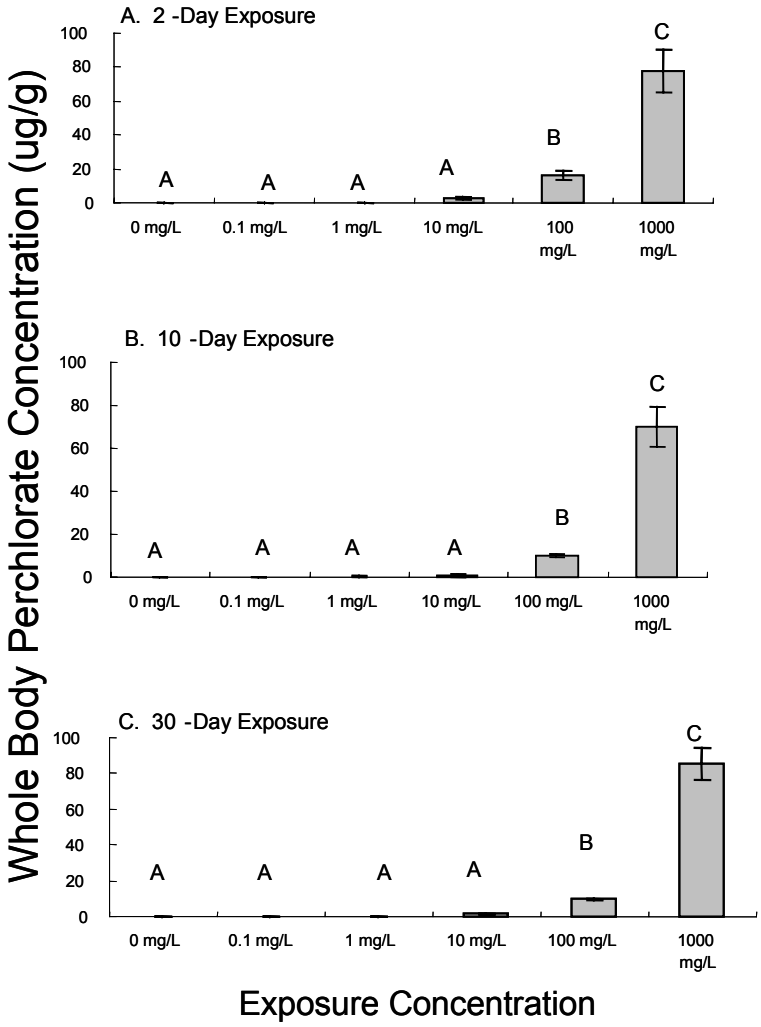

Figure 5-147
Perchlorate Uptake in Mosquitofish Exposed to Various Concentrations of Sodium Perchlorate for 2 days (A), 10 days (B), and 30 days (C)

Perchlorate concentrations are based upon the wet weight of the sample. Bars labeled with different letters are significantly different ($P < 0.05$, Kruskal-Wallis test). (Bars are the mean ± standard error, n = 5).

Bioconcentration factors for the uptake of perchlorate into whole mosquitofish were determined. Mosquitofish had very low BCF across all exposure concentrations. The highest BCF were in fish exposed to 10 and 100 mg/L sodium perchlorate with BCF of 0.15 and 0.12, respectively. BCF for all other exposure concentrations were less than 0.01.

Because the data for the uptake of perchlorate into mosquitofish were non-normally distributed and no transformations could be found that would normalize the data or homogenize the variances, differences between the concentrations in whole body mosquitofish were analyzed by the Kruskal-Wallis test. There was no difference in the uptake of perchlorate depending upon the exposure time ($P = 1.000$), which leads to the assumption that perchlorate does not bioconcentrate in fish. There was, however, a

difference in perchlorate uptake depending upon the concentration of the exposure dose ($P < 0.001$).

Comparisons of the treatments with the control group within each time period indicated there were differences in uptake in fish exposed to the 2 highest concentrations of perchlorate. Fish exposed to 100 mg/L sodium perchlorate in all 3 time periods had a significantly higher uptake than the control group ($P = 0.05$). Fish exposed to 1000 mg/L also had significantly higher uptake compared to the control group ($P = 0.01$).

Comparisons of treatment medians within each time period showed that mosquitofish exposed to 1000 mg/L sodium perchlorate in all 3 treatment time periods had a significantly greater uptake of perchlorate into the tissues ($P = 0.05$) compared to mosquitofish exposed to 0, 0.1, 1 mg/L sodium perchlorate. Fish exposed to 100 mg/L sodium perchlorate also had a significantly higher uptake of perchlorate into the tissues after the 2 day and 10 day exposures ($P = 0.10$) as well as after 30 days of exposure ($P = 0.05$) compared to fish exposed to the lower concentrations.

5.4.1.3.3.2 Uptake of Perchlorate in Catfish

In catfish, the largest concentrations of perchlorate were found in the head and in the fillet, while perchlorate was not detected in the gonad tissue. The gastrointestinal tract, liver, kidney, and gill all took up less perchlorate relative to the head and fillet. Based upon the results of this first experiment, the exposure was repeated with 5 catfish in order to look at sections of the head and the muscle and the skin separately in order to determine if there is a specific region in which perchlorate accumulates. **Figure 5-148** shows the uptake of perchlorate in catfish.

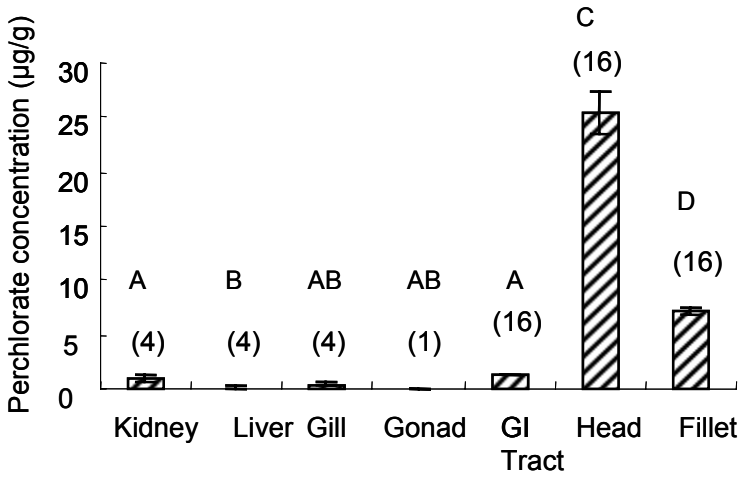

Figure 5-148
Uptake of Perchlorate in Tissues of Catfish Exposed to 100 mg/L Sodium Perchlorate for 5 days

Sample sizes are given in parentheses above each bar. (Bars are the mean of the actual concentrations ± standard error). Sample sizes < 16 indicate that tissues were pooled for that individual tissue to provide enough material for analysis. Bars labeled with the same letter are not statistically significantly different ($P > 0.05$, Kruskall-Wallis test).

The BCF's for all tissue types indicate that bioconcentration was very low. The highest BCF was in the head of the catfish with a BCF of 0.21, and the 3 head sections had BCF's of about 0.14, indicating that the differences in water concentration may have attributed to the differences in the head concentration compared to the head sections. The next highest BCF was in the fillet with a BCF of 0.060, and the skin and muscle tissues had a BCF of 0.14 and 0.03, respectively. All other BCF's were less than 0.01 (kidney, liver, gill, gonad, and gastrointestinal tract).

Because of the large variances in the uptake of perchlorate in catfish tissues, data were logged transformed and then analyzed by a 1-way ANOVA. Results from the 1-way ANOVA on the logged transformed data indicated there was a difference in perchlorate uptake into the various catfish tissues, depending upon which tissues were examined ($P < 0.001$). A comparison of the treatment means showed that most tissues had significantly different uptakes relative to all other tissues examined.

5.4.1.3.3.3 Perchlorate Elimination Rate in Catfish

The measured concentration of perchlorate, 83,843 ± 4,165 ppb in aquaria during exposure periods was close to the nominal concentration of sodium perchlorate, 100 ppm. In depuration period, the measured concentrations of perchlorate eliminated by the fish into the aquarium water were 69.7 ± 82.8 ppb, 121 ± 110 ppb, 138 ± 115 ppb, 107 ± 91 ppb, 71.2 ± 59.3 ppb, 56.0 ± 44.7 ppb, and 40.5 ± 29.5 ppb at 0, 1, 2, 3, 5, 7, and 10 days. The concentration of perchlorate in aquarium water during the first two days of the depuration period increased. No water change was performed during this period. Thereafter, the perchlorate concentration in aquarium water decreased due to the rapid elimination of perchlorate from body and the water changes, which occurred every other day. Accumulated perchlorate concentrations in each tissue during 2-day exposure period were showed at 4.1%, 21.1%, 13.3%, 14.2%, and 21.3% of exposure concentration in fillet, liver, GI tract, gills, and head, respectively (**Figure 5-149**).

The uptake rate constants (K_1) for 2 day exposure calculated in this study showed highest value in head and liver among the tissues, and fillet showed the lowest value (see **Table 5-39**).

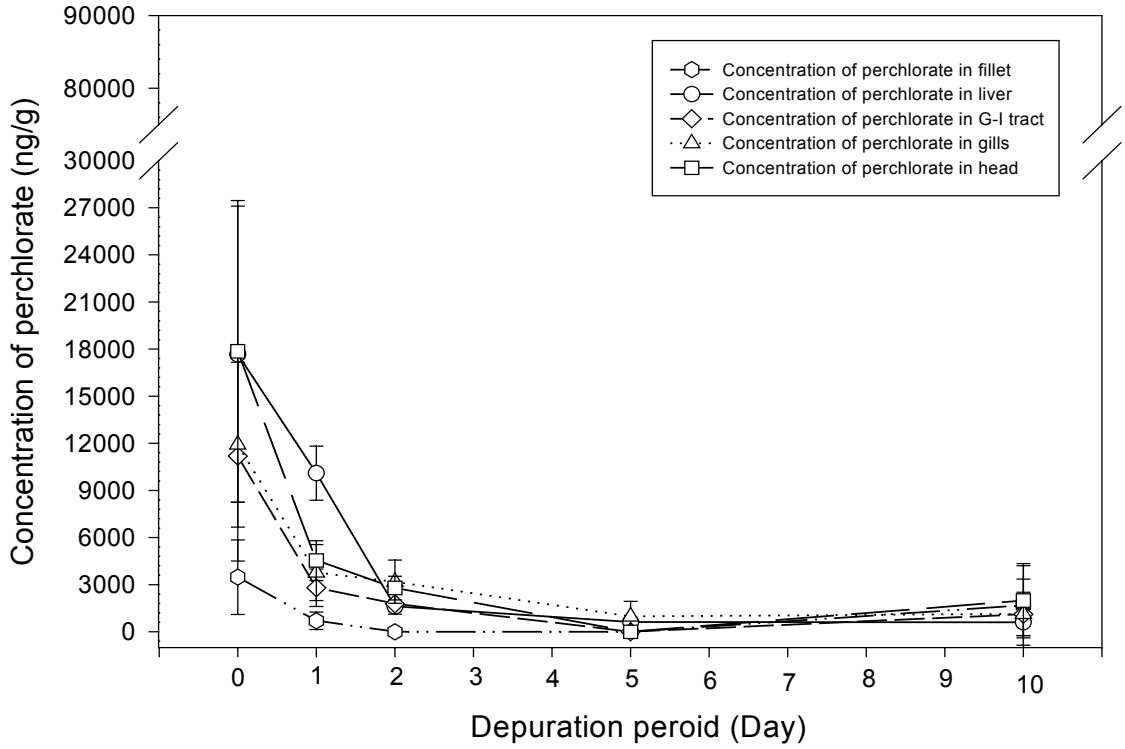

Figure 5-149
Perchlorate Contents (µg/g, Wet Weight) in each Tissue of Catfish (*Ictalurus punctatus*) during Recovery Period (10 Days) after Exposure to 100 g/L Sodium Perchlorate for 2 Days in a Static Design System (n=4, except Liver (n=2))

Table 5-39
Perchlorate Toxicokinetic Indexes in Tissues of the Channel Catfish (*Ictalurus punctatus*) and Whole Body of Mosquitofish (*Gambusia holbrooki*)

Tissues	Uptake Rate Constant K_1 (day^{-1})	Elimination Rate Constant K_2 (day^{-1})	Half-life $T_{1/2}$ (day)
Catfish Fillet	0.07	1.67	0.41
Liver	0.17	0.79	0.88
G-I tract	0.16	1.22	0.57
Gills	0.13	0.88	0.79
Head	0.26	1.22	0.57
Mosquitofish	0.10	0.76	0.91

After one day of elimination, more than 50% of perchlorate was eliminated from the fillet without skin (80%), head (75%), G-I tract (74%), gills (68%), and liver (43%) (**Figure 5-149**). However, perchlorate was still present in all tissues in at least some individuals

after 10 days. The uptake (K_1), elimination rate (K_2) and the half-life ($T_{1/2}$) of perchlorate were calculated and are presented in table 1. The elimination rate constant was most rapid from fillet and slowest from liver tissue. The elimination of perchlorate residues from fillet was approximately two times faster than liver. That would be related to a low half-life in fillet (0.41 day). In all tissues of catfish, perchlorate concentrations significantly varied with time (significance levels were $P < 0.05$), except in liver ($P = 0.14$). Observed concentrations of ClO_4^- in tissues of catfish decreased with time (**Figure 5-149**).

Because the perchlorate was not totally eliminated after 10 days, we expanded the depuration period to 20 days. The measured concentration of perchlorate during 2-day exposure periods was 78,274 ± 6,199 ppb, which is also close to nominal concentration of sodium perchlorate (100ppm). In depuration period, the measured concentrations of perchlorate eliminated into the water were 1.5 ± 3.4 ppb, 70.2 ± 28.4 ppb, 89.3 ± 33.8 ppb, 11.3 ± 5.0 ppb, 6.1 ± 3.6 ppb, 0.8 ± 1.8, and 0 ± 0 ppb, at 0, 1, 2, 6, 10, 14, and 18 days. At day 20, perchlorate was not detected at fillet, gill, and head; however, there were still detections of perchlorate in liver and G-I tract of catfish at 2 and 0.6 mg/L, respectively.

5.4.1.3.3.4 Perchlorate Elimination Rate in Mosquitofish

Analysis of perchlorate in aquarium water during a 2 day exposure period showed that the actual concentration of perchlorate was 73,720 ± 2,005 ppb and was close to the nominal concentration of sodium perchlorate (100 ppm). In depuration period, the measured concentrations of perchlorate eliminated into the water were 0 ± 0 ppb, 12.4 ± 0.9 ppb, 16.2 ± 1.3 ppb, 9.8 ± 0.8 ppb, 0 ± 0 ppb, 0 ± 0 ppb, 0 ± 0 ppb, and 0 ± 0 ppb at 0, 1, 2, 5, 10, 14, 18, and 20 days, respectively. The concentration of perchlorate in the water also increased during the first two days of the depuration, indicating that perchlorate was depurated from the body into water. No water change occurred during this period. The kinetics of perchlorate elimination in mosquitofish is presented in **Figure 5-150**.

Perchlorate concentrations in fish at the end of the 2-day exposure period were 9,880 ± 1,440. The accumulation of perchlorate after 2-day exposure was showed at 13.4%. The uptake (K_1), elimination rate (K_2) and the half-life ($T_{1/2}$) of perchlorate were calculated and are presented in **Table 5-39**. Perchlorate concentrations in fish also varied significantly with time ($P < 0.05$) and observed concentration of ClO_4^- in mosquitofish also decreased with time (**Figure 5-150**).

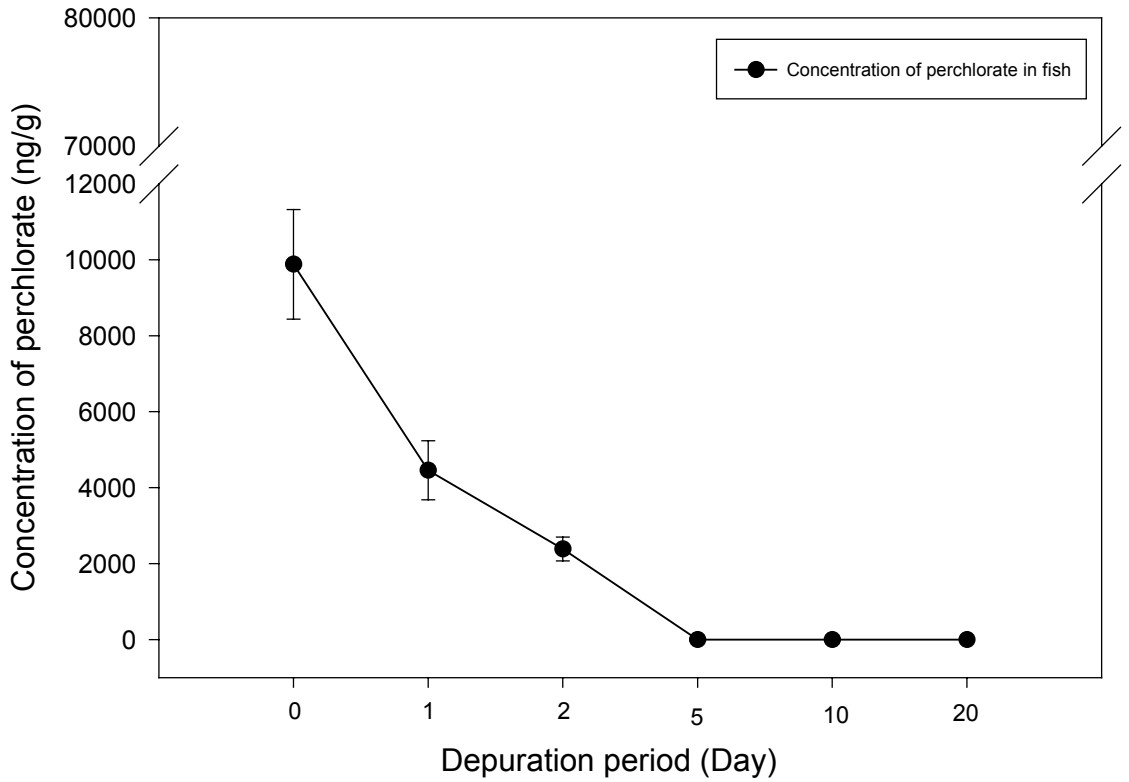

Figure 5-150
Perchlorate Contents (µg/g, Wet Weight) in Mosquitofish (*Gambusia holbrooki*) during Recovery Period (20 Days) after Exposure to 100 g/L Sodium Perchlorate for 2 Days in a Static Design System (n=5)

5.4.1.3.4 Discussion

In general, these results indicate that perchlorate is taken up and reaches steady state relatively rapidly, in accordance with one of the initial hypotheses. However, perchlorate was detected in fish only at elevated exposure concentrations and was not detected in fish that were exposed to environmentally relevant concentrations of perchlorate. Fish that were exposed to perchlorate in the laboratory did not bioaccumulate perchlorate; the tissue concentration of perchlorate was much lower than the exposure concentration of perchlorate. Contrary to this study, perchlorate concentrations in wild fish may be greater than those in the water.

Results from the catfish exposure to perchlorate were consistent with field studies. Under laboratory conditions, the highest perchlorate concentrations were found in the head, with the fillet having the next highest concentration. Perchlorate concentrations in the head were more than 3 times higher than the perchlorate concentrations in the fillets, and other tissues had very small amounts of perchlorate in comparison to the concentrations found in the head. Field studies were conducted to determine the amount of perchlorate present in the heads and fillets of several species of fish. These studies also found that the head

had much higher concentrations of perchlorate than did the fillets, at least for black crappie (Section 5.4.1.1).

The results of this study indicate that the head and fillet are the most important tissues to analyze when determining the uptake of perchlorate into fish. Although perchlorate did accumulate in other tissues in the catfish (kidney, liver, gill, and gastrointestinal tract), these tissues had very low concentrations of perchlorate compared to the head and the fillet. This may have important implications for dietary exposure of perchlorate to humans eating contaminated fish, because the muscle tissue usually constitutes the majority of tissue consumed. This information is necessary for determining ecological and human health exposure assessments. Information gained from uptake studies is also important for environmental fate and effects modeling.

The current study showed that ClO_4^- was absorbed, eliminated and distributed among tissues quickly. Rapid elimination of ClO_4^- in both species was observed when the animals were transferred to clean water, with half-lives of ClO_4^- in both species of less than 1 day. Mosquitofish had lower elimination rate and higher half-life than any tissue in the catfish. This fact could be considered as the effect of body size on elimination of ClO_4^- between two species because it has been reported that elimination of water-soluble chemicals in fish is affected by allometric (size) relationship (Newman, 1995). Alternatively, this could be due to species-specific elimination mechanisms. In channel catfish, the head showed the highest uptake ($K_1 = 0.26$) of ClO_4^-.

Many water-soluble xenobiotics are taken into the body by passive diffusion through semi-permeable membranes such as gills, lining of the mouth, or gastrointestinal tract. Fish gills are especially vulnerable to foreign chemicals because their design maximizes diffusion (Spacie and Hamelink, 1999). Due to the high water solubility of ClO_4^-, the respiratory surfaces, gills, of the fish can be the main routes of ClO_4^- uptake (Evans, 2002; Marshall, 2002). Because the body burden of ClO_4^- in gills was relatively higher among tissues in this study, the main uptake of ClO_4^- via gills might play an important role in the uptake to the organs through blood system, resulting in high uptake rates in head, liver, and G-I tract. Further examination of the concentration of ClO_4^- in the blood is needed to test this assumption. For future studies, it would be important to distinguish between the primary routes of elimination in fish, because the route can be affected differently by temperature, tissue damage, pre-exposure to toxicants or the presence of competing chemicals.

The high uptake rate of ClO_4^- in head ($K_1 = 0.26$ day^{-1}) and GI tract (0.16 day^{-1}) and low uptake rate in fillet (0.07 day^{-1}) are similar to those of Yu et al., (2002) who investigated the uptake of $^{36}ClO_4^-$ (3.3 mg/kg) over a 48 hour period in rat tissue. These authors reported that uptake of ClO_4^- showed the highest concentration in thyroid and GI tract, while the lowest $^{36}ClO_4^-$ concentration was detected in muscle. Both the head and liver of catfish had the highest uptake rate, but perchlorate concentration was more easily eliminated from the head ($K_2 = 1.22$ day^{-1}; $T_{1/2} = 0.57$ day) than liver (0.79 day^{-1}; 0.88 day). This pattern was also consistent with the findings at Yu et al., (2002), and may be related to the fact that the liver is a principal organ of detoxication and excretion of many

toxicants. In the rat, the perchlorate half-life ranged from 8 to 20 hour (Wolff, 1998). Tissue clearance of ClO_4^- in catfish was slower compared to rats when the chemical was given (Yu et al., 2002). Although species, concentration and uptake period of ClO_4^- were different between two investigations, the trend of elimination was similar, low half-life in fillet and high in head, liver and G-I tract. Compared to rats, the longer half-life in fish is probably the result of lower metabolic rate in fish compared to rats.

There was also a high degree of variability in elimination of perchlorate between individuals. For example, at day 20 of elimination, a few catfish had detectable levels of perchlorate in their tissues, while most others did not. If perchlorate exposure in natural water bodies is episodic, then fish in the wild may experience periods of rapid uptake when perchlorate concentrations in the water are high, followed by a depuration period when perchlorate levels drop. Such a pattern, when combined with high inter-individual variability in elimination rates, may explain the findings that perchlorate concentrations in field-caught fish are higher than in the water, but perchlorate is usually only detected in a few individuals of each species.

In conclusion, results from the current study indicated that perchlorate is rapidly eliminated from channel catfish and mosquitofish tissue and the elimination rate constants differ between the species and between tissue types. Perchlorate kinetics in thyroid and different tissues have well been investigated in mammals, to date and to the best of our knowledge, no study has been conducted to determine the elimination of perchlorate in teleost fish. These results may be critical and used to develop models of fate, effects, and transport of ClO_4^- in natural water systems, as well as to assess ecological risk in a contaminated ecosystem.

5.4.1.4 Pharmacokinetic/Population Modeling of Perchlorate in Fish

5.4.1.4.1 Introduction

Predicting the effects of perchlorate on individual animals, much less populations of any wildlife species is a daunting task. Mathematical modeling and computer simulation may be the only possible way to gain an understanding of the dynamics of perchlorate exposure and effects in the environment. We developed a model to assist in the estimation of risk to fish populations in Lakes Belton and Waco from exposure to perchlorate. Computer simulations were conducted of the effects of perchlorate on fish populations in Lake Belton and Lake Waco in the Bosque and Leon Rivers Watersheds in Central Texas.

5.4.1.4.2 Methodology

A physiologically based toxicokinetic (PBTK) model was developed to simulate the movement of perchlorate within the channel catfish. Contaminant movement was governed by a series of mass-balanced differential equation programmed in Matlab®. Model compartments and blood flow can be seen in **Figure 5-151**. General equations used in the model were taken from PBTKs developed by Nichols et al. for rainbow trout (1990 and 1991) and channel catfish (1993). The rate of change for each of the seven compartments (skin, kidney, muscle, GI tract, liver, fat, and thyroid) at individual time

steps is obtained by solving the differential equations simultaneously using numerical integration. Fish gill physiology was kept biologically accurate by accounting for countercurrent chemical flux, including both flow and diffusion limitations (Erickson and McKim, 1990b). Flow-limited distribution was assumed i.e. chemical equilibrium existed between the tissues and blood leaving the compartment. Additionally portal blood flow was incorporated into the kidney and liver from poorly perfused tissue and richly perfused tissue respectively. Portal blood flow to the kidney was set as 60% of blood flow to skin and muscle compartments, with portal flow to the liver equal to blood flow to the GI tract (Nichols et al., 1990).

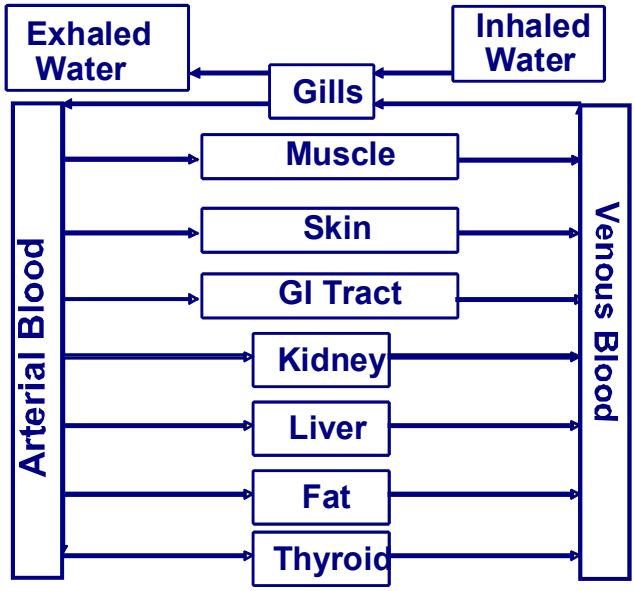

Figure 5-151
Flow Diagram of the PBTK Model for Perchlorate Inhalation in Fish

Physiological parameters for blood flow and cardiac output in the channel catfish were obtained from existing literature (Erickson and McKim, 1990a; Erickson and McKim, 1990b; Hughes, 1984; Nichols et al., 1990; 1993). Blood flow, cardiac output, and ventilation parameters were based on a 1 kg adult catfish, significantly larger than the 60g fishes used for tissue volumes and calibration. This difference was accounted for by scaling tissue blood flow by body weight to the 0.75 power. Tissue masses were measured in undosed juvenile channel catfish (Bradford, 2002), with their averages used to determine their fractional mass (**Table 5-40**).

Table 5-40
Physiological Parameters Used in the PBTK Model for Channel Catfish

Weight (kilograms)	BW = 0.0605
Oxygen Consumption Rate (mg/h)a	VO = 71.9
Oxygen Uptake efficiency (%)a	UE = 48.9
Ventilation volume (l/h)a	QV = 17.7
Cardiac output (l/h per kg)a	CO = 2.5
Tissue group volumes (%)b,c	
Fractional volume of kidney	VK = 0.0074
Fractional volume of liver	VL = 0.019
Fractional volume of thyroid	VT = 0.0941
Fractional volume of GI tract	VG = 0.0795
Fractional volume of skin	VS = 0.03971
Fractional volume of muscle	VM = 0.17785
Fractional volume of fat	VF = 0.066
Arterial blood flow to tissues (%)[d]	
Fractional blood flow to kidney	QK = 0.06
Fractional blood flow to liver	QL = 0.036
Fractional blood flow to GI tract	QG = 0.16
Fractional blood flow to thyroid	QT = 0.1908
Fractional blood flow to skin	QS = 0.0805
Fractional blood flow to muscle	QM = 0.3606
Fractional blood flow to fat	QF = 0.112

aValues given are for a 1 kg catfish.
bTissue volumes are calculated as a product of the fractional volume and body weight(l).
cFractional volumes are calculated as the average of measured tissue mass for catfish used in calibration.
dTissue blood flow (l/h) calculated as follows: $qi = Qi*(CO*BW^{0.75})$
eAll partitioning coefficients (Pij) were derived through calibration.

Route of exposure for perchlorate in the PBTK model was uptake through inspired water, which was simulated by using an equation for chemical flux at the gills (Erickson and McKim, 1990b). This method accounts for counter-current exchange with flows separated by a diffusion barrier made up of the gill epithelium and stagnant boundary layers in adjacent blood and water channels (Nichols et al., 1993). For purposes of this model, diffusion resistances were considered negligible in flow channels. By integrating diffusive flux on the gill surface, we arrive at an equation that relates total flux (Fg in µg/h) to an exchange coefficient and the difference in chemical activities between the venous blood and inspired water:

$$F_g = k_x \times \left(C_{insp} - \frac{C_{ven}}{P_{bw}} \right)$$

Exchange coefficient (k_x) is determined by the chemical capacities of water (k_w) and blood (k_b) flowing to perfused gill lamellae, and the average resistance to chemical diffusion (k_d), each parameter's sensitivity can fluctuate based on the chemical and organism (Erickson and McKim, 1990b):

$$k_x = \frac{e^{-k_d/k_b} - e^{-k_d/k_w}}{\frac{e^{-k_d/k_b}}{k_w} - \frac{e^{-k_d/k_w}}{k_w}}$$

Chemical capacity of water flowing to the perfused gill lamellae is equal to the effective respiratory volume (Qw), with chemical capacity calculated as follows:

$$k_b = Q_c \times P_{bw}$$

Diffusional resistance was calculated with the following equation:

$$k_d = D \times \frac{A}{T}$$

where: D = molecular diffusivity (m^2/h)
A = total lamellar gill area (m^2)
T = diffusion barrier thickness (m)

Because of limited data on catfish gill morphometry it was necessary to use values for total gill area and epithelial thickness reported for other fish species exhibiting intermediate levels of activity (Hughes, 1984).

Total lamellar gill area was scaled to body weight as follows:

$$A = \frac{(600 \times BW^{0.8})}{1000}$$

Erickson and McKim (1990b) calculated diffusion barrier thickness as the sum of the thickness of the epithelial layer plus one-third the combined thickness of the water and blood layers. An epithelial thickness of 4 µm was used by Nichols et al. (1993) as an average of the values reported by Hughes (1984), resulting in a total diffusion barrier of 12 µm.

Molecular diffusivity of perchlorate in water was calculated using an equation developed by Wilke and Chang (1955), and estimated as 0.0561 cm2/s:

$$D_{BW} = \frac{7.4 \times 10^{-8} (\varphi_W M_W)^{1/2} T}{\eta_W V_B^{0.6}}$$

where φW = solvent association factor
MW = molecular weight of water (g/mol)
T = temperature (K)
ηW = viscosity of water (cP)
VB = molar volume of solute B (cm^3/mol)

Reduced permeability across the gill epithelia was accounted for by multiplying the molecular diffusivity by 0.75 to obtain combined diffusivity (Erickson and McKim, 1990b). See **Table 5-41** for an explanation of abbreviations and model parameter symbols.

Table 5-41
Abbreviations and Symbols Used

F^G = flux of perchlorate across the gills, $mg \cdot kg^{-1} \cdot h^{-1}$
k_X^G = exchange coefficient
f_W = ratio of free chemical in exposure water to total concentration
f_B = ratio of free to total perchlorate in blood
$C_W^{aff,G}$ = total concentration of perchlorate in exposure water, $mg \cdot kg^{-1}$
$C_B^{aff,G}$ = concentration of perchlorate in the blood afferent to the gills, $mg \cdot kg^{-1}$
$C_B^{eff,G}$ = concentration of perchlorate in the blood efferent to the gills, $mg \cdot kg^{-1}$
$C_B^{eff,i}$ = concentration of perchlorate in the blood efferent to the tissue compartment, $mg \cdot kg^{-1}$
Q_B^i = blood flow rate from the tissue, $L \cdot h^{-1}$
Q_B^G = total cardiac output, $L \cdot h^{-1} \cdot kg$
A^i = amount of perchlorate in the tissue, mg
V^i = volume the tissue compartment, kg
K_B^i = tissue/blood partitioning coefficient
$\frac{dA^i}{dt}$ = rate of change in perchlorate concentration in the tissue compartment, $mg \cdot kg^{-1} \cdot h^{-1}$

Water intake (dose) is governed by the gill flux equation below :

$$F^G = k_X^G \left(f_W C_W^{aff,G} - f_B C_B^{aff,G} \right)$$

The concentration of perchlorate in the blood afferent to the gills, also known as the venous blood, was calculated as follows:

$$C_B^{aff,G} = \frac{\sum \left(C_B^{eff,i} * Q_B^i \right)}{Q_B^G}$$

The concentration of perchlorate in blood efferent to each tissue compartment was calculated as follows:

$$C_B^{eff,i} = \frac{\left(\frac{A^i}{V^i}\right)}{K_B^i}$$

The rate of change in perchlorate concentration for each tissue compartment is defined by the differential equation:

$$\frac{dA^i}{dt} = Q_B^i (C_B^{eff,G} - C_B^{eff,i})$$

Finally, the concentration of perchlorate in the blood efferent to the gills was calculated as follows:

$$C_B^{eff,G} = C_B^{aff,G} + \frac{F^G}{Q_B^G}$$

For our purposes the PBTK model was calibrated by altering the partitioning coefficients, the only parameters that were not taken from the published literature or directly measured. The calibration data were taken from an 11-day elimination study in channel catfish (Theodorakis et al., 2003). Juvenile channel catfish were exposed to 84 ± 4 ppm perchlorate for two days, followed by movement to a clean tank for an 11-day elimination period. By fitting the model to the elimination data as well as the uptake data we were able to account for the lack of a urinary excretion term (**Figure 5-152** through **Figure 5-158**).

Model calibration was tested by calculating the root mean squared (RMS) for the gill, GI, muscle, liver and thyroid compartments and conducting a sensitivity analysis. The purpose of using the RMS was to create a quantifiable measure of calibration "goodness" while accounting for simulated values greater than or less than the calibration values. A mean error calculation would not differentiate between the two, potentially leading to a false low value that was achieved by positive and negative errors canceling each other out.

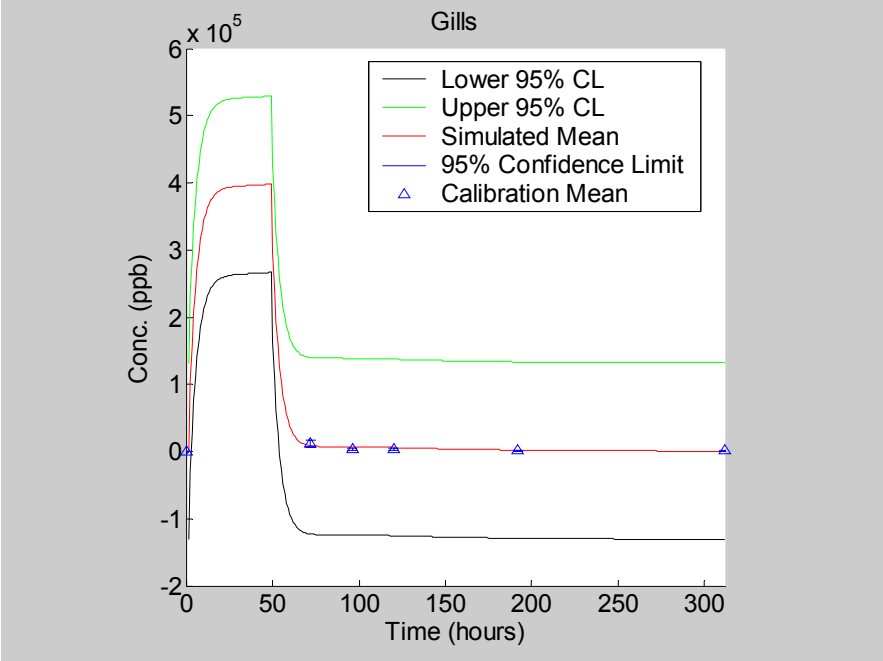

Figure 5-152
Calibration Curve for Gill Compartment Using Elimination Data

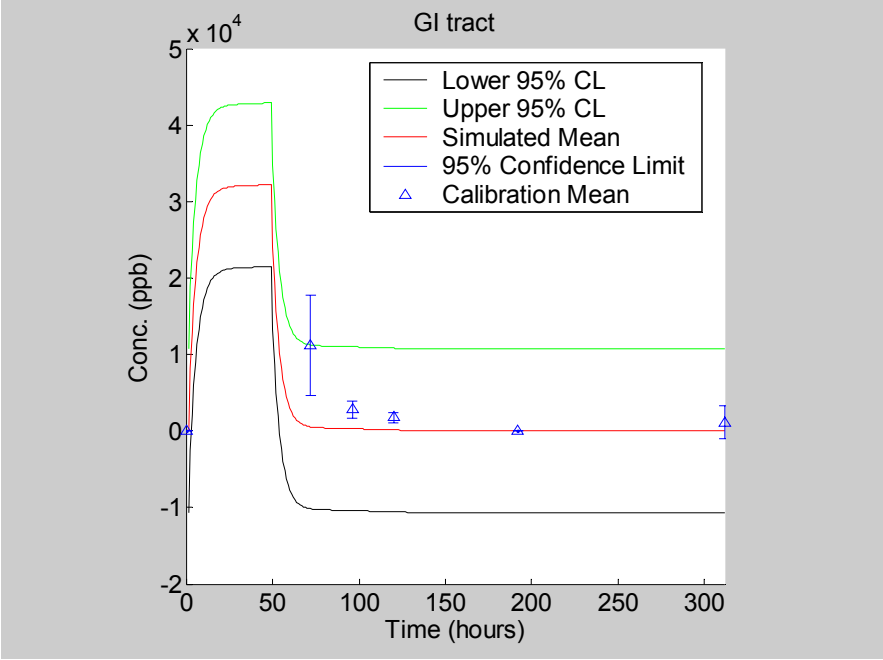

Figure 5-153
Calibration Curve for GI Compartment Using Elimination Data

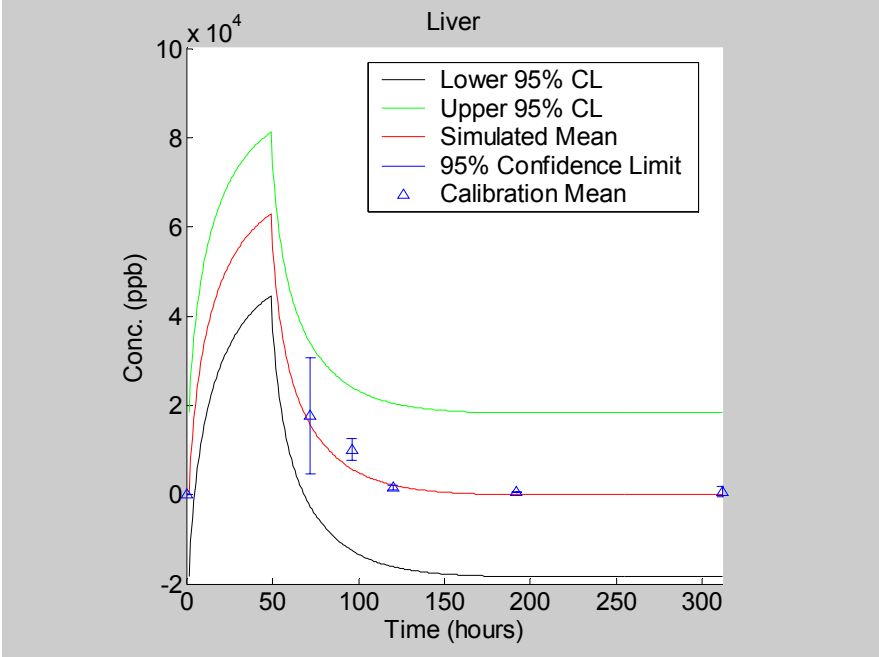

Figure 5-154
Calibration Curve for Liver Compartment Using Elimination Data

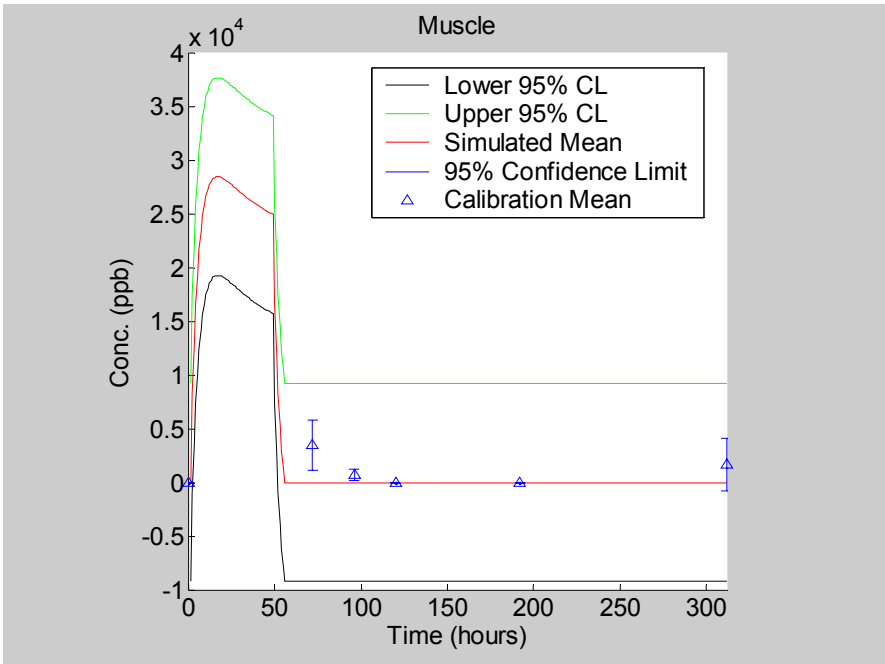

Figure 5-155
Calibration Curve for Muscle Compartment Using Elimination Data

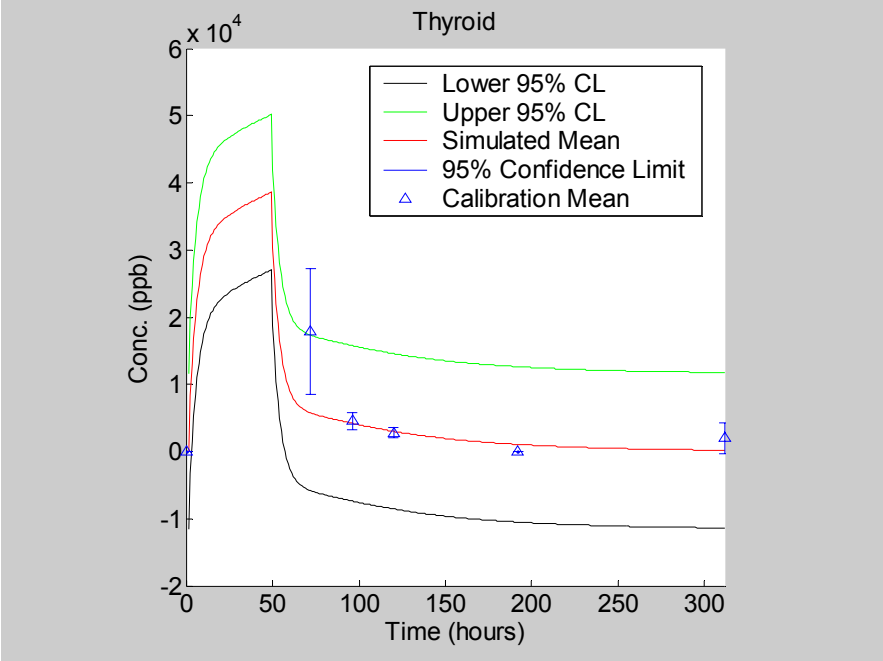

Figure 5-156
Calibration Curve for Thyroid Compartment Using Elimination Data

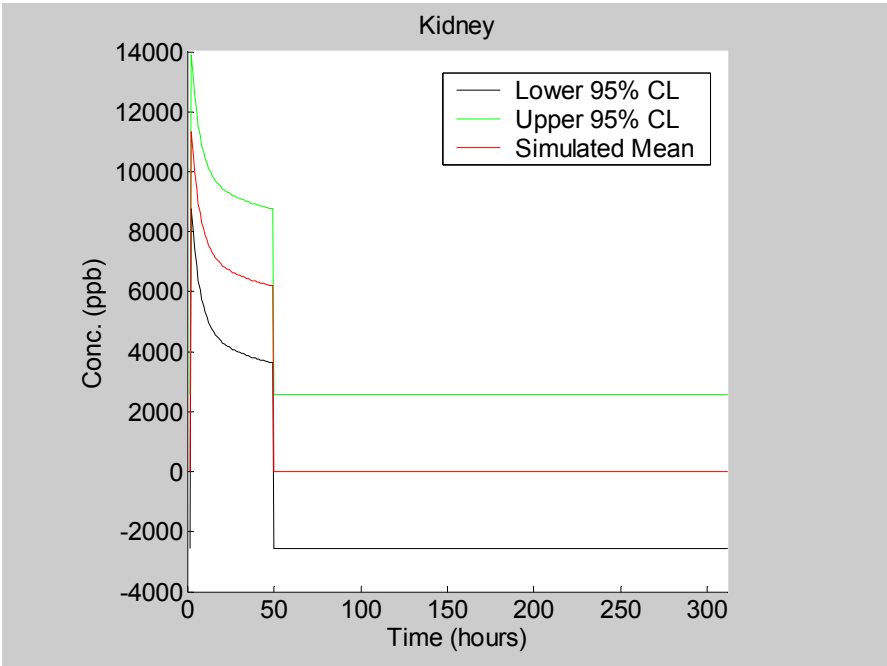

Figure 5-157
Partial Calibration Curve for Kidney Compartment Using Dosing Data

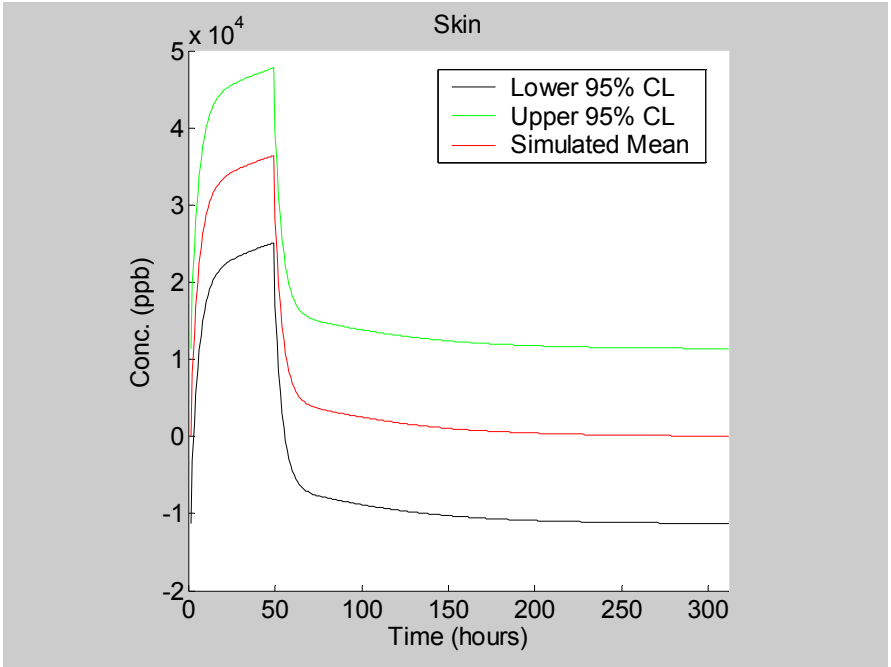

Figure 5-158
Partial Calibration Curve for Skin Compartment Using Dosing Data

For the sensitivity analysis, each partitioning coefficient was adjusted individually by ± 15% and the parameter sensitivity recorded (**Table 5-42**). This information provides a measure of uncertainty in the model and determines how greatly this uncertainty can affect model output. Sixty-eight of the 98 permutations were sensitive, indicating the need for accurate partitioning coefficients. Varying the coefficients by ± 15% is a very conservative range since measured partitioning coefficients provide an exact number. The area of greatest sensitivity in model output is as tissue concentration approaches equilibrium and complete elimination in the first 72 hours. This should be expected because of the very steep slope (**Figure 5-159**). A compartment was considered sensitive to a parameter adjustment if its sensitivity coefficient was greater than one:

$$S = \frac{\dfrac{(X_a - X_n)}{X_n}}{\dfrac{(P_a - P_n)}{P_n}}$$

where X_a = adjusted variable
X_n = nominal variable
P_a = adjusted parameter value
P_n = nominal parameter value

Table 5-42
Variable Sensitivity to Parameter Adjustment (+ Sensitive, - Sensitive)

	Gill	GI	Kidney	Liver	Muscle	Skin	Thyroid
kbw –15%	+	+	+	+	+	+	+
kbw +15%	+	+	-	+	+	+	-
kg –15%	+	+	+	+	+	+	+
kg +15%	+	+	-	+	+	-	-
kk –15%	+	+	+	+	+	+	+
kk +15%	-	+	-	+	-	-	-
kl –15%	+	+	+	+	+	+	+
kl +15%	+	-	-	+	-	-	-
km –15%	+	+	-	-	+	-	-
km +15%	+	+	+	+	+	+	+
ks –15%	+	-	-	-	-	+	-
ks +15%	+	+	+	+	+	+	+
kt –15%	+	+	+	+	+	+	+
kt +15%	+	-	-	-	-	-	+

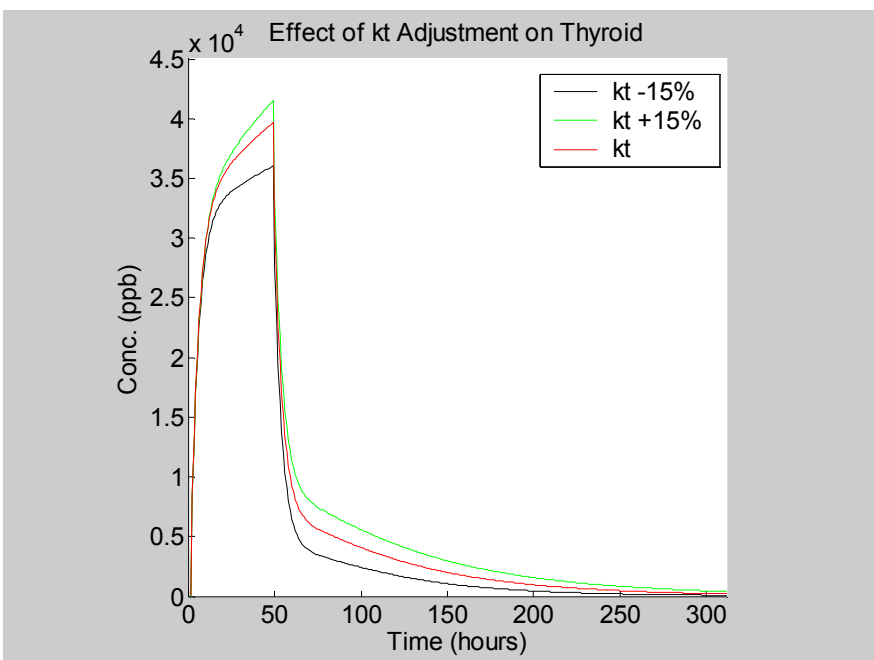

Figure 5-159
Effect of Increasing or Decreasing Thyroid-Blood Partitioning Coefficient (kt) by 15% on Concentration of Perchlorate in the Thyroid Compartment

Thyroid Model Description. A six-compartment model of combined T_3 and T_4 kinetics originally developed for mammals (DiStefano, 1986; Bianchi et al., 1987; Pilo et al., 1990; Hershman et al., 1986) and subsequently applied to rainbow trout (Sefkow et al., 1996) was used to simulate the secretion of T_3 and T_4 in channel catfish, as well as the

impacts of perchlorate on secretion rates. The six compartments represented included: T_4 slow exchange tissue pool, T_4 rapid exchange tissue pool, T_4 plasma pool, T_3 slow exchange tissue pool, T_3 rapid exchange tissue pool, and T_3 plasma pool. Skeletal muscle makes up the majority of the slow tissue pool. The rapid tissue pool consists primarily of the liver, but also the kidney and gill, all of which possess deiodinating capacity (MacLatchy and Eales, 1992; Eales and Brown, 1993). While total T_3 production is lower in the rapid pool, compared to the slow pool, its rapid efflux (12x greater) makes it an essential component in the acute adjustment of plasma T_3 levels (Sefkow et al., 1996).

Model Parameters and Equations. Parameters in the model were either uniquely identifiable (**Table 5-43**) or determined within a range using the method of interval identifiability analysis (DiStefano, 1983). Interval identifiability analysis uses estimates of identifiable parameter combinations to determine bounded intervals. An example of this is:

$$k_{21}^{\min} \equiv \frac{\gamma_2 - \xi_2 / k_{55}}{-k_{22}} \leq k_{21} \leq -k_{11} - k_{31}^{\min} \equiv k_{21}^{\max}$$

Feng and DiStefano (1995) used interval analysis to obtain finite bounds for all combinations of kij. These relationships in turn were used to bound the unidentifiable steady-state compartment masses and percentage of T_3 converted to T_4 (Sefkow et al., 1996).

Table 5-43
Parameters Used in Thyroid Model

k_{ij} = Flow rate to compartment i from compartment j (min^{-1})
Qi = Hormone concentration in compartment Ii (ng/ml)
[T4]p = Endogenous T4 plasma concentration at steady state (ng/ml)
[T3]p = Endogenous T3 plasma concentration at steady state (ng/ml)
Vp = Plasma volume (ml)
SR3 = T3 secretion rate (ng/hr·100g BW)
SR4 = T4 secretion rate (ng/hr·100g BW)
CR_{4-3}^{slow} = T4 to T3 conversion rate in the slow compartment (ng/hr)
CR_{4-3}^{fast} = T4 to T3 conversion rate in the fast compartment (ng/hr)
ξi = uniquely identifiable parameter combination (min^{-3})
γi = uniquely identifiable parameter combination (min^{-2})

Only the plasma compartments have uniquely identifiable steady-state masses. The remaining compartments are defined within a given interval based on the minimum and maximum values of a unique unidentifiable parameter within each compartment. Steady-state equations were developed by Sefkow et al. (1996) based on data from fasted rainbow trout injected with radiolabeled T_3 and T_4.

Steady-state mass in T_4 plasma compartment (Q_1):

$$Q_1 = [T_4]_p V_p$$

Steady-state mass in T_3 plasma compartment (Q_4):

$$Q_4 = [T_3]_p V_p$$

Steady-state mass in T_4 rapid exchange compartment (Q_2):

$$Q_2 = \left(\frac{Q_1}{-k_{22}} \right) k_{21}$$

Steady-state mass in T_3 rapid exchange compartment (Q_5):

$$Q_5 = \left(\frac{Q_4}{-k_{55}} + \frac{\xi_2 Q_1}{\gamma_5 k_{22} k_{55}} \right) k_{54}$$

Steady-state mass in T_4 slow exchange compartment (Q_3):

$$Q_3 = \left(\frac{Q_1}{-k_{33}} \right) k_{31}$$

Steady-state mass in T_3 slow exchange compartment (Q_6):

$$Q_6 = \left(\frac{Q_4}{-k_{66}} + \frac{\xi_3 Q_1}{\gamma_6 k_{33} k_{66}} \right) k_{64}$$

Secretion rates were found by assuming a rate of change equal to zero and solving for exogenous constant-rate inputs into the plasma compartments:

$$SR_4 = \left(-k_{11} + \frac{\gamma_2}{k_{22}} + \frac{\gamma_3}{k_{33}} \right) Q_1$$

$$SR_3 = \left(-k_{44} + \frac{\gamma_5}{k_{55}} + \frac{\gamma_6}{k_{66}} \right) Q_4 - \left(\frac{\xi_2}{k_{22} k_{55}} + \frac{\xi_3}{k_{33} k_{66}} \right) Q_1$$

T_4 to T_3 conversion rates in the slow and fast pool compartments were solved using a similar approach:

$$CR_{4-3}^{slow} = k_{63}Q_3 = \frac{\xi_3 k_{64}}{-k_{33}\gamma_6}Q_1$$

$$CR_{4-3}^{fast} = k_{52}Q_2 = \frac{\xi_2 k_{54}}{-k_{22}\gamma_5}Q_1$$

Model Calibration. Since the steady state values reported by Sefkow et al. (1996) were calculated from laboratory data for rainbow trout, it was important to make adjustments to hormone levels to better represent channel catfish. It is also important to note that the steady-state values are not based on a mass-balance model as used in this research. Compared to the T_3 and T_4 levels of 7.1 x 10^{-4} ng/mL and 1.04 x 10^{-3} ng/mL respectively, reported by Sefkow et al (1996), Gaylord et al (2001) reported plasma T_3 levels of ~7.6-13.3 ng/mL and T_4 levels of ~2.3-4 ng/mL in channel catfish. Additionally the regular fluctuations in hormone levels within fish make a "steady-state" value misleading. T_3 levels are relatively stable with an average of 0.4 ± 0.1 peaks/24 hour period significantly greater than baseline with an average amplitude of 1.7 ± 0.2 ng/mL. T_4, however, exhibited 2.5 ± 0.2 peaks/24 hour period with an average amplitude of 3.0 ± 0.4 ng/mL (Gomez et al., 1997).

Initially, the model had to be run long enough for the compartments to equilibrate from a starting concentration of 0 ppm. The number of time steps (hours) necessary to achieve a baseline value ranged from 500 to 1000 depending on the compartment. Once our baseline (steady-state) had been achieved we could proceed with calibration. The first step in model calibration for the thyroid model was to compare the mass-balance model results to those reported by Sefkow et al (1996), as stated previously the values would not be expected to be similar because of the different techniques utilized. Instead the percentage of hormone in each compartment was used for comparison, as mass-balance ratios should remain constant. All compartments were within 1% of the percentages as reported in the literature, indicating good model structure (**Table 5-44**). Plasma T_3 and T_4 compartments were then adjusted, keeping the same percentage distribution with other compartments, to meet levels reported by Gaylord et al. (2001) in channel catfish (**Figure 5-160** through **Figure 5-165**). Alteration of plasma T_3 and T_4 levels automatically adjusts hormone concentrations in the remaining compartments based on mass-balance.

Table 5-44
Comparison of Simulated and Reported Values for Hormone Distribution in Model Compartments

	Sefkow et al. (1996)	Simulated Percentage
Plasma T_3	13.7%	13.7%
Fast Pool T_3	12.6%	12.2%
Slow Pool T_3	73.7%	74.7%
Plasma T_4	18.5%	17.8%
Fast Pool T_4	16.2%	15.9%
Slow Pool T_4	65.3%	66.3%

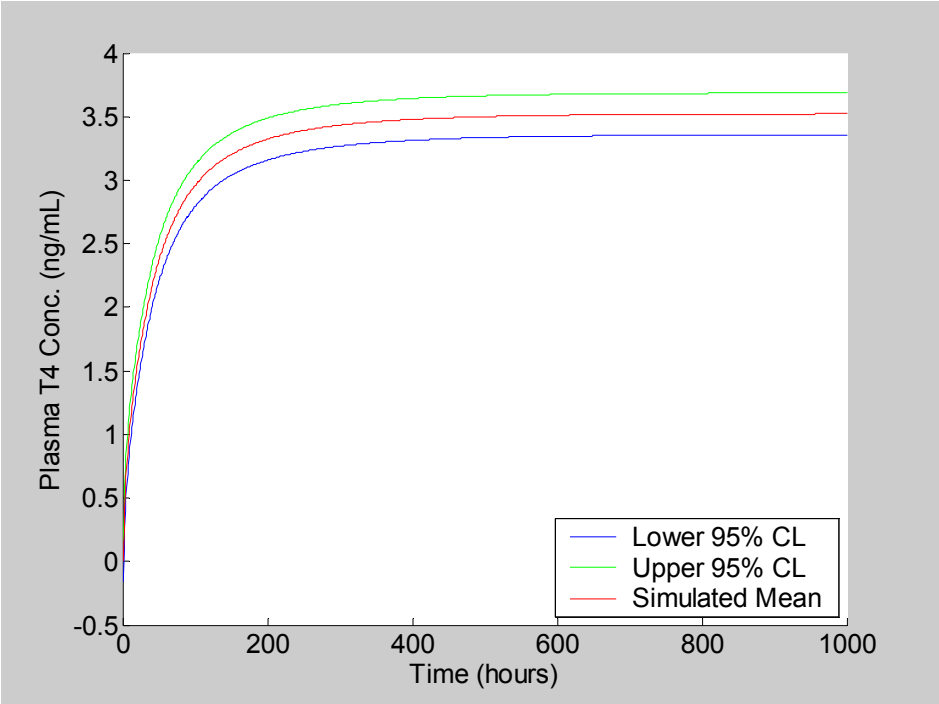

Figure 5-160
Calibrated T_4 Plasma Compartment Curve

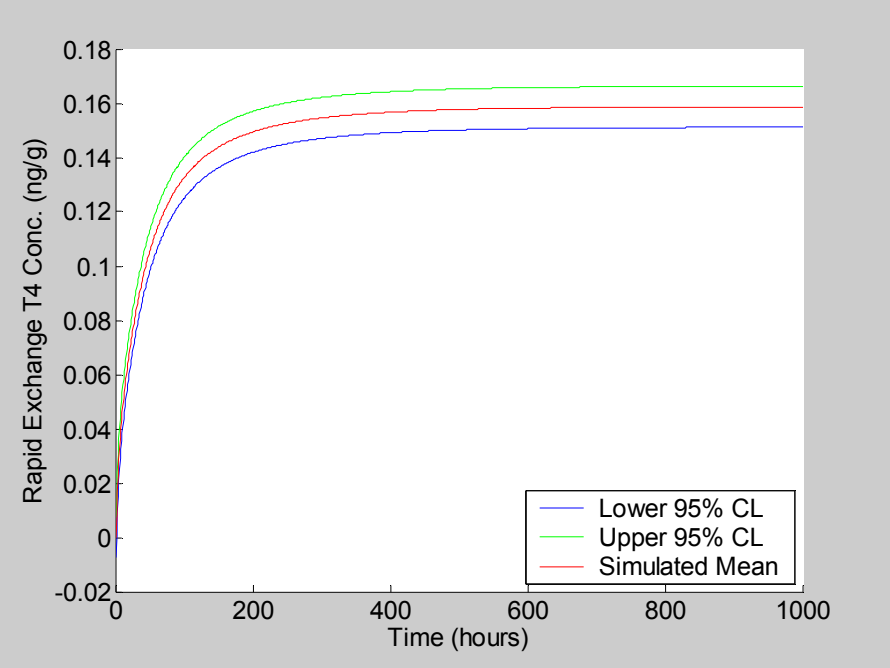

Figure 5-161
Calibrated T_4 Rapid Exchange Compartment Curve

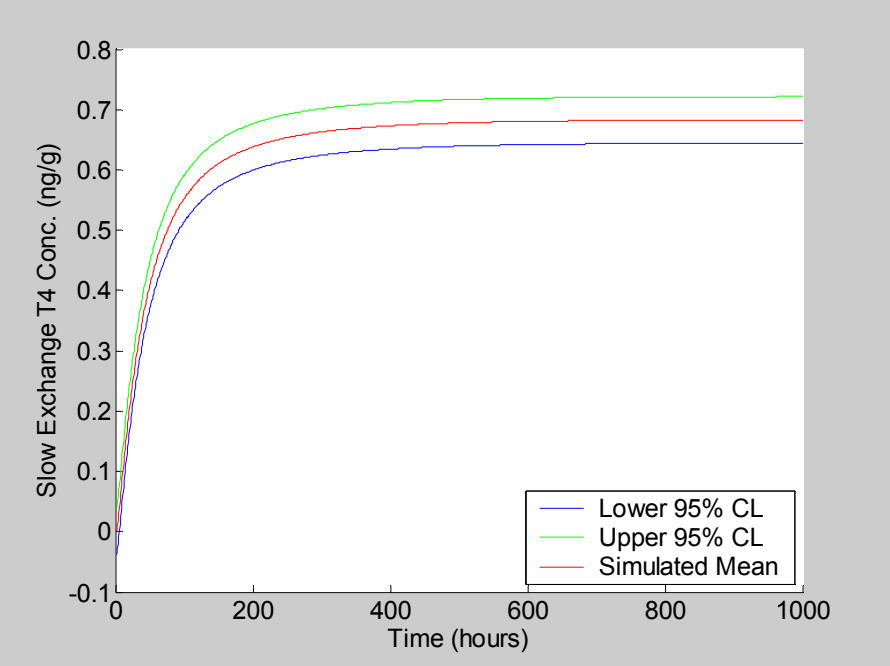

Figure 5-162
Calibrated T_4 Slow Exchange Compartment Curve

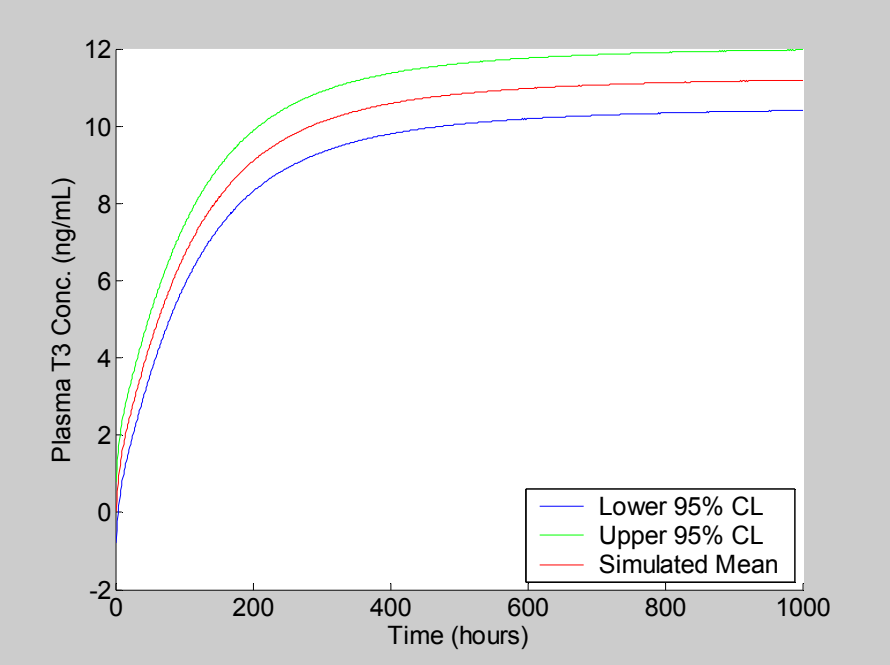

Figure 5-163
Calibrated T_3 Plasma Compartment Curve

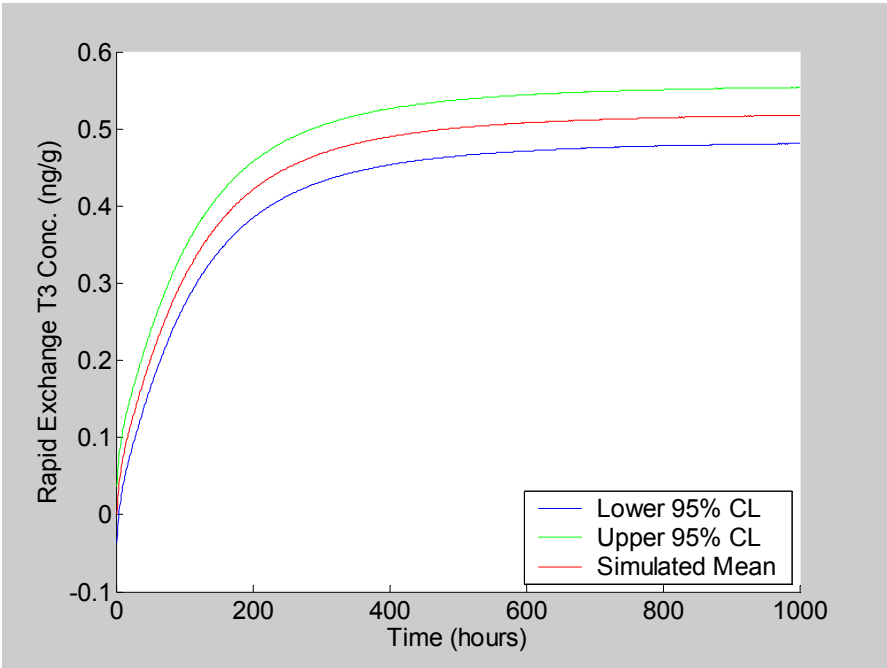

Figure 5-164
Calibrated T_3 Rapid Exchange Compartment Curve

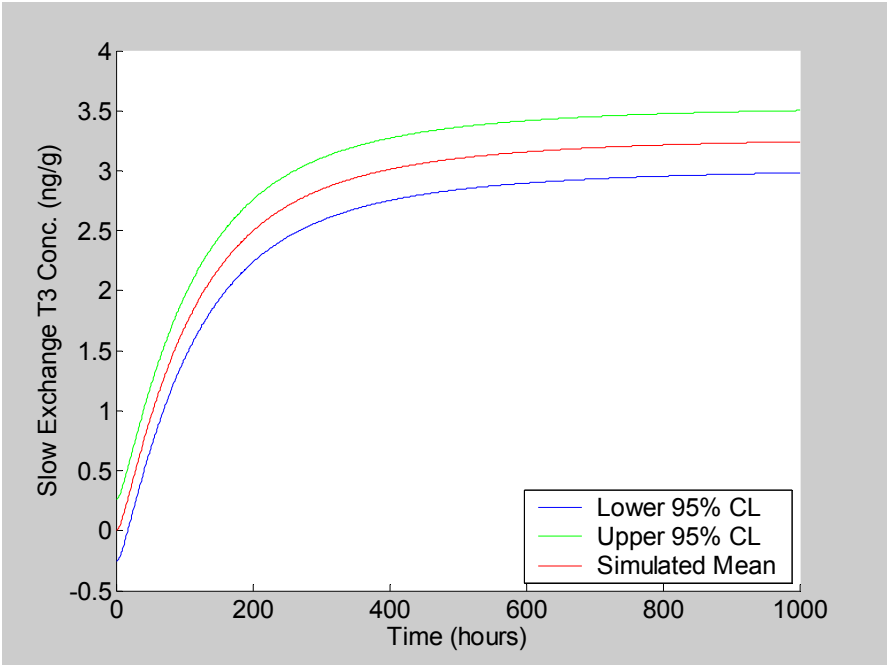

Figure 5-165
Calibrated T_3 Slow Exchange Compartment Curve

Hormone Inhibition. Hormone inhibition by perchlorate was calculated from data on mosquitofish dosed with sodium perchlorate for 2,10, or 30 days at doses of 0, 0.1, 1, 10, 100, and 100mg/L (ppm) (Bradford, 2002). Whole body T_4 concentrations were determined by radioimmunoassay for pooled groups of fish. A regression curve was fit to the data to derive the inhibition equation based on the concentration of perchlorate in the thyroid tissue (DT):

$$APfactor = 0.686 - 0.1047 \times \log(DT)$$

This equation was in turn applied to the three T_4 compartments in the model (plasma, rapid exchange and slow exchange) through adjustment of the T_4 secretion rate term. While this was the only data available for perchlorate related hormone inhibition in fish, it is not robust enough to draw a definitive conclusion as to the inhibition properties of perchlorate. A sensitivity analysis performed on the inhibition term (APfactor), as described above, identified all compartments as insensitive to an increase or decrease in the APfactor of 15% (**Figure 5-166**).

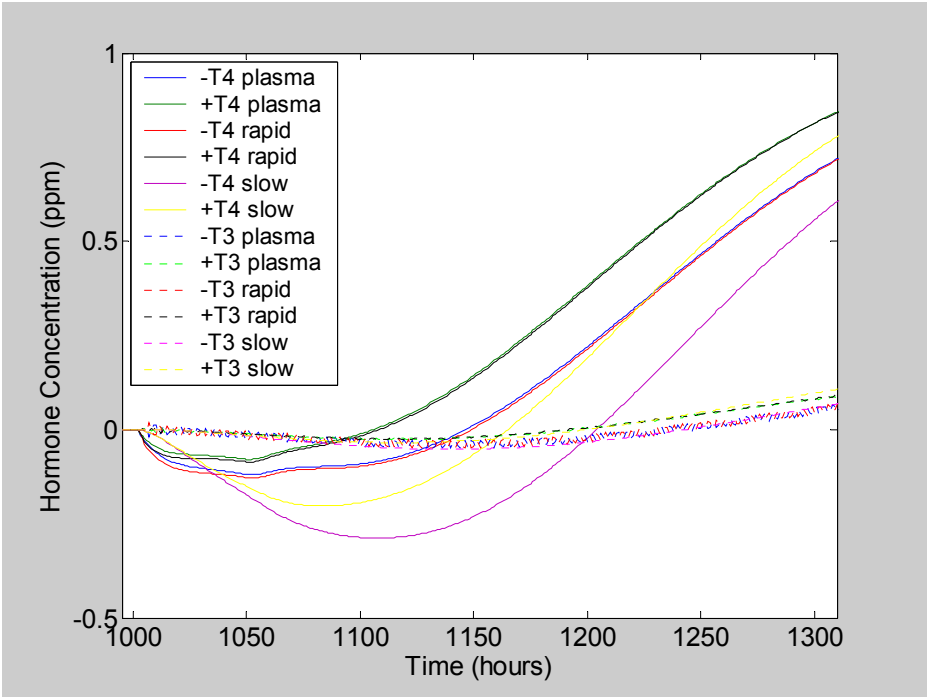

Figure 5-166
Sensitivity Analysis for Thyroid Hormone Compartments Based on ± 15% Change to AP Factor

5.4.1.4.3 Data

5.4.1.4.3.1 Exposure Scenario

A "worst-case" scenario was developed, in which individual catfish were exposed to the highest measured field water concentrations until their tissues reached equilibrium. Field values varied widely, with the highest reported value being 540 ppb in S Creek at Highway 317 (T15) (Todd Anderson, personal communication). Most tissue compartments took approximately 120 hours to equilibrate, with the gill and thyroid compartment taking 250 and 500 hours respectively (**Figure 5-167**, **Figure 5-168**). If we assume that 540 ppb is the highest level any fish is exposed to then all measured field tissue concentrations should be less than or equal to those simulated. The highest measured muscle concentration, based on tissue wet weight, was 60 ppb. Simulated muscle concentrations were ~150 ppb, indicating model validity. Since thyroid tissue cannot be collected in fish, the head was used as an approximate measure. Field measurements had a high wet weight concentration of 850 ppb compared to an initial simulated 300 ppb. The model was re-calibrated with the field data resulting in a mean simulated thyroid concentration of 880 ± 120 ppb (mean ± 95% CI) after 500 hours. The thyroid concentration data were then used in the hormone secretion model to determine the level of hormone inhibition. Perchlorate inhibition was initiated after 1000 hours to allow the hormone compartments to reach steady-state prior to insult (**Figure 5-169**). T_3 hormone levels decreased 18.7-22% and T_4 levels decreased 56.5% (**Table 5-45**).

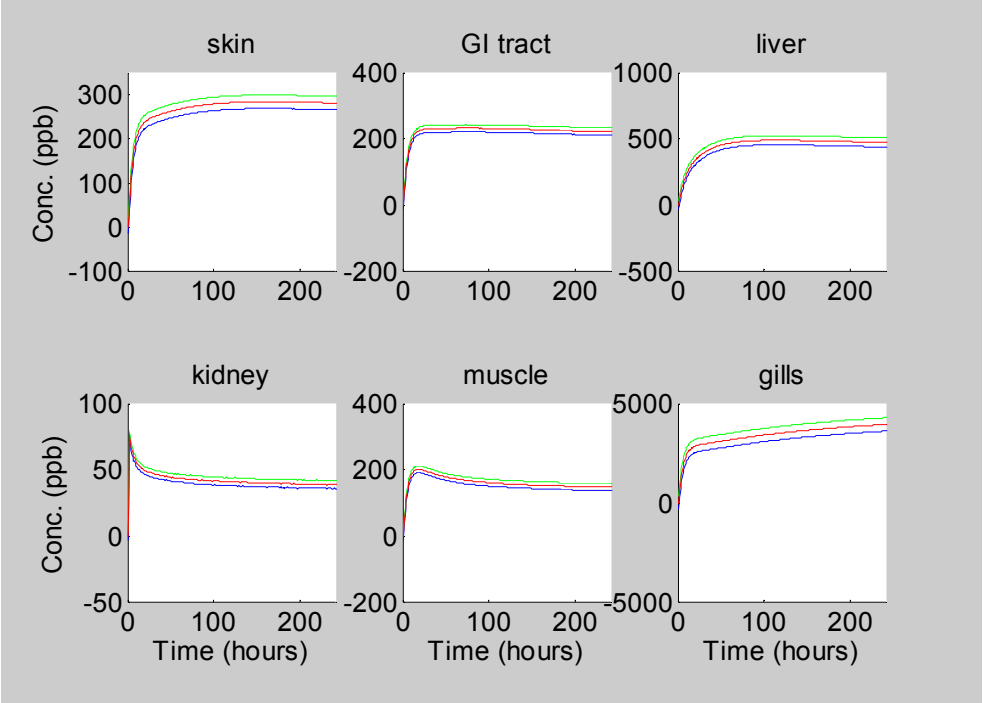

Figure 5-167
Simulated Tissue Concentrations Based on a 540 ppb Perchlorate Exposure

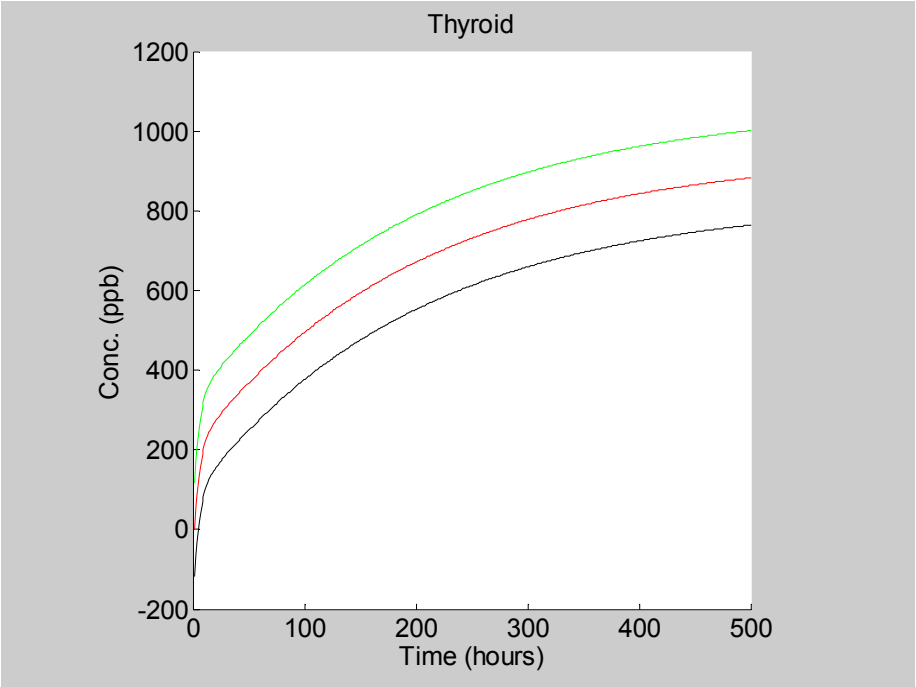

Figure 5-168
Simulated Thyroid Tissue Concentrations Based on a 540 ppb Perchlorate Exposure

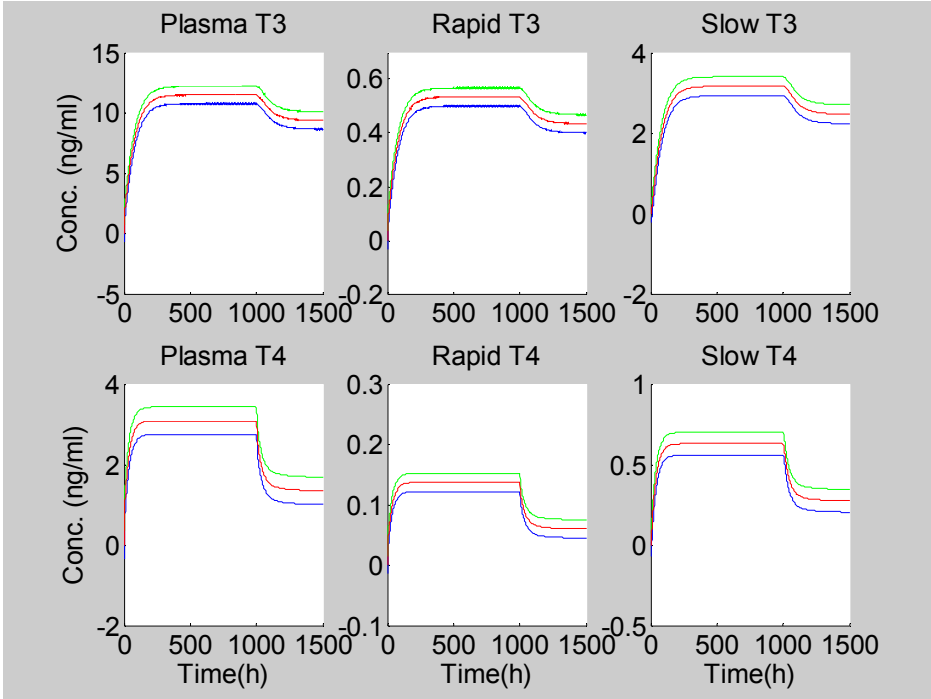

Figure 5-169
Simulated Hormone Inhibition Based on 540 ppb of Perchlorate Exposure Starting at Hour 1000

Table 5-45
Thyroid Hormone Inhibition Based on 540 ppb Perchlorate

	Steady State ng/mL	Simulated ng/mL (% decrease)
Plasma T_3	11.5	9.3557 (18.7%)
Fast Pool T_3	0.5347	0.4342 (18.8%)
Slow Pool T_3	3.1605	2.4652 (22%)
Plasma T_4	3.0908	1.3440 (56.5%)
Fast Pool T_4	0.1373	0.0597 (56.5%)
Slow Pool T_4	0.63	0.2741 (56.5%)

5.4.1.4.4 Discussion

The PBTK model was able to reproduce field results indicating model validity. Because of the complexity of real world systems, it is not uncommon for additional calibration of the model to occur. The limited data sets available for initial model calibration resulted in a simplification of the thyroid compartment. A singular thyroid compartment may be an oversimplification of the kinetic behavior of perchlorate. Chow and Woodbury (1970) determined that a three-compartment model (stroma, follicle, and lumen) was necessary to properly model the behavior of perchlorate in rat thyroids. It is unknown if fish would follow a similar kinetic behavior since they lack the centralized thyroid gland of

mammals. The simplification of the thyroid compartment, in conjunction with the use of 100 ppm dosing data for calibration, may have underestimated uptake at low doses.

The rapid, and substantial, decrease in hormone levels should be viewed cautiously. We do not know if channel catfish respond in the same way to perchlorate exposure as the mosquitofish used for the inhibition term. Additionally we cannot fully characterize the system without the data necessary to determine a T_3 inhibition term, hence the reason T_3 levels decreased substantially less than T_4. At present the simulated results are only a "best guess" and should not be viewed as a definitive answer. It is also important to note that fish are very adept at altering thyroid hormone levels based on various environmental and dietary conditions. As a result of this ability, fish tend to rapidly reestablish normal hormone levels once the inhibitory condition is removed. In addition, various studies have shown that different species rapidly eliminate perchlorate and recover from exposure, once they are removed from a contaminated system.

5.4.2 Frogs

Environmental perchlorate could be a potential threat to vertebrate wildlife because it blocks iodide uptake by thyroid follicular cells. Because the mechanism of iodide uptake and thyroid hormone synthesis is essentially identical in all vertebrates, the extensive data set on perchlorate mode of action that has collected over the last 50-60 years can be used to predict potential effects in wildlife. One of the groups at greatest risk are frogs (amphibians), as they require normal thyroid hormone secretion to complete the transformation from tadpole to frog. If tadpoles do not complete this transformation they cannot reproduce. If metamorphosis is delayed, tadpoles are at risk of greater exposure to predators and pond drying. Finally, because metamorphosis is such a robust and dramatic morphological change, the effects of perchlorate can be observed using non-invasive endpoints such as limb emergence, tail resorption, or limb length.

Previous work (Goleman et al., 2002a,b; Carr et al., 2003) indicates that exposure to environmentally relevant concentrations of perchlorate delays the completion of metamorphosis and alters sex ratio in African clawed frogs. The effects of perchlorate on metamorphosis are coincident with increases in thyroid gland hypertrophy and reductions in whole-body thyroid hormone concentrations. These effects are not restricted to laboratory studies; examination of tadpoles inhabiting perchlorate contaminated sites at Longhorn Army Ammunition Plant in East Texas suggests that similar effects are observed in tadpoles exposed to perchlorate under natural conditions (Carr et al., 2003).

The goal of this study was to examine naturally occurring frogs inhabiting the Lake Waco and Lake Belton watersheds for evidence of thyroid disruption. Our approach was twofold. First, we collected adult and larval frogs from reference and contaminated sites in and around McGregor, Texas and preserved these specimens for subsequent histological assessment of thyroid function. Second, we collected water samples from reference and contaminated sites in the vicinity of McGregor Texas, and transported the water samples back to the laboratory. Larval frogs were raised in the water samples to determine if thyroid-disrupting agents, including perchlorate, were present. Our working

hypothesis was that frogs exposed to biologically active concentrations of perchlorate in the environment would exhibit evidence of thyroid disruption based on thyroid histopathology endpoints, and that samples of surface water bodies contaminated with perchlorate would cause changes in developmental rate in laboratory exposed tadpoles.

5.4.2.1 Thyroid Histology of Native Frogs

5.4.2.1.1 Methodology

Seven trips were made to sites (see **Table 5-46**) in the NWIRP area (7/19/01, 10/10/01, 11/09/01, 5/7/02, 7/23/02, 4/21/03, and 6/16/03). GPS coordinates (UTM) were recorded for each using a Garmin GPS III Plus receiver. Pictures were taken of each site with a Nikon COOLPIX 995 digital camera. Frogs and tadpoles were collected in dip nets along the bank or in shallow water and immediately euthanized by immersion in MS-222 (3-aminobenzoic acid ethyl ester and $NaHCO_3$, each mixed in distilled water at 3 g/L). The animals were then stored in 10% neutral-buffered formalin (NBF). Sampling of most sites took approximately 1 hour and was conducted by 1-2 people. Whenever possible each site was visited in the morning and later in the afternoon. All animals were identified to species and measured for snout-vent length, hindlimb length, total length, and weight. Sex was determined by direct visual inspection of the gonads. Tadpoles were staged (Gosner, 1960), weighed, and measured for snout-vent length, hindlimb length, total length, and tail length and height. Water samples for perchlorate analysis were collected in 20 mL glass scintillations vials and placed in ice chests. See **Table 5-46** and **Figure 5-170** for a list of collection sites.

Table 5-46
Field Sites Visited for the Amphibian Work

Site ID	GPS (UTM)	Site Description	Water Collected for Perchlorate Analysis
NBSB (T14)	14R 0652873E 3476602N	South Bosque, south of water treatment plant, 3.3 miles south of Hwy 84	Y
HC84E (T34)	14R 0659507E 3483449N	Harris Creek at Highway 84 near the Executive Airport	Y
HCOOE (T17)	14R 0649179E 3478959N	Harris Creek east on Old Oglesby Rd 0.9 mi south of Hwy 84.	Y
HCGHA	14R 0649006E 3479236N	Harris Creek on Gladys Hollan's land, NE of old barn	Y
CROOR (T18)	14R 0649223E 3479242N	Spring on Old Oglesby Road, 0.2 mile south of Hwy 84	Y
ONCRN (T24)	14R 0645519E 3472070N	Onion Creek North of Hwy 107	Y
OLLO (T35)	14R 0664172E 3484662N	South Bosque at Highway 84 (Old Lorena Road)	Y
SBIT (T33)	14R 0655016E 3476016N	South Bosque at Indian Trail	Y
SBSBE	14R 0657527E 3477420N	South Bosque on unknown road	Y
MNSP (T28)	14R 0645313E 3465379N	Leon River (at Mother Neff Park)	Y
MGRB (T29)	14R 0650101E 3473922N	Creek on McGruffey RD	Y
MCGR (T13)	14R 0651011E 3480195N	Harris Creek at Highway 317	Y
PC317 (T3)	14R 0650195E 3483242N	Pecan Creek at Hwy 317	Y
SBSBL	14R 0654534E 3475300N	South Bosque at McGregor South Loop	Y
SOBO (T16)	14R 0653811E 3473890N	South Bosque at Highway 317, 4 miles south of Hwy 84	Y
SC107 (T23)	14R 0642978E 3471315N	Station Creek at Hwy 107	Y
SCOOR (T6)	14R 0642334E 3477056N	Station Creek at Oglesby road	Y
UN317 (T15)	14R 0653656E 3474994N	S Creek at Hwy 317	Y
SOBOW	14R 0647801E 3472718N	South Bosque on Hwy 2671-west side of road	Y
UB2671	14R 0646966E 3469992N	Unknown creek on Hwy 2671 0.75 mi south of McClennen Rd	Y
UCGHA	14R 0648944E 3479174N	Water from pipe draining into Harris Creek	Y

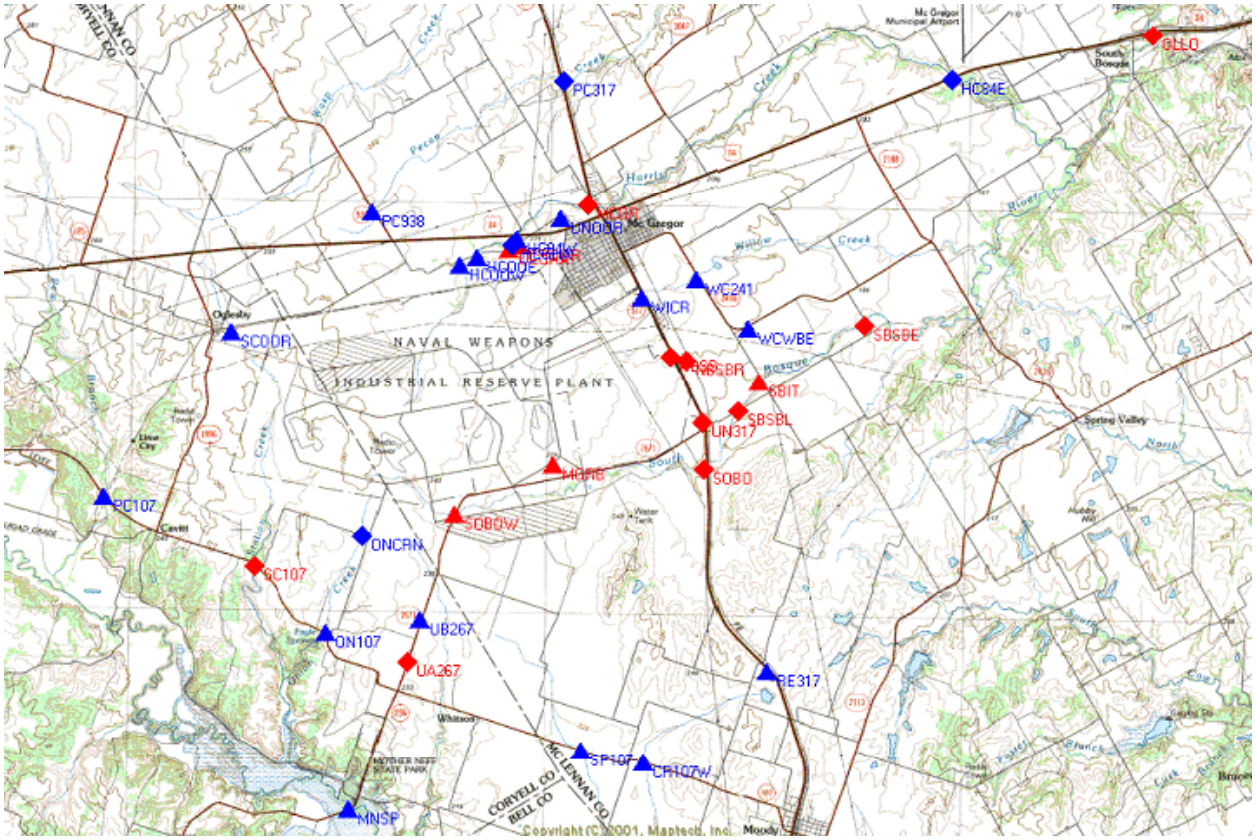

Figure 5-170
Locations Where Larval and Adult Frogs Were Collected

(Diamonds represent areas where both frogs and surface water samples were collected. Triangles represent areas where surface water only was collected. Blue – no perchlorate detected in water. Red – perchlorate detected in water.)

The lower jaws of all adult frogs collected in the field and whole tadpoles were dehydrated in a graded series of alcohols and processed for routine paraffin embedding. Serial transverse sections (10 µm) through the jaw were mounted on glass slides and stained using Harris's progressive hematoxylin and eosin procedure and coverslips were mounted. The right thyroid was located for each animal and the beginning and end sections were marked. The fifth, middle, and fifth from the last sections were examined for colloid depletion, follicular cell hypertrophy, and follicular cell hyperplasia as described by Carr et al. (2003) as shown below:

Colloid Depletion

- 0 No reduction.
- 1 Pale, lacey, vacuolated, or granular appearance; slight to moderate reduction.
- 2 Large reduction or absence.

Follicle Cell Hypertrophy

0 No hypertrophy – follicles lined by squamous to cuboidal epithelium.

1 Follicles lined by tall cuboidal to columnar epithelium; cytoplasm; nucleus ratio increased.

2 Follicular epithelial cells distinctly larger than normal; follicular lumen severely decreased or obliterated.

Follicle Cell Hyperplasia

0 No hyperplasia – follicles lined by a single layer of normal appearing squamous to short cuboidal epithelium.

1 Two or more follicles exhibiting stratification of follicular epithelium, usually 2-3 cells thick, protruding into lumen (NOTE: follicles exhibiting stratification near gland periphery are not counted).

2 Greater number of affected follicles exhibiting stratification of follicular epithelium, usually more than 3 layers thick, protruding into lumen; area of hyperplasia may also have microfollicular formation within them.

Data were analyzed using either parametric or nonparametric statistics. Data were tested for homogeneity of variance using Bartlett's test. If the assumptions of parametric statistics were met, then Student's two-tailed t-test or ANOVA followed by the Tukey-Kramer multiple comparison test were used. If the assumptions of parametric tests were not met, then the Mann-Whitney nonparametric t-test or the Kruskal-Wallis (KW) ANOVA by ranks followed by Dunn's multiple comparisons test were used. All statistical analyses were performed using InStat (v. 2.05a, GraphPad Software, San Diego, CA) or SPSS (v. 11, SPSS Inc., Chicago, IL).

5.4.2.1.2 Data

The species and numbers of adult and larval frogs collected are reported in **Table 5-47**. By far the most abundant collected species was Blanchard's cricket frog, *Acris crepitans Blanchardi*. Adult *A. crepitans* were collected routinely throughout the study period at several sites, whereas larval *A. crepitans* were collected at fewer sites and only on the last trip in the spring of 2003. Because adult *A. crepitans* were collected at reference sites, as well as sites known to have measurable perchlorate in surface waters, we used these animals for the thyroid histopathological analysis. Morphological parameters and sex ratios for the adult *Acris crepitans* are reported below in **Table 5-48**. In general, male and females were equally represented at each site, and there was no evidence at the gross morphological level of intersexual gonads (Reeder et al., 1998; Carr et al., 2003).

Table 5-47
Adult and Larval Amphibians Collected by Species Between June 2001 and June 2003 in the Lake Waco and Lake Belton Watersheds

Site	Species							
	A. crepitans		*R. catesbeiana*		*R. blairi*		*Bufo valliceps* (adult only)	*Hyla versicolor or H. chrysoscelis* (larvae only)
	adults	larvae	adults	larvae	adults	larvae		
HC84E	2	10		17				
HCGHA	1							
MCGR	8							
NBSB	13							
NBSBR	5							
OLLO	39				2		3	3
ONCRN					2			
PC317	4	48		14				
SBSBE	7			2				
SBSBL	8							
SC107	2				1			
SCOOR								
SOBO	52	124		1	3			
SOBOW								
UA267				28				
UB267					10			
UN317	1						1	
Total	**142**	**182**		**62**	**18**		**4**	**3**

Table 5-48
Mean (± Standard Error) Snout-Vent Length (mm), Hindlimb Length (mm), Body Weight (g), and Sex Ratios in Field-Caught Adult Cricket Frogs (*Acris crepitans* blanchardi)

Site	N	SVL[a]	HLL[b]	Body Weight	Sex Ratio (M/F)	Perchlorate (µg/L)[c]
HC84E	2	21.5 ± 6.50	32.0 ± 9.00	0.76 ± 0.43	0/2	0-4
HCGHA	1	23.0	39.0	1.03	1/0	0
MCGR	8	17.3 ± 1.15	30.25 ± 2.33	0.56 ± 0.10	3/3	0-16
NBSB	18	25.0 ± 2.08	40.3 ± 2.78	1.99 ± 0.51	6/12	0-232
OLLO	29	23.0 ± 0.55	39.9 ± 1.06	2.07 ± 0.28	18/11	0-7
PC317	3	24.7 ± 1.76	42.0 ± 4.16	1.24 ± 0.45	2/1	0
SBSBE	6	21.2 ± 1.72	42.0 ± 2.63	1.12 ± 0.33	1/5	0-2.25
SBSBL	4	21.9 ± 3.2	36.75 ± 4.77	1.27 ± 0.51	1/3	0-3.3
SC107	2	22.0 ± 5.00	34.0 ± 2.89	0.96 ± 0.45	1/1	0-149
SOBO	39	22.7 ± 0.61	38.9 ± 1.08	1.24 ± 0.09	14/20	0-31.3
UN317	1	20	34.0	0.9095	0/1	62-540

[a]SVL, snout-vent length
[b]HLL, Hindlimb length
[c]Range of values measured between March 2001 and June 2003

Average perchlorate concentrations at the study sites used for thyroid histopathological analyses are shown in **Table 5-49**. The perchlorate concentrations were averaged from individual measurements performed between March 2001 and June 2003. Average perchlorate concentrations in surface water were statistically greater at site NBSB than all other sites. Average perchlorate concentrations at site MCGR were greater than SOBO and OLLO. The rank order for perchlorate concentrations at the study sites was NBSB > MCGR > SOBO > OLLO > SBSBL > SBSBE. There was no evidence of colloid depletion or follicle cell hyperplasia in any of the 86 animals studied. There was evidence for moderate follicle cell hypertrophy in animals from NBSB and MCGR, the two sites with the greatest measured perchlorate concentrations. Follicle cell hypertrophy was statistically greater in NBSB animals than animals from all other sites (**Figure 5-171**). Follicle cell hypertrophy was greater in animals from MCGR than in animals from OLLO and SOBO. The rank order for follicle cell hypertrophy based on collection sites was NBSB > MCGR > SBSBL > OLLO > SOBO > SBSBE. These findings suggested an association between follicle cell hypertrophy and average perchlorate concentrations at the study site. Hypertrophy data were plotted against mean perchlorate concentration and analyzed by Spearman's rank correlation. There was a significant positive correlation ($P < 0.0001$) between hypertrophy score and average perchlorate concentration in surface water, although the relationship explained only 36% of the variation in thyroid hypertrophy score amongst the animals studied (**Figure 5-172**).

Table 5-49
Mean (± Standard Error) Perchlorate Concentrations (ng Perchlorate/mL) in Surface Water and Thyroid Histopathology Scores in Adult *Acris crepitans* Collected in Lake Waco and Lake Belton Watershed

Site	Perchlorate	Colloid Depletion	Hypertrophy	Hyperplasia
SBSBE	$0.95 \pm 0.39^{a,b}$ (n=7)	0.00 ± 0.00 (5)	$0.10 \pm 0.07^{a,b}$ (5)	0.00 ± 0.00 (5)
SOBO	1.40 ± 0.86^{a} (n=40)	0.00 ± 0.00 (32)	0.15 ± 0.05^{a} (32)	0.00 ± 0.00 (32)
OLLO	1.20 ± 0.43^{a} (n=28)	0.00 ± 0.00 (25)	0.22 ± 0.07^{a} (25)	0.00 ± 0.00 (25)
SBSBL	$1.01 \pm 0.55^{a,b}$ (n=7)	0.00 ± 0.00 (3)	$0.28 \pm 0.25^{a,b}$ (3)	0.00 ± 0.00 (3)
MCGR	5.95 ± 0.93^{b} (n=32)	0.00 ± 0.00 (7)	0.65 ± 0.13^{b} (7)	0.00 ± 0.00 (7)
NBSB	25.9 ± 8.32^{c} (n=37)	0.00 ± 0.00 (14)	0.71 ± 0.05^{c} (14)	0.00 ± 0.00 (14)

Values with different superscripts are significantly different based on Kruskall-Wallis nonparametric ANOVA ($p<0.05$).

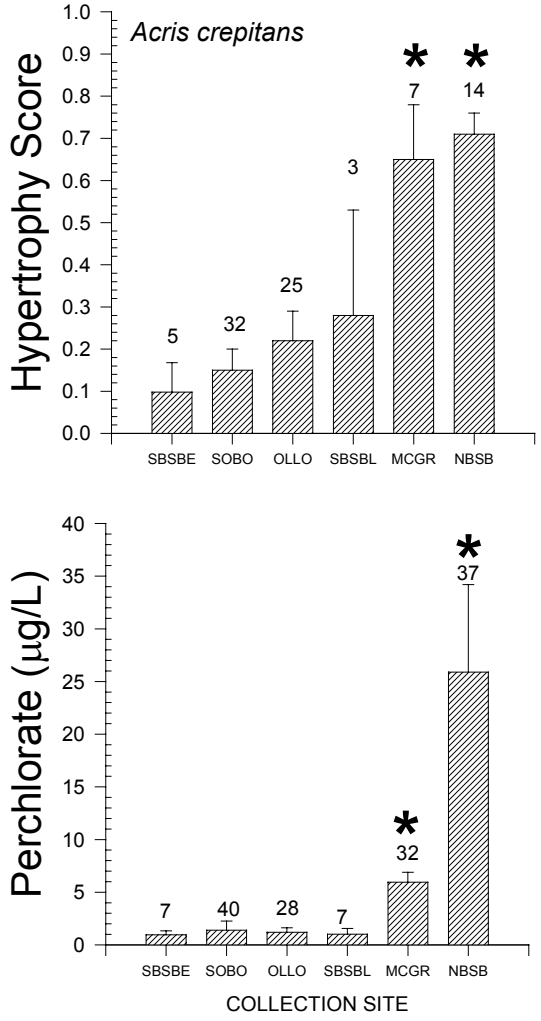

Figure 5-171
Thyroid Follicle Cell Hypertrophy Score (Upper Panel) and Average Perchlorate Concentration at Sites Where Adult *Acris crepitans* Were Collected

Bares are the mean + S.E.M. Numbers above the bars indicate sample size.

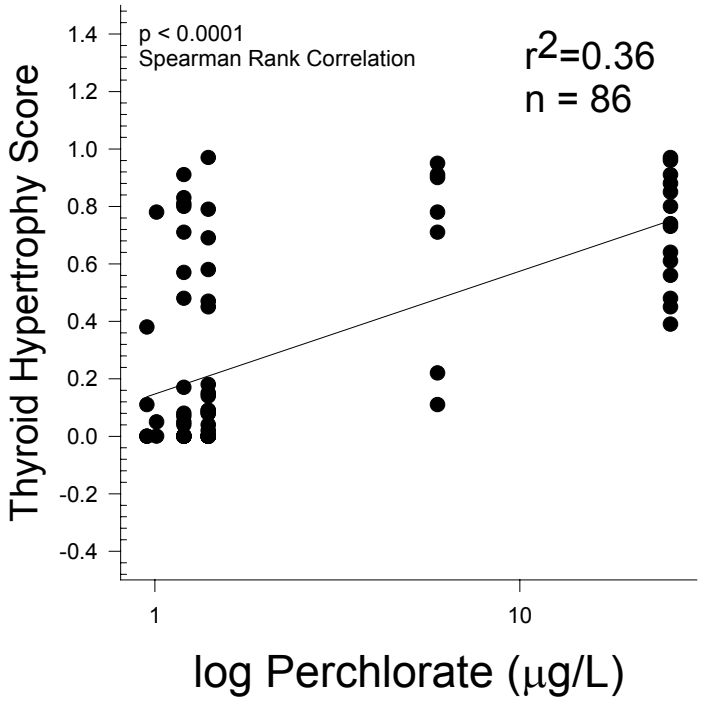

Figure 5-172
Thyroid Hypertrophy Score Plotted Against the Log of Mean Perchlorate Concentration for Sites at Which *Acris crepitans* Adults Were Collected

Each point represents the thyroid hypertrophy score for a single animal

Developmental stage and morphological characteristics of larval *Acris crepitans* collected sites in the Lake Waco/Lake Belton watersheds are shown in **Table 5-50**. Tadpoles at site PC317 were at a significantly earlier developmental stage, and were significantly smaller based upon snout-vent length (SVL), total length, and body weight, than tadpoles collected at HC84E or SOBO. None of the sites had appreciable perchlorate contamination. Perchlorate ranging from 3 to 4 µg/L was detected at HC84E in only 2 of 19 samples. Perchlorate was not detected in three samples collected from PC317 at the time of animal collection. Perchlorate was detected in 5 of 40 samples from SOBO ranging from 1.35 µg perchlorate/L to 31 µg perchlorate/L. Although hindlimb length, a sensitive indicator of perchlorate exposure under laboratory conditions, was smaller in animals collected from PC317, snout-vent length also was smaller in these animals. Under laboratory conditions perchlorate exposure specially affects hindlimb growth but does not influence overall somatic growth as gauged by snout-vent length (SVL).

Table 5-50
Mean (± Standard Error) Gosner Stage, Snout-Vent Length (SVL), Total Length, Hindlimb Length (HLL), Tail Length, Tail Height, and Body Weight in Larval *Acris crepitans* Collected in the Lake Waco and Lake Belton Watersheds

Site	N	Gosner Stage	SVL (mm)	Total Length (mm)	HLL (mm)	Tail Length (mm)	Tail Height (mm)	Body Weight (g)
HC84E	10	34.0 ± 1.52[a,b]	13.2 ± 1.11[a]	31.7 ± 2.80[a,b]	4.14 ± 0.93[a,b]	18.7 ± 1.89[a]	8.20 ± 0.51[a]	0.49 ± 0.08[a]
PC317	49	31.6 ± 0.54[b]	10.2 ± 0.26[b]	26.6 ± 0.82[b]	1.65 ± 0.17[b]	16.4 ± 0.72[a]	6.98 ± 0.17[a]	0.28 ± 0.02[b]
SOBO	124	33.0 ± 0.37[a]	11.2 ± 0.22[a]	30.0 ± 0.81[a]	4.57 ± 0.50[a]	18.9 ± 0.60[a]	7.21 ± 0.17[a]	0.46 ± 0.02[a]

[a]Calculated as the number dead at completion of the experiment divided by the number of animals placed in the tank at the start of the experiment.
[b]Number reaching forelimb emergence (both forelimbs) divided by the number of animals placed in the tank at the start of the experiment.
[c]Number completing tail resorption divided by the number of animals placed in the tank at the start of the experiment.

5.4.2.1.3 Discussion

In the laboratory, perchlorate exposure throughout larval development results in thyroid colloid depletion, follicle cell hypertrophy, and follicle cell hyperplasia (Goleman et al., 2003; Hu et al., 2003). Full larval period exposures to concentration as low as 59 µg perchlorate/L result in follicle cell hypertrophy. Furthermore, tadpoles (*Pseudacris triseriata*) exposed to greater than 9 ppm perchlorate in native ponds demonstrate colloid depletion and follicle cell hypertrophy. Our data indicate that frogs collected from NBSB and MGRB showed evidence of follicle cell hypertrophy but not colloid depletion of follicle cell hyperplasia. These findings are of interest because both NBSB and MCGR contained the greatest mean perchlorate concentrations of all of the sites studied. Qualitative histological analysis revealed that the follicle cell epithelium of the frogs collected from NBSB did not exhibit the gross alterations in size characteristic of exposure of *Xenopus laevis* tadpoles to mg/L concentrations of perchlorate under laboratory conditions. Furthermore, linear regression analysis of all of the hypertrophy scores against mean perchlorate concentration revealed that mean perchlorate concentrations accounted for only 36% of the variation in hypertrophy score, suggesting that other factors may also be involved. Dioxins and numerous other synthetic contaminants can influence thyroid function (Howdeshell et al., 2002), and the possibility that other contaminants may be involved has not been addressed.

Laboratory studies indicate that ammonium perchlorate delays metamorphosis and reduces hindlimb growth, a thyroid hormone dependent process in larval amphibians. Although tadpoles could not be collected at all of the study sites, significant numbers of tadpoles were collected at sites HC84E, PC317, and SOBO. Based on gross morphological measurements, including hindlimb length, there was no evidence that tadpoles from any of these sites were exposed to enough perchlorate to alter thyroid-hormone dependent features of metamorphosis.

One point that should be made is that comparing data from field collected specimens to data from numerous laboratory studies on perchlorate-inhibition of metamorphosis can be problematic, as laboratory studies generally employ a long and constant exposure period (in our lab studies a 70-day exposure) and data from field sites around Lake Waco indicate that perchlorate in surface waters varies considerably from month to month and year to year. For example, NBSB consistently had some of the greater perchlorate concentrations reported during the study period. Of 37 water samples from this site, there were several samples with no detectable perchlorate and many with perchlorate concentrations less than 10 µg/L (**Figure 5-173**) . Levels in October of 2001 were greater than 200 µg/L however. Thus, developing frogs inhabiting the most contaminated sites in this study may have been exposed to considerably varying perchlorate concentrations and periods of no perchlorate exposure at all. This is significant as perchlorate effects on metamorphosis are reversible (Goleman et al., 2002b).

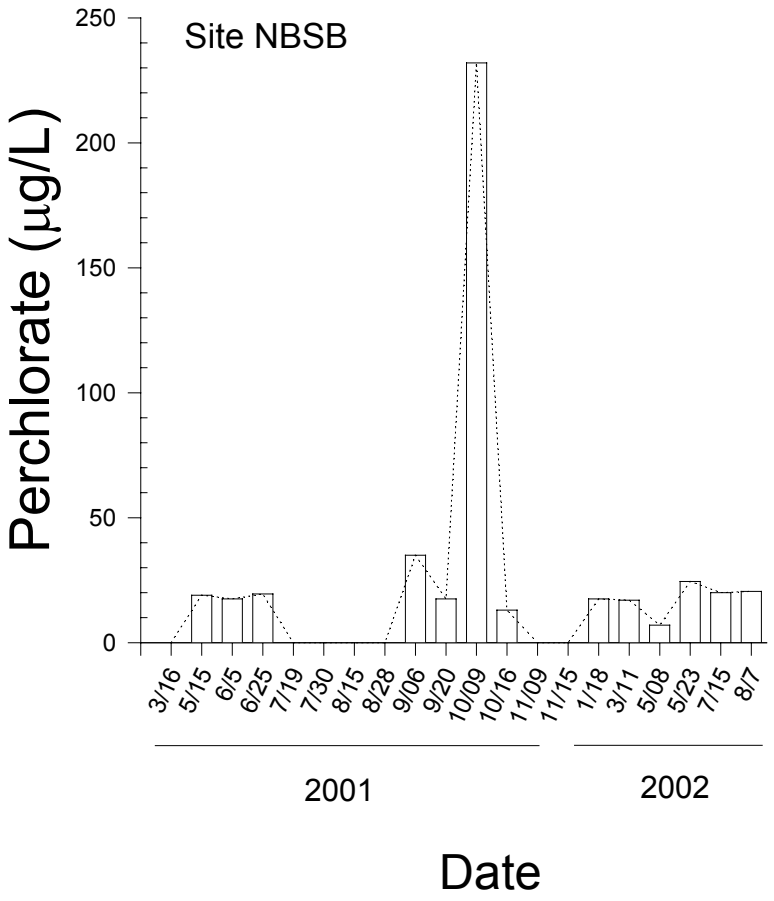

Figure 5-173
Perchlorate Concentrations Measured in Surface Water from NBSB Plotted Against Collection Date

5.4.2.2 Developmental Assays Using Xenopus laevis, the African Clawed Frog

5.4.2.2.1 Methodology

Sexually mature male and sexually mature oocyte positive HCG tested female *Xenopus laevis* were purchased from Xenopus Express and Nasco. Frogs were maintained in dechlorinated tap water in flow-through 160 L tanks at 19 °C at a density of 20 frogs per tank on a 12L:12D light regimen. Frogs were fed frog brittle (Nasco, Ft. Atkinson, WI, USA) three times weekly. Ammonia, pH, and specific conductance of the tank water were monitored every 7 days. Dissolved oxygen was monitored every 2 days, and water temperature monitored once daily. A YSI® model 85 meter (Yellow Springs, OH, USA) was used to monitor water temperature, percent saturation with dissolved oxygen, specific conductivity, and salinity for each tank. Free ammonium ion concentration and pH of the water in each tank were determined with a Hach® spectrophotometer model DR/2000 (Loveland, CO, USA) and an Oakton® pH meter (Gresham, OR, USA), respectively.

Breeding pairs were allowed to acclimate in 40-L glass aquaria containing 18 L FETAX medium (Dawson and Bantle, 1987) for 7 days prior to breeding. Naturally fertilized eggs were obtained from 5-8 pairs of adults as previously described (Goleman et al., 2002). Viable embryos were identified by visual observation with a binocular dissecting microscope. Embryos and tadpoles up to 5 days old were held in 9 L of FETAX medium in 21-L glass tanks acclimated to 22 °C (± 2 °C) on a 12L:12D regimen. Five–day old larvae were transferred to 45-L glass tanks containing 18 L FETAX or test solution. Tadpoles were fed 0.4 g of powered frog brittle (Nasco) every 48 hours. Tanks were checked daily for depletion of food and additional food supplied as necessary. All procedures involving *X. laevis* were approved by the Texas Tech Animal Care and Use Committee.

Water samples for developmental exposure of *X. laevis* tadpoles were collected in 4-L amber glass jugs. Eight-liters of water were collected from each of four or more sites. Water was collected in the middle of the stream or pond approximately 0.3 m off the bottom (in some cases the water was so shallow this was not possible). The water was kept as cool as possible in the field. Upon arrival in Lubbock the samples were stored at 4 °C. See **Table 5-46** for sites. Water quality measurements were recorded for temperature, dissolved oxygen, conductivity, salinity, and pH using a YSI® model 85 meter (Yellow Springs, OH, USA) and an Oakton® pH meter (Gresham, OR, USA).

Initially the experiment was performed using NF stage 55 tadpoles (Nieuwkoop and Faber, 1994). Eight liters of water were collected from each of four sites on two trips (OLLO, SBIT, SOBO, and NBSB) and from five sites on the third trip (OLLO, SBIT, SOBO, MGCR, and SC107). One site (NBSB) was dry on the last trip. Samples from each site were analyzed for ClO_4 ; samples were also collected from each beaker before the exposure and tested for ClO_4. The larvae were allowed to develop to NF stage 55 (Nieuwkoop and Faber, 1994) and snout-vent length (SVL), and tail length and height were recorded. Thirteen 2-L glass beakers containing 1-L of field water were setup. Six 2-L glass beakers containing 1-L of FETAX were setup as controls. Twenty tadpoles

were placed in each beaker. A 50% water change was made every 2 days and 0.5-L of site water was added back. Water was maintained at 22 °C ± 2 °C with a 12L:12D regimen. Tadpoles were exposed until they reached stage NF 66. Mortality, number showing deformities, number showing abnormal swimming behavior, and number metamorphosed animals were recorded daily. Dead tadpoles were removed daily and preserved in 10% neutral-buffered formalin (NBF). At the end of the exposure each animal was staged (Nieuwkoop and Faber, 1994), and SVL, tail length and height, hindlimb length, total length, and weight were recorded. Every 7 days ammonia, pH, and specific conductance, and salinity were recorded. Water temperature was monitored daily. A YSI® model 85 meter (Yellow Springs, OH, USA) and an Oakton® pH meter (Gresham, OR, USA) were used. Free ammonium ion levels were determined with a Hach® spectrophotometer model DR/2000 (Loveland, CO, USA). Dissolved oxygen was measured for tadpoles every 2 days with the YSI® model 85 meter.

The EDSTAC Tier I frog metamorphosis has been criticized for lacking sensitivity, as the NF stage 60 animals that are used for this procedure already exhibit elevated blood levels of thyroid hormones; as a result the assay may not sensitively detect alterations in thyroid hormone synthesis. Data of ours collected from studies unrelated to the present study indicated that even stage 55 tadpoles may not be sensitive to the disrupting effects of perchlorate. To ensure that our laboratory exposures utilized tadpoles that would be sensitive to the perchlorate, we repeated the exposure studies using NF stage 49 tadpoles. The thyroid gland is not yet fully developed in stage 49 tadpoles and theoretically they would respond maximally to any disruption of thyroid hormone synthesis. For this assay larvae were allowed to develop to NF stage 48-49. One hundred eighty larvae were placed in six 10-L glass tanks (thirty larvae per tank), each containing 4-L of FETAX or field water from Trip 7. Two days later this was repeated. Medium was changed every three days. Larvae were exposed to the medium in each tank for 28 days. Each tank was maintained at 21 °C ± 1 °C with a 12L:12D regimen. Every 7 days, water temperature, ammonia, pH, specific conductance, and salinity were recorded.. A YSI® model 585 meter (Yellow Springs, OH, USA) was used. Free ammonium ion levels were determined with a Hach® spectrophotometer model DR/2000 (Loveland, CO, USA. Water temperature was also monitored daily by use of surrogate tanks. Before placement in the test solutions larvae were staged (Nieuwkoop and Faber, 1967) and snout-vent length and tail length were recorded. Each day the number of dead tadpoles, number showing deformities, and number displaying abnormal swimming behavior were recorded. At the end of the exposure (Day 28) the NF stage, snout-vent length, tail length and height, hindlimb length, and total length were recorded for each tadpole. After 28 days of exposure the tadpoles were euthanized by immersion in MS-222, and placed in Bouin's fixative. Water samples were taken from each carboy and analyzed for perchlorate.

Data were analyzed using either parametric or nonparametric statistics. Data were tested for homogeneity of variance using Bartlett's test. If the assumptions of parametric statistics were met, then Student's two-tailed t-test or ANOVA followed by the Tukey-Kramer multiple comparison test were used. If the assumptions of parametric tests were not met, then the Mann-Whitney nonparametric t-test or the Kruskal-Wallis (KW) ANOVA by ranks followed by Dunn's multiple comparisons test were used. All

statistical analyses were performed using InStat (v. 2.05a, GraphPad Software, San Diego, CA) or SPSS (v. 11, SPSS Inc., Chicago, IL).

5.4.2.2.2 Data

Surface water samples were collected from a number of sites in the Lake Waco/Lake Belton watersheds (**Table 5-51**). The number of experimental replicates and total number of tadpoles surviving for the 38-39 day exposure are also shown in **Table 5-52**. The majority of animals completed metamorphosis (NF stage 66) in all treatments (**Table 5-53**), including in the NBSB treatment which had the greatest perchlorate concentrations prior to testing. Mortality was low (3%) in the control FETAX medium exposure. All of the animals exposed to water from the SBIT4 site died within a day or two of exposure in all three replicates. Mortality also was high (60%) in the MGRB and SC107 sites. There were no obvious signs of edema in any of the exposed animals, although ten percent of the animals exposed to MGRB water (the same water that produced 60% mortality) exhibited abnormal swimming. Hindlimb growth (a sensitive indicator of thyroid disruption in tadpoles) did not differ amongst animals from any of the treatments relative to controls.

Table 5-51
Site Collection Data for the Frog Metamorphosis Assay

Site	Total N*	Replicates	GPS (UTM)	Location	Collection Date	Perchlorate (µg/L)**
CONT	97	5	NA	NA	NA	FETAX[a]
MGRB	8	1	650101E 3473922N	Unidentified Tributary at bridge on McGruffy Rd.	11/01	0
NBSB	28	2	652853E 3476589N	Unnamed Tributary near Wastewater Treatment Plant	8/01-10/01	0-232
OLLO	55	3	664177E 3484767N	South Bosque at Highway 84	8/01-11/01	0-3.61
SBIT	39	2	655034E 3476011N	South Bosque at Indian Trail	8/01-10/01	0-9.56
SBIT 4	0	3	655034E 3476011N	South Bosque at Indian Trail	11/01	5.82-8.76
SC107	9	1	642988E 3471304N	Station Creek at Highway 107 (T23)	11/01	10.27-22.07
SOBO	56	3	653810E 3473908N	South Branch of South Bosque at Highway 317 (T16)	8/01-11/01	0-31.3

*Total surviving, 20 animals per replicate. All animals in SBIT-4 died before the end of the experiment.
** Measured in the surface water samples used for testing.
[a]No perchlorate detected in FETAX medium.

Table 5-52
Mean ± Standard Error Mortality, Forelimb Emergence, Tail Resorption, Edema, Bent Tails, and Abnormal Swimming in *Xenopus laevis* Tadpoles Exposed to Surface Water for 38-39 Days Beginning at Stage 55

Site	Mortality[a] (%)	Forelimb Emergence[b] (%)	Tail Resorption[c] (%)	Edema (%)	Bent Tails (%)	Abnormal Swimming (%)
CONT	3.00 ± 1.23	97.0 ± 3.00	89.0 ± 4.58	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 1.23
MGRB	60.0	35.0	30.0	0.00 ± 0.00	0.00 ± 0.00	10.0 ± 0.00
NBSB	30.0 ± 10.0	80.0 ± 15.0	60.0 ± 20.0	0.00 ± 0.00	0.00 ± 0.00	15.0 ± 5.00
OLLO	8.33 ± 4.41	98.3 ± 1.67	90.0 ± 5.00	0.00 ± 0.00	0.00 ± 0.00	0.00
SBIT	2.50 ± 2.50	100	97.5 ± 2.50	0.00 ± 0.00	0.00 ± 0.00	0.00
SBIT 4	100	0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
SC107	55.0	45.0	45.0	0.00 ± 0.00	0.00 ± 0.00	0.00
SOBO	6.67 ± 3.33	96.7 ± 3.33	91.7 ± 4.41	0.00 ± 0.00	0.00 ± 0.00	0.00

[a]Calculated as the number dead at completion of the experiment divided by the number of animals placed in the tank at the start of the experiment.
[b]Number reaching forelimb emergence (both forelimbs) divided by the number of animals placed in the tank at the start of the experiment.
[c]Number completing tail resorption divided by the number of animals placed in the tank at the start of the experiment.

Table 5-53
Mean ± Standard Error Developmental Stage, Snout-Vent Length (SVL), Hindlimb Length, Total Length, Tail Length, Tail Height, Body Weight, and Sex Ratio in *Xenopus laevis* Tadpoles Exposed to Surface Water for 38-39 Days Beginning at Stage 55

Site	Nieuwkoop -Faber Stage	SVL (mm)	HLL (mm)	Total Length (mm)	Tail Length (mm)	Tail Height (mm)	Body Weight (gm)	Sex Ratio (M/F)
CONT	65.6 ± 0.18	16.7 ± 0.17	18.8 ± 0.42	17.8 ± 0.58	1.14 ± 0.58	0.21 ± 0.09	0.46 ± 0.01	50.0%
MGRB	64.5 ± 1.24	14.8 ± 0.43	15.0 ± 2.22	15.0 ± 2.23	4.75 ± 4.34	0.63 ± 0.63	0.38 ± 0.05	50.0%
NBSB	65.5 ± 0.32	16.8 ± 0.29	19.0 ± 0.81	18.2 ± 1.12	1.35 ± 1.09	0.29 ± 0.18	0.49 ± 0.02	80.0%
OLLO	65.8 ± 0.16	16.1 ± 0.26	18.5 ± 0.43	16.7 ± 0.67	0.64 ± 0.63	0.10 ± 0.09	0.42 ± 0.02	37.9%
SBIT	66.0 ± 0.03	16.8 ± 0.25	19.1 ± 0.34	16.8 ± 0.25	0.03 ± 0.03	0.03 ± 0.03	0.46 ± 0.02	55.6%
SBIT 4	NA	NA	NA	NA	NA	NA	NA	NA
SC107	66.0 ± 0.00	16.3 ± 0.45	19.4 ± 1.03	16.3 ± 0.45	0.00 ± 0.00	0.00 ± 0.00	0.48 ± 0.04	33.3%
SOBO	65.8 ± 0.16	16.2 ± 0.20	18.4 ± 0.46	16.8 ± 0.58	0.54 ± 0.54	0.09 ± 0.09	0.45 ± 0.02	51.9%

Recently, Opitz et al. (2002) have proposed an alternative to the flawed EDSTAC frog metamorphosis assay. In the XEMA assay (*Xenopus* metamorphosis assay), NF stage 49 tadpoles are used. In theory these animals would be more sensitive the thyroid disrupting agents, as exposures would begin before NF stages 51-52, when the thyroid gland is fully formed. In the Fall of 2003 we performed some preliminary trials using the XEMA assay to test water samples collected from CROOR, MNSP, NBSB, PC317, and UN317. In these tests we spent a considerable amount of time making sure that NF stage 49 tadpoles only were used in the tests. Unfortunately we encountered very high mortality (greater

than 80%) in all of these trials. This mortality is unacceptably high, and occurred even in the controls (contrast this to mortality in the EDSTAC experiments which was 3%).

5.4.2.2.3 Discussion

As it was originally described, the EDSTAC Tier I frog metamorphosis assay (Fort and Stover, 1997; Federal Register, 1998) was designed to use NF stage 66 tadpoles in a 14- or 21-day exposure. The overriding problem with such an approach is that the thyroid-hormone dependent gene program that initiates metamorphosis is already well-under way in NF stage 60 tadpoles, and plasma thyroid hormones levels are near maximal in these animals (Leloup and Buscaglia, 1977). Thus, one would not predict that the frog metamorphosis assay as it is designed would be sensitive enough to detect chemicals that alter thyroid hormone synthesis. For this reason, the EDSTAC procedure was modified so that it began at NF stage 55 rather than NF stage 60. The data on metamorphosis as well as the measurement of perchlorate in the surface water samples tested together suggest that none of the samples had sufficient perchlorate concentrations to alter the rate of metamorphosis. Animals completed metamorphosis in all of the tested samples except for MCGR, which showed slightly slower metamorphosis, although a high percentage of the animals exposed to water from this site died before the end of the testing period. Water from one site SBIT4 killed all of the test animals in all three trials. Given that the minimal perchlorate concentration capable of fully blocking metamorphosis in *X. laevis* in the laboratory is 147 mg/L under constant 70-day exposure (Goleman et al., 2002a), it is likely that none of the sites studied would lead to a complete blockade in metamorphosis.

Data collected from the 28-day exposure using stage 49 larvae revealed high but delayed mortality in all treatments, most likely due to the handling required to accurately stage the NF stage 49 larvae. These handling effects resulted in none of the animals, including controls, developing normally during the 28-day exposure. The mortality was traced to the methods employed for selection of NF stage 49 animals. These larvae were held on ice-chilled FETAX medium to reduce movement so that they could be observed under the dissecting microscope to identify stage. At present there are no accepted and standardized guidelines for the 28-day partial life cycle test. A solution for future studies would be to use age-matched tadpoles whose stage on average was stage 49 for that post-hatching date.

5.4.2.3 Gonadal Effects on Native and Non-Native Frogs

5.4.2.3.1 Methodology

Sex ratios were determined by direct visual inspection of field-collected animals or *X. laevis* from the laboratory exposure studies as described previously (Goleman et al., 2002a). Previously fixed specimens were rinsed in deionized water and pinned to a dissecting dish of hardened paraffin wax placed under a binocular dissecting microscope with a high intensity illuminator as a light source. The abdominal cavity was opened and the intestines removed. The kidneys were located in the retroperitoneal region. Appearance of the gonads depended upon the age of the animal and the species, but in general the ovaries were long and lobular, with small areas of dark pigmentation visible.

Testes were shorter and lacked lobes, but varied in pigmentation based upon the species. Gonadal abnormalities were scored using the following criteria: Intersex gonads were categorized as: left/right intersex (a testis on one side and an ovary on the opposite side), rostral/caudal intersex (testicular characteristics rostrally and ovarian characteristics caudally or vice versa), or mixed sex (mixed testicular/ovarian tissue). For purposes of analysis all three subcategories were combined and referred to as intersex.

After assessment of gonadal morphology, the dorsal wall of the abdominal cavity with kidneys and gonads attached was processed for routine paraffin embedding. Gonads were sectioned at 10 µM, stained with hematoxylin and eosin, and gonadal morphology examined using a light microscope.

Sex ratio and intersex were reported as incidence per treatment and were transformed (arcsine of the square root) before ANOVA in the case on intersex data or analyzed by Chi-square for sex ratio data.

5.4.2.3.2 Data

Phenotypic sex of field caught *Acris crepitans* was determined by gross morphological assessment of gonadal tissue and confirmation of a subset of animals by histology. There were no unambiguous sex (i.e. intersex) animals noted based on gross morphological assessment. The ratio of females to males was approximately 2:1 at NBSB, the site where the greatest perchlorate concentrations were observed. However, X^2 analysis revealed that this distribution was not significant, probably due to the rather small sample size.

In the frog metamorphosis assay, the sex ratio was distributed equally among 97 animals in 5 replicates, although exposures in this study began at stage 55, past the window of sex differentiation. In other words, neither the EDSTAC frog metamorphosis assay or our modification of the assay is designed to detect changes in phenotypic sex ratio because exposures begin after the critical window for gonadal sex differentiation, which is NF stage 44-50 in *Xenopus laevis* (Villalpando et al., 1990). This is not to say that the exposures could not affect gonadal growth or development in the study, however gonadal size was not evaluated in the current study and there are no reports on the effects of perchlorate exposure on gonadal growth. The unusual sex ratio observed for NBSB (80% males) is most likely due to a high percentage of males in one test tank prior to the onset of exposures. There is no evidence that perchlorate or thyroid disruption in general causes masculinization in *Xenopus laevis* or any other frog species.

5.4.2.3.3 Discussion

There was no evidence of intersexuality in captured *A. crepitans* from any of the field sites and no obvious trends between incidence of females and perchlorate in surface water. In *X. laevis*, exposure to 59 µg perchlorate/L for 70 days alters the sex ratio toward more females (Goleman et al., 2002b). In the current study there were no sites with perchlorate consistently detectable at greater than 50 µg perchlorate/L with the exception of Station Creek SC107 and UN317. There was no evidence in any of our trips that either of these sites was inhabited by amphibians. Moreover, it is unclear whether thyroid disruption would alter sex differentiation in this species as it does in *Xenopus laevis*.

5.5 TERRESTRIAL ANIMALS

5.5.1 Small Mammals and Birds

5.5.1.1 Perchlorate Residues in Native Small Mammals and Birds

5.5.1.1.1 Introduction

Small mammals and birds are routinely used as sentinel species to assess environmental exposure to chemicals (movement of chemicals from the environment into organisms) and the possible effects resulting from any exposure. Their utility is often tied directly to their diet, relative small home ranges or foraging areas, and position in the food chain. For example, small mammals, such as most rodents, consume a variety of plant and animal matter, and rarely venture over an area much larger than a hectare. Birds also have a variable diet, and although they may venture over large areas during parts of their life, during the breeding season their home ranges are often quite restricted. Thus many small mammals and birds collected from a specific location can be viewed with confidence as living and feeding in the area of collection. In addition, their position near the bottom of most food chains makes them excellent conduits for chemical movement into top-level mammalian, reptile, and avian predators. The combination of these characteristics of small mammals and birds make them useful for monitoring exposure and effects to a variety of chemicals, including perchlorate.

Perchlorate, although a very water soluble compound, has been detected in a variety of matrices, including soil, water, and plants. Small mammals and birds routinely consume free standing water, plants, and the insects living in association with water, soil, and plants. Thus, the potential is high for small mammals and birds to consume some dietary component that contains perchlorate. However, potential for exposure does not always equate to actual exposure, although perchlorate has been detected in birds and small mammals at other sites contaminated with perchlorate (Smith et al., 2001). The purpose of this phase was two-fold. First, to assess actual exposure of small mammals and birds to perchlorate residues as a function of living near contaminated environments and consuming food and water from those contaminated sites. We hypothesized that small mammals and birds would harbor detectable concentrations of perchlorate as a function of living and feeding on contaminated areas. Results of this phase of the study were used to assess the degree of exposure that occurs in wildlife populations of animals and answer the basic question of whether perchlorate, a water-soluble compound, can accumulate to detectable levels in small vertebrates. Second, we wanted to assess the distribution of perchlorate among different tissues in a small mammal model given perchlorate under controlled conditions.

Perchlorate exerts its primary effect on animals by inhibiting uptake of iodide into the thyroid gland (Stanbury, 1952). This inhibition of iodide uptake in turn inhibits the production of thyroid hormones that are subsequently sent into the peripheral blood (Wolff, 1998). Therefore, analyzing the concentration of thyroid hormones in blood (specifically plasma) samples from animals is one effects measurement indicative of

exposure to perchlorate. We hypothesized that small mammals exposed to perchlorate would exhibit alterations (decreases) in thyroid hormone concentrations.

5.5.1.1.2 Methodology

5.5.1.1.2.1 Field Collections

Small mammals and birds were collected from one or more sites distributed throughout the Lakes Waco and Belton watersheds, as shown on **Figure 5-174**. All of these sites represented riparian areas or drainage ditches associated with suspected perchlorate-contaminated areas that drained into local creeks. These areas include the groundwater spring south of Oglesby Road (T18) (HCS), the spring fed tributary that feeds into Harris Creek (HCT), Harris Creek at Highway 84 West of McGregor (T19) (HC84), Station Creek at Highway 107 (T23) (SC107), the unnamed tributary near the waste water treatment plant at Highway 317 (T14) (TF), and S Creek at Highway 317 (T15) (NB317).

5.5.1.1.2.2 Animal Collections

A variety of passerine bird species (n = 12) and small mammals (n = 35) were collected from the locations near the NWIRP site near McGregor, Texas. Mist nets were placed along creeks and ditches, and monitored continuously while open and all captures euthanized and frozen at -20 °C in the field. Snap traps were set in similar locations to capture small mammals. Traps were set in the evening and checked the following morning, and all captures frozen at -20 °C in the field. Birds and rodents were placed in plastic bags, labeled with location, species, and date. Samples were transported back to TIEHH for analysis.

5.5.1.1.2.3 Water Collections

Water samples were collected into clean vials from just below the water surface wherever possible. All water samples (5 mL) were filtered and either analyzed for perchlorate ion directly, or diluted with distilled, deionized water and then analyzed. Soil samples were taken from the top 5 cm of soil. Soil samples were weighed, placed in glass jars, and extracted (mechanical agitation) with distilled, deionized water (2:1 water:soil). Water extracts were filtered and either analyzed for perchlorate ion directly, or diluted with distilled, deionized water and then analyzed. Vegetation, invertebrate, and vertebrate diet samples were collected from areas adjacent to where animals were collected. Samples were removed from soil, sediment, or water and placed in plastic bags. Prior to extraction, samples were air-dried, and weighed.

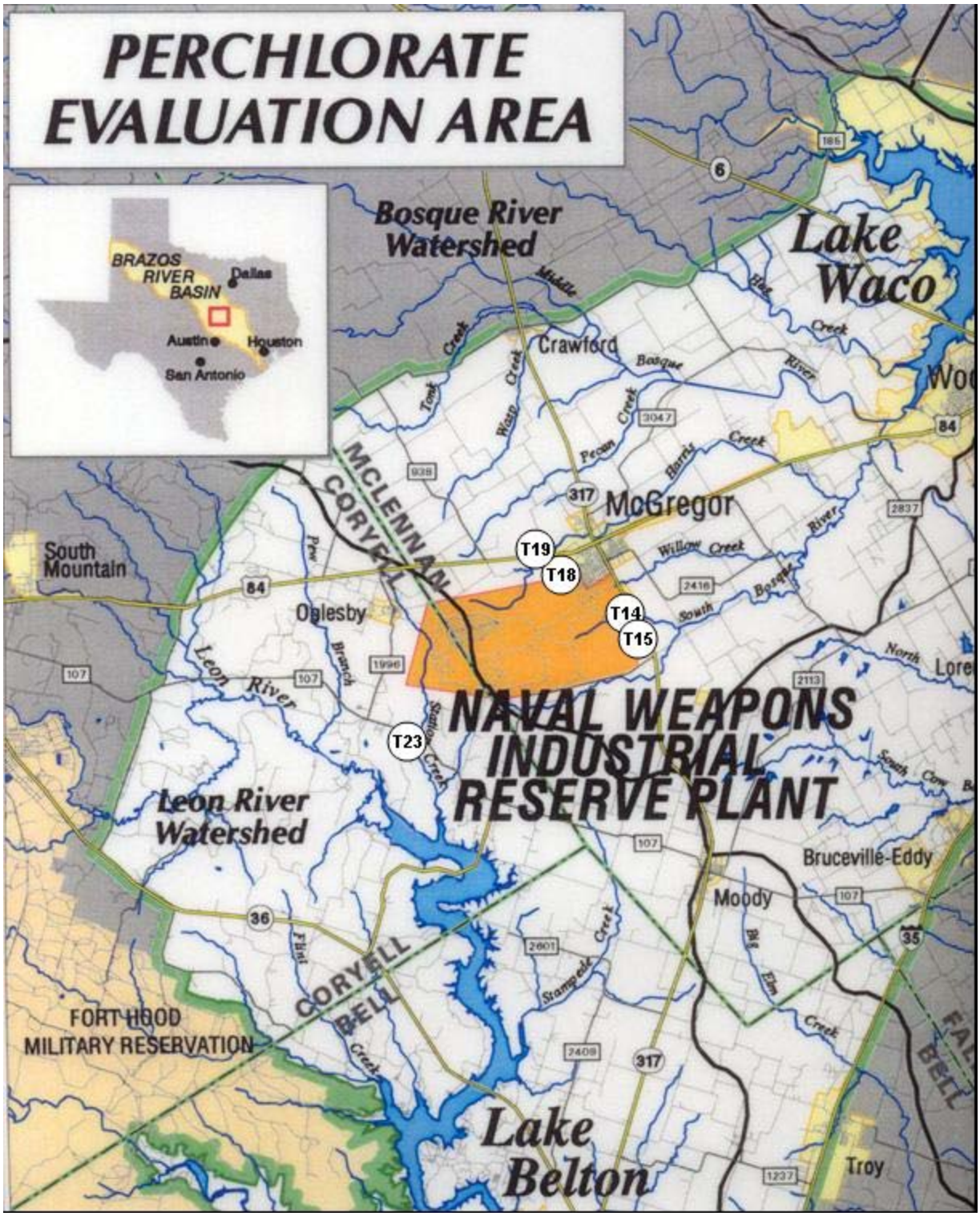

Figure 5-174
Map of Study Area Illustrating the Approximate Locations where Small Mammal and Bird Samples Were Collected

5.5.1.1.2.4 Tissue Distribution Study

Ten male voles were taken from our breeding colony housed at Texas Tech University for use in this study. These voles were randomly placed into 10 different metabolic cages (labeled 1-10) at 8:00 pm on September 1, 2003 with no food or water. Reference urine and feces were collected from the metabolic cages between 7:30 am and 8:00 am on September 2, 2003. At 8:00 am, upon completion of collection of reference materials, all voles were given 250 ppm magnesium perchlorate dissolved in water. The water bottles were weighed prior to putting them on the cages. The 10 voles were then randomly assigned to either a 4 hour or 8 hour dosing period. Vole 10, 6, 3, 5, and 9 were dosed from 8:00 am to 12:00pm. At the end of the 4 hours the water bottles were removed from the metabolic cages and weighed. These voles were euthanized using a carbon dioxide chamber and necropsied where blood, liver, kidney and thyroids were collected. Once all five voles were necropsied, the blood was spun down to collect plasma. All samples were stored on dry ice until returning to The Institute of Environmental and Human Health where they were stored in a -80 °C freezer until analysis. Urine and feces were collected from the cages upon completion of the necropsy. Vole 4, 7, 1, 2, and 8 were dosed from 8:00 am to 4:00 pm and then processed as described above for the 4 hour group.

5.5.1.1.2.5 Sample Analysis

Livers and kidneys were analyzed for perchlorate content using standard tissue extraction and analysis techniques developed in the analytical core of this project (**Appendix X**).

5.5.1.1.2.6 Statistical Analysis

Chi-square analysis was used to compare frequency distributions of quantifiable versus pooled trace and non-detectable concentrations of perchlorate in small mammals across sites and species. Linear regression was used to compare the relationship between concentrations of perchlorate in rodent tissues and water from collection sites. Two-tailed T-tests were conducted to test differences in water consumption, urine production, and perchlorate recovery in voles in the tissue distribution study. All summary statistics are reported as means and standard errors.

5.5.1.1.2.7 Detection Limits

All positive indications of perchlorate concentrations below the detection limits (**Table 5-54**), below the 2.5 ppb standard's area, or with less than a 2:1 signal to noise ratio were reported as trace amounts. A non-detect (ND) indicates that no positive indication of perchlorate was found in the sample. Minimum detection limit concentrations were estimated considering the chromatograph minimal detection area for each set of standards in conjunction with conservative extract volume and sample weight values of similar sample types. The samples were divided into categories based on tissue type and animal class, for example: mammalian kidneys and avian livers were two of the categories examined. Based on this examination and estimation minimum detection limits were determined.

Table 5-54
Minimum Detection Limits of Perchlorate in Mammal and Avian Tissues

Mammalian Kidney	5 ppm
Mammalian Liver	3 ppm
Avian Kidney	8 ppm
Avian Liver	6 ppm

5.5.1.1.3 Data

5.5.1.1.3.1 Field Collections

A total of 35 small mammals and 12 birds were collected (**Table 5-55**). Most small mammals and birds were collected from the Harris Creek site (HC84) and an adjacent site, HCT. Small numbers of mice and one bird were collected from the remaining sites. The lack of captures was due to sparse habitat at most sites and a concomitant lack of animals.

Table 5-55
Number of Individual Small Mammals and Birds Captured at the Six Field Site Locations in the Waco/Belton Watershed Near McGregor, Texas

Species	**HC84 (T19)**	**HCT (T18-T19)**	**TF (T14)**	**NB317 (T15)**	**HCS (T18)**	**SC107 (T23)**
Small Mammals						
House mouse	6	0	0	0	0	0
Peromyscus sp.	3	1	3	2	2	4
Harvest mouse	1	0	0	0	0	0
Cotton rat	13	0	0	0	0	0
Birds						
Eastern phoebe	0	3	0	0	0	0
Lincolns sparrow	0	1	0	0	0	0
Mockingbird	0	1	0	0	0	0
Northern cardinal	0	3	0	1	0	0
Song sparrow	0	1	0	0	0	0
White-crowned sparrow	0	2	0	0	0	0

1 See methods for descriptions of field site abbreviations.

Perchlorate was detected in kidney and liver samples from small mammals and birds (**Table 5-56**). In general, perchlorate was detected in quantifiable amounts more often in kidney than liver tissues. Quantifiable concentrations ranged from 4 ppm to 64 ppm in kidney samples and 7 ppm to 40 ppm in liver samples in small mammals. Frequency distributions of quantifiable concentrations of perchlorate in small mammals differed from trace/non-detectable perchlorate residues in both kidney and liver samples (kidney: $X_2 = 10.9$, d.f. = 1, $P < 0.005$; Liver: $X_2 = 5.3$, d.f. = 1, $P < 0.025$). This analysis compared frequency distributions among four sites; HC84, HCS and HCT combined, SC107, and TF and NB317 combined. Sites were combined based on proximity to one

another and existing in common drainages. Results for perchlorate in kidney indicated that quantifiable perchlorate was detected a less than expected number of times in mice on site HC84, and higher than expected on site SC107. Results differed for liver samples, with a higher than expected number of quantifiable detections of perchlorate in mice from HCS/HCT and TF/NB317, and a lower than expected number in mice from HC84.

Table 5-56
Perchlorate Concentrations in Tissues from Small Mammals and Birds Collected from Riparian Areas in the Waco/Belton Watershed Near McGregor, Texas

Biota	Site	Common Name	Kidney		Liver	
			Perchlorate (ppm)	Dry Wt. (g)	Perchlorate (ppm)	Dry Wt. (g)
Mammals	HC84 (T19)	House mouse	Trace*	0.1026	ND	0.3849
			Trace*	0.0569	ND	0.2248
			Trace*	0.0604	ND	0.2408
			Trace*	0.0542	ND	0.2549
			Trace*	0.0711	11	0.2732
			27*	0.0835	ND	0.3928
		Deer mouse	Trace*	0.0989	ND	0.3654
			Trace*	0.1046	ND	0.4735
			Trace*	0.0703	ND	0.3288
		Harvest mouse	Trace*	0.0462	ND	0.1613
		Cotton Rat	Trace*	0.1778	Trace*	1.1635
			4*	0.1276	7*	0.6775
			10*	0.2002	ND*	1.0321
			8*	0.2949	ND*	1.6693
			17*	0.0581	Trace*	0.2386
			Trace*	0.2243	10*	1.1098
			Trace*	0.3485	Trace*	1.9194
			Trace*	0.1738	Trace*	0.9738
			Trace*	0.164	ND*	0.7288
			Trace*	0.1503	Trace*	0.7989
			Trace*	0.264	Trace*	0.9692
			Trace*	0.3008	Trace	2.0421
			Trace*	0.2463	Trace	1.215
	HCS (T18)	Deer mouse	38*	0.0732	ND	0.2436
			Trace*	0.0809	14	0.2484
	HCT (T18-T19)	Deer mouse	26*	0.0898	40	0.3359
	SC107 (T23)	Deer mouse	23*	0.0809	12	0.2668
			7*	0.1042	ND	0.4732
			28*	0.1055	Trace	0.3768
			34*	0.0918	ND	0.3629
	TF (T14)	Deer mouse	28*	0.0757	10	0.3396
			64*	0.0773	Trace	0.1809
			45*	0.0928	ND	0.4306
	NB 317 (T15)	Deer mouse	ND	0.0539	17	0.2317

Biota	Site	Common Name	Kidney		Liver	
			Perchlorate (ppm)	Dry Wt. (g)	Perchlorate (ppm)	Dry Wt. (g)
			ND	0.0452	ND	0.2411
Birds	HCT (T18-T19)	Eastern Phoebe	86*	0.0457	Trace	0.1575
			28*	0.0509	ND	0.1205
			17*	0.1395	9	0.3905
		Lincoln's Sparrow	37*	0.037	ND	0.1747
		Mockingbird	18*	0.156	ND	0.9404
		Northern Cardinal	10*	0.119	7	0.4701
			31*	0.137	ND	0.3474
			17*	0.0921	ND	0.3545
		Song Sparrow	32	0.1127	47	0.2659
		White Crowned Sparrow	42*	0.0813	ND	0.3441
			19*	0.0751	7	0.4132
	NB 317 (T15)	Northern Cardinal	ND	0.1023	ND	0.3691

* indicates extraction with EtOH instead of water

Mean concentrations of perchlorate in kidney samples of rodents were also compared to mean concentrations of perchlorate in water samples collected from the different sampling areas (**Figure 5-175**). Variation in perchlorate in water samples explained 27% of the variation in perchlorate concentrations in kidney samples across four sampling areas.

Frequency distributions of quantifiable perchlorate concentrations in tissues also differed among rodent species pooled across sites. House mice, deer mice, and cotton rats, but not harvest mice (n = 1) were compared to assess the distribution of quantifiable versus trace and non-detectable perchlorate in kidney and liver samples. The frequency of quantifiable perchlorate differed across species in kidney samples (X^2 = 4.3, d.f . = 1, P < 0.05) but not for liver samples (X^2 = 1.5, d.f. = 1; P > 0.100). The number of quantifiable perchlorate concentrations in kidney samples was higher than expected in deer mice.

These same analyses could not be performed for birds given the low number of captures across sites (all but one bird was captured at HCT). However, perchlorate concentrations in birds at HCT tend to agree with the elevated concentrations of perchlorate found in small mammals from the same site or same general area (e.g., HC84, HCS, and HCT).

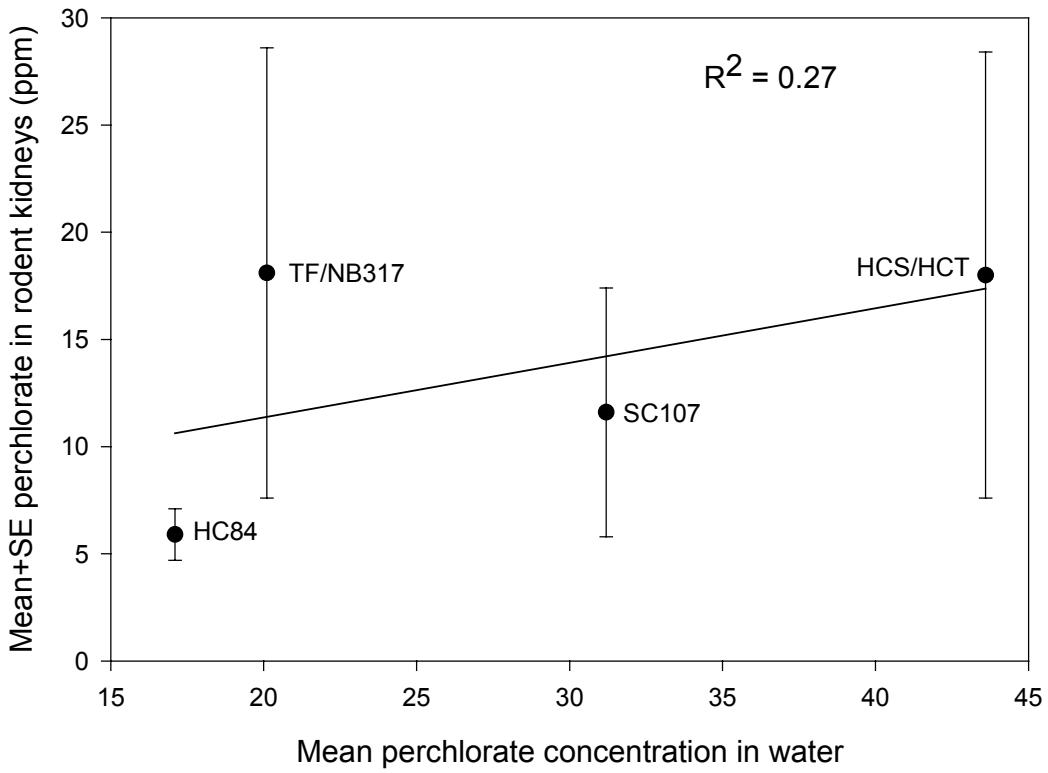

Figure 5-175
Linear Regression of Perchlorate Concentrations in Rodent Kidneys Versus Perchlorate Concentration in Water Samples from Study Sites in the Lake Waco and Lake Belton Watersheds near McGregor, TX

5.5.1.1.3.2 HC84 (T19)

Peromyscus spp. (deer mice or white-footed mice), and one harvest mouse contained trace concentrations or concentrations below our minimum detection limit (ND). However, two house mice and five cotton rats contained elevated perchlorate concentrations in kidneys and/or livers. Yet individuals of both species were also collected that contained no quantifiable perchlorate residues. Interestingly, higher concentrations were detected in kidneys than livers in general, perhaps indicating a recent or intermittent exposure since kidneys remove, store, and eliminate ions from blood so as to maintain osmotic balance. Quantifiable kidney concentrations ranged from 4-17 ppm and 7-10 ppm in liver samples.

5.5.1.1.3.3 HCS (T18)

Two *Peromyscus* were collected at this location, both containing elevated tissue concentrations of perchlorate. Few rodents were collected at this site despite extensive sampling effort. This site provides surface water year-round, potentially serving as a source of exposure to birds and mammals even during drought periods.

5.5.1.1.3.4 HCT (Between T18 and T19)

This site offered opportunities to erect and monitor mist nets intended for bird capture and collection. Among the avian species collected were migratory and part-time resident species. All birds collected from this area contained elevated perchlorate concentrations in kidneys, and many contained quantifiable concentrations of perchlorate in liver tissues. Highest tissue concentrations were found in the kidneys of an Eastern phoebe (86 ppm) and a liver sample from a Song sparrow (47 ppm). These tissue concentrations exceed the highest ever reported for any wildlife species (see Smith et al., 2001). Currently, there are no data available to evaluate potential effects related to these tissue concentrations. One *Peromyscus* collected from this area also contained elevated kidney and liver concentrations.

5.5.1.1.3.5 SC107 (T23) and TF (T14)

Four *Peromyscus* were collected from SC107 and three from TF, all of which contained elevated tissue perchlorate residues. As seen in other rodents and birds collected during this sampling period, perchlorate was detected more frequently in kidneys than livers. TF produced rodents containing the highest tissue concentrations among all rodents collected. One *Peromyscus* contained 65 ppm perchlorate in kidneys, again considerably higher than values reported in the scientific literature.

5.5.1.1.3.6 NB317 (T15)

Two deer mice and one Northern cardinal were collected from NB317. All but one of the tissues from these animals were non-detectable for perchlorate. One sample, a liver from a deer mouse had 17 ppm perchlorate. This value generally agrees with other perchlorate concentrations in liver samples from rodents in this study.

5.5.1.1.3.7 Tissue Distribution Study

Ten voles were used in this study, although one vole was excluded from analysis due to exceedingly high intake of water (10.31 mL) that was about two-fold greater than the means for either group (**Table 5-57**). The remaining nine voles were used to calculate summary statistics and test differences between means.

Table 5-57
Mean ± SE Values for Water Consumption, Urine Production, and Perchlorate Recovery in Voles Dosed with 250 ppm Perchlorate in Drinking Water for Either 4 Hours or 8 Hours Following a 12 Hour Fasting Period

Group	Water Consumption (mL)	Urine Volume (mL)	Percent Perchlorate Recovered			
			Urine	Blood	Kidney	Liver
4 hour	4.8 ± 0.6	1.3 ± 0.5	33 ± 12	2.0 ± 0.4	0.03 ± 0.02	0.19 ± 0.10
8 hour	5.6 ± 0.3	2.6 ± 0.2	86 ± 7	1.6 ± 0.2	0.02 ± 0.01	0.22 ± 0.05

Water consumption did not differ between the 4 and 8 hour groups (P = 0.8190). In contrast, urine production was two-fold greater in the 8 hour group than in the 4 hour group. This difference was not statistically significant (P = 0.0653), but may indicate

biological significance. The lack of statistical significance in this test was undoubtedly driven in part by low sample size.

Most of the perchlorate was accounted for in the urine, with 33% and 86% recovered from voles in the 4 hour and 8 hour groups, respectively (**Table 5-57**). Likewise, recovery of perchlorate differed between groups (P = 0.0149). Conversely, little perchlorate was recovered in either blood, kidney, or liver samples in either group. Also, perchlorate recoveries did not differ between groups for any of these matrices (P > 0.400).

As expected, most of the perchlorate consumed by the voles was expelled in the urine. This was especially evident in the 8 hour group. Little perchlorate was observed in blood, kidney, or liver samples, indicating that these tissues are not efficient sinks for perchlorate under the constraints of this study. Of interest was our inability to account for all of the perchlorate in the four matrices examined, especially in the 4 hour dose group. This may be a function of time of exposure, with voles in the 4 hour group experiencing a latent period where much of the consumed perchlorate still resides in the intestinal tract. Voles in the 8 hour dose group have likely had more time to equilibrate to the exposure, with more of the consumed perchlorate passing out of the intestinal tract, into circulation, then passed in the urine. This idea is supported by the lack of differences in water consumption in conjunction with a two-fold increase in urine output in the 8 hour versus the 4 hour group.

The results of this study confirm previous work demonstrating that large portions of ingested perchlorate are expelled in the urine. However, we have observed high concentrations of perchlorate in kidney samples, and to a lesser degree, liver samples, in small mammals and birds. The short-term exposure and recovery period for perchlorate in this study does not mimic the potential long-term and continuous exposure experienced by wildlife in the field. Long exposure periods may result in accumulation not seen under laboratory conditions.

5.5.1.1.4 Discussion

These data should be interpreted with caution due to small sample sizes (both in terms of numbers and physical dimension), sampling design (collections from areas of known contamination only), reporting of dry weight tissue concentrations, and a lack of reference samples collected from non-impacted areas. Nevertheless, these data demonstrate significant exposure among migratory avian species and rodents inhabiting areas containing impacted waterways.

The rodent data suggest that perchlorate concentrations in tissues follow a gradient of potential exposure, with concentrations increasing in tissues with increasing concentrations in water. These data are weak however in that sample sizes were low for all sites except HC84. A more robust data set would add to the resolution in the relationship between environmental concentrations and tissue concentrations. Nonetheless, the evidence supports differential exposure among sites in terms of concentration gradients and frequency of quantifiable perchlorate in rodents.

Different species of wildlife vary in their inherent life-history traits. Differences in foraging strategies (e.g., diet, food processing), habitat preferences, home ranges, and others all play a role in how an individual species interacts with its environment, and thus dictate the exposure scenario in a contaminated environment. Observed differences among rodent species in the frequency distribution of quantifiable concentrations of perchlorate are not overtly surprising. House mice, deer mice, and cotton rats, although occupying the same general habitats, can differ in diet composition and how they process forage. Species in the genus *Peromyscus* (e.g., deer mice, white-footed mice) often consume diets consisting of relatively high percentages of invertebrates, although diets do vary tremendously (Lackey et al., 1985). By comparison, cotton rats typically consume a high percentage of plant material, particularly monocots (grasses; Kincaid and Cameron, 1982). House mice often have omnivorous diets, consuming a variety of plant and invertebrate material (Miller and Webb, 2000). However, these apparent differences must be viewed with caution as diets of many small mammals are notoriously flexible and dependent on availability of the different diet components. The differences observed in the frequency of quantifiable concentrations of perchlorate among small species in this study may be directly attributable to differences in diet composition and food processing techniques.

Similar variation among species would likely occur in birds as well, with perchlorate exposure varying among species depending on diet. Birds such as northern cardinals and different species of sparrows consume diets dominated by seeds, whereas mockingbirds and phoebes consume copious amounts of invertebrates. Unfortunately the bird collections in this study were essentially restricted to site HC84, minimizing our overall sample size. At site HC84, perchlorate concentrations in kidneys of insectivores (phoebes and mocking birds; 37.3 ± 16.4 ppm) did not differ from granivorous birds (cardinals and sparrows; 26.9 ± 4.4 ppm; $P = 0.5794$). However, the trend toward higher concentrations in insectivorous birds is evident.

Results from small mammals and birds in this study demonstrate that wildlife receptors are being exposed to perchlorate, that exposure may differ among species as a function of their diet, and that exposure varies across sites, possibly in a dose-dependent manner.

5.5.1.2 Thyroid Hormones in Native Small Mammals and Birds

5.5.1.2.1 Introduction

Perchlorate exerts its primary effect on animals by inhibiting uptake of iodide into the thyroid gland (Stanbury, 1952). This inhibition of iodide uptake in turn inhibits the production of thyroid hormones that are subsequently sent into the peripheral blood (Wolff, 1998). Therefore, analyzing the concentration of thyroid hormones in blood (specifically plasma) samples from native animals is one effects measurement indicative of exposure to perchlorate. We hypothesized that small mammals exposed to perchlorate would exhibit alterations (decreases) in thyroid hormone concentrations.

5.5.1.2.2 Methodology

Hormone analysis was not conducted in field captured mice and rats. Rodents captured in the field were collected with snap-traps to minimize or eliminate depuration of perchlorate from the body. This trapping method precludes collection of plasma. Therefore, in order to estimate the effects of perchlorate on native rodents, laboratory studies were conducted on deer mice (*Peromyscus maniculatus*) and prairie voles (*Microtus ochrogaster*).

Thirty nine adult male deer mice were dosed with perchlorate in food and drinking water for 44 days. Concentration of perchlorate was 5.3 ppm in food and 2.2 ppm in water, with 0 ppm controls. Mice were terminated after 44 days and plasma samples collected and stored frozen until analysis of thyroid hormones.

One hundred and twenty adult prairie voles were dosed with perchlorate in food and drinking water for 21 to 63 days. Concentration of perchlorate was 2.1 ppm in food and 0.99 ppm in water, with 0 ppm controls. At the end of each exposure period, voles were terminated and plasma and tissue samples collected for hormone, residue, and histological analyses. Plasma and tissues for residues were stored frozen until analysis. Tissues collected for histological analysis were stored in fixative until sectioned and examined under a light microscope.

Thyroid hormone analysis was conducted using Diagnostic Products Coat-A-Count Total kits (TKT31 and TKT45 respectively; Diagnostic Product Corporation, Los Angeles, CA). If plasma volume was insufficient for both assays, T_3 was run first, followed by T_4. When there was not a sufficient volume of plasma to run T_3 analysis (250 µL), only T_4 analysis (70 µL) was performed. The kits detected both free and protein-bound hormone (T_3 and T_4). Total T_3 and T_4 concentrations were calculated from the relative binding of a known amount of radio-labeled hormone to the antibodies on the tube and the binding of the unknown amount of hormone in the sample. Radioimmunoassays were conducted using standard procedures described in the manufacturers' instructions. RIA test kits used for hormone analysis were optimized for detection of T_3 and T_4 concentrations in the rodent plasma.

Thyroids from all voles were fixed in a triple aldehyde electron microscopy fixative (Tandler, 1990) for 10 minutes and preserved in formalin for histological processing. A Tissue-Tek V.I.P. 2000 Processor (Miles Scientific, Naperville, IL) was used to process tissues which were then embedded in paraffin. Paraffin blocks containing thyroid tissue were cut into sections at 7 µm with a microtome (Cut 4055, Olympus America Inc., Melville, NY). Ribbons of tissue sections were then mounted on glass microscope slides and stained following a basic hematoxylin and Eosin Y staining technique (Hinton, 1990).

Slides were examined with an Olympus BX52 compound light microscope and photographed with an attached Olympus DP1-L digital camera (Olympus Optical Co., Ltd, Tokyo, Japan) at 40X magnification. A section from the beginning, the middle, and the end of each specimen was selected from slides containing thyroid tissue to assess

overall histological status of each thyroid. Thyroid tissue from both lobes (right and left) of the thyroid was examined. Thyroid tissue was examined and blindly scored for the presence and severity of hyperplasia, hypertrophy, and colloid depletion based on guidelines established by the Pathology Working Group (Mann, 2000). Each trait was given a score of zero (0) for no indication of damage, 1 for slight damage (minimal), or 2 for severely damaged (severe) (Mann, 2000).

5.5.1.2.3 Data

Deer mice. Concentrations of thyroid hormones varied among treatment groups. Mean concentration of T_4 was approximately 50% greater in mice dosed via food than control mice ($P = 0.0108$). However, mice dosed via water showed similar T_4 concentrations as control mice. T_3 concentrations did not differ among treatment groups ($P > 0.05$).

Prairie voles. Concentrations of perchlorate in liver and kidney were determined in 150 adult voles. Only six voles showed detectable concentrations in liver samples, ranging from 0.183 ppm to 1.9 ppm. Likewise, perchlorate was detected in only five kidney samples, ranging from 1.0 ppm to 5.6 ppm.

Thyroid hormone concentrations varied little among treatment groups (**Table 5-58**). Concentrations of T_3 did not differ among treatments ($P = 0.7133$); likewise, T_4 concentrations were similar among treatments ($P = 0.3700$).

Table 5-58
Mean (± SE) Concentrations for T_3 and T_4 Hormones in Adult Prairie Voles Dosed with Perchlorate in Food and Water, and Controls

	n	Thyroid Hormone Concentration T_3 (µg/dL)	n	Thyroid Hormone Concentration T_4 (µg/dL)
Control	29	104.4 ± 8.9	27	2.3 ± 0.2
Food	42	108.9 ± 5.0	40	2.5 ± 0.2
Water	43	102.0 ± 5.7	35	2.6 ± 0.2

Histological examination of the thyroid gland of exposed and non-exposed voles was used to augment hormone data, as histological lesions are often considered a less transient effect of exposure to perchlorate. Thyroid glands of 102 adult voles were examined for follicular cell hyperplasia/hypertrophy and follicular cell colloid depletion (**Table 5-59**).

Table 5-59
Occurrence of Follicular Cell Hyperplasia/Hypertrophy and Follicular Cell Colloid Depletion (Incidence/Number of Thyroid Glands Examined) in Prairie Voles Dosed with Perchlorate in Water, Food, and Controls

Endpoint		Male			Female		
		Control	Water	Food	Control	Water	Food
Hyperplasia/ Hypertrophy	Minimal	0/15	2/19	1/19	0/18	2/15	0/16
	Severe	0/15	0/19	0/19	0/18	0/15	0/16
	Total	0/15	2/19	0/19	0/18	2/15	0/16
Colloid depletion	Minimal	4/15	8/19	4/19	3/18	8/15	2/16
	Severe	0/15	0/19	0/19	0/18	0/15	0/16
	Total	4/15	8/19	4/19	3/18	8/15	2/16

(See methods for description of Scores (minimal and severe)

Hyperplasia of the follicular cells was observed concurrent with hypertrophy. These alterations were observed only in the voles dosed with perchlorate, but at a low frequency (7.9% of the dosed animals). Conversely, colloid depletion of follicular cells was observed in all groups, including controls, and at a higher rate than for hyperplasia and hypertrophy (**Table 5-59**). Colloid depletion was observed at two to four times greater incidence in voles exposed to perchlorate in water than food exposed or control voles ($X^2 = 8.66$, d.f .= 2, $P < 0.025$).

5.5.1.2.4 Discussion

Results from native rodents indicate that exposure to perchlorate resulted in alterations in either thyroid hormones or thyroid structural pathology. However, differences did exist between species, with deer mice exhibiting altered hormone concentrations, which was not observed in voles. But in voles, thyroid histology did show alterations even in the absence of hormone alterations. Dosing designs differed between the two species, with deer mice exposed to higher concentrations of perchlorate for a consistently longer period of time. This higher level of exposure overall could account for the observed differences in thyroid hormones that was not observed in voles. Deer mice, although not showing hormone alterations, do exhibit classic signs of perchlorate effects with an increased incidence of colloid depletion in follicular cells. This result could indicate that although perchlorate is acting on the target cells in the thyroid, effects on actual hormone production have not been reached and/or animals are still operating on reserve hormones stored in the thyroid.

In comparison to field captured rodents, the laboratory dosed animals appear to show much lower exposure. This is evidenced by the low, and rarely observed, concentrations of perchlorate in either liver or kidney samples from voles. It is difficult to estimate the daily exposure of perchlorate to wild rodents, or the duration and frequency of exposure in the field. However, given the known doses in the laboratory studies and subsequent tissue burdens, it is safe to assume that rodents captured in the field are easily exposed to equal or greater amounts of perchlorate, as they routinely show parts per million levels of perchlorate. Given this observation and the laboratory results (Thuett et al., 2002), we

estimate that the field exposed animals would likely show alterations in hormone concentrations and/or thyroid histology.

The exact ramification of these lesions is difficult to determine. Recent work in our laboratories show that deer mice and/or voles exposed to perchlorate at environmentally relevant doses show alterations in metabolic activity and/or reproduction. Metabolic activity increased, and then began decreasing over a 30 to 40 day exposure period in deer mice exposed to perchlorate in water and food. These results suggest that continued exposure to perchlorate at environmentally relevant doses could alter metabolic function, leading to alterations in energy regulation and thermogenesis. These alterations in normal homeostasis may have significant effects on animals during periods of growth, reproduction, and varying climate extremes.

5.5.1.3 Pharmacokinetic/Population Modeling of Small Mammals and Birds

5.5.1.3.1 Introduction

A mathematical model was developed and computer simulations were conducted of the effects of perchlorate on populations of small mammals in the Bosque and Leon Rivers Watersheds in Central Texas. Compartments in the small mammal model include blood plasma, liver, kidneys, gut wall, gut contents, and the thyroid gland. The primary exposure pathway in small mammals is ingestion of food and water contaminated with perchlorate. Random variables include perchlorate concentrations in food and water. Using a time-step of one hour, the model currently runs simulations of one year (8760 hours) for each individual animal. Several individuals are simulated to obtain mean and 95% confidence limits on perchlorate concentrations in each compartment.

A second mathematical model was developed and computer simulations were conducted of the effects of perchlorate on populations of Bobwhite quail in the Bosque and Leon Rivers Watersheds in Central Texas. The modeling objectives were to (1) predict the uptake and distribution of perchlorate in avian body tissues, (2) predict maternal transfer of perchlorate to eggs, and (3) predict the effects of ammonium perchlorate on the thyroid hormone system. The model contains random variables for the concentrations of perchlorate in drinking water and in other dietary components, as well as the number of eggs produced by female quail.

5.5.1.3.2 Methodology

5.5.1.3.2.1 Model Testing

The modeling approach was to develop and apply mathematical models, either through modification of off-the-shelf models or de novo development, as appropriate, and parameterize the models using data from laboratory studies above.

Software tests are intended to challenge the application software and other parts of the overall system functionally and structurally. Functional testing demonstrates only that the system outputs appear to be correct. It does not allow an assessment of whether the software is actually performing according to specifications and requirements. A complete

functional test of every combination of inputs may not be feasible except for very small programs. Functional testing is essentially a subset of structural testing.

Structural testing is designed to exercise all modules and branches of the software and their interrelationships with the hardware and peripheral devices. Structural testing is performed to ensure that all relevant functions in the software perform as intended.

Each of the testing types described below was conducted. Performing only one type of test will not prove that the system is working properly.

Normal testing includes cases that test the functional and structural integrity of the computerized system. The input data for these test cases all fall within the range the user considers to be normal. Performing enough test cases can give a reasonable level of confidence that the system behaves as intended under normal conditions.

Boundary testing is performed using values that force the system to discern whether the input is valid or invalid, or to make a decision as to which branch of the program to execute. Boundary test values are set at the edges (i.e., slightly below and above) of valid input ranges. Boundary testing does not mean making the computerized system "crash" or involuntarily stop.

Special Case testing, also known as "exceptional case testing", documents the system's reactions to specific types of data or lack of data and is intended to ensure that the computerized system does not accept unsuitable data. These tests should be designed to document what happens when values that are not included in the ranges defined in the specifications are entered. Use of test cases with no data entry in a field will assist in establishing software system defaults.

Parallel testing is one of the most common types of tests performed by software developers. Parallel testing is performed by running two systems in parallel and comparing the outputs (e.g., two software application versions or software compared with a manual procedure). The comparison of the outputs from the same software release on different systems or different releases on the same system is part of parallel testing plans. Parallel testing can be a valuable tool when it is used in conjunction with other testing types for validation, or to train personnel to use a new computer system.

The model was tested with each of the structural tests described above and passed each test.

5.5.1.3.2.2 Model Development

A mathematical model was developed and computer simulations were conducted of the effects of perchlorate on populations of small mammals and bird species in the Bosque and Leon watersheds near Waco, Texas. The model consists of two sub-models: (1) a physiologically based toxicokinetics (PBTK) model of the uptake and distribution of perchlorate in mammal and bird body tissues for each individual in the population, including maternal transfer of perchlorate from mother to egg, and (2) a model of the

thyroid hormone secretion as affected by the perchlorate concentration at the thyroid. The model is stochastic in that it contains random variables for the concentrations of perchlorate in drinking water and in other dietary components, as well as the number of eggs produced by female quail. These random variables provide the capability to conduct Monte Carlo simulations.

5.5.1.3.2.3 Model Description

The PBTK model includes compartments for blood plasma, kidney, liver, skin and fat, thyroid, heart, gut contents, gut wall, and egg (**Figure 5-176**).

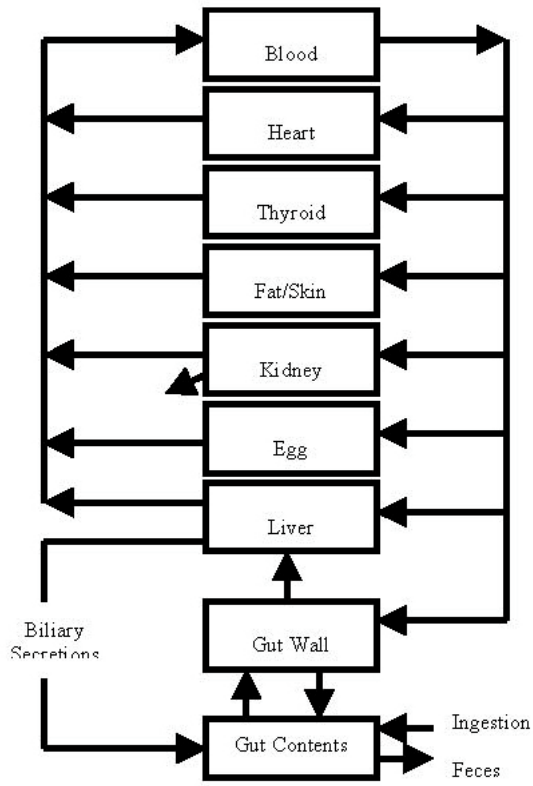

Figure 5-176
Flow Diagram of Ammonium Perchlorate PBTK Model

The organ compartment concentrations of perchlorate in an individual between time *t* and time *t+1* results from the rates of ingestion and elimination of perchlorate in the interval. The dose at time *t+1*, Q_{t+1}, then can be described by the general difference equation (1):

$$Q_{t+1} = Q_t - \left(\frac{F \bullet (Q_t / v)}{P} \right) + \left(\frac{F \bullet (Qplasma_t / v)}{P} \right) \qquad (1)$$

where,

Q_t = perchlorate compartment burden at time *t*, mg • L $^{-1}$

Q_{t+1} = perchlorate compartment burden at time $t+1$, mg • L $^{-1}$
F = Blood flow rate into the compartment, L/hour
v = Volume of compartment, L
P= Compartmental partitioning coefficient for perchlorate
$Qplasma_t$ = AP plasma burned at time t, mg • L $^{-1}$

Equation 1 holds for the heart, thyroid, fat, and egg compartments. The plasma compartment burden is described by the difference equation (2):

$$Qplasma_{t+1} = Qplasma_t - \sum\left(\frac{F \bullet (Qplasma_t / v)}{P}\right) + \sum\left(\frac{F \bullet (Q_t / v)}{P}\right) \qquad (2)$$

where,
$Qplasma_t$ = perchlorate plasma burden at time t, mg • L $^{-1}$
$Qplasma_{t+1}$ = perchlorate plasma burden at time $t+1$, mg • L $^{-1}$
Q_t = individual perchlorate compartment burden at time t, mg • L $^{-1}$
F = Blood flow rate into the individual compartments, L/hour
v = Volume of individual compartments, L
P= Individual compartmental partitioning coefficients for perchlorate

The kidney burden may be described as (3):

$$Qkidney_{t+1} = Qkidney_t - \left(\frac{F \bullet (Qkidney_t / v)}{P}\right) - U \bullet Qkidney_t \ldots + \left(\frac{F \bullet (Qplasma_t / v)}{P}\right) \qquad (3)$$

where,
$Qkidney_t$ = perchlorate kidney burden at time t, mg • L $^{-1}$
$Qkidney_{t+1}$ = perchlorate kidney burden at time $t+1$, mg • L $^{-1}$
F = Blood flow rate into the kidney, L/hour
v = Volume of kidney, L
P = Compartmental partitioning coefficient for perchlorate,
U = Coefficient for the loss of perchlorate through urinary secretion
$Qplasma_t$ = perchlorate plasma burned at time t, mg • L $^{-1}$

The perchlorate liver burden may be determined by (4):

$$Qliver_{t+1} = Qliver_t - \left(\frac{F \bullet (Qliver_t / v)}{P}\right) - B \bullet Qliver_t \ldots + \left(\frac{FG \bullet (Qgutwall_t / v)}{P}\right) + \left(\frac{F \bullet (Qplasma_t / v)}{P}\right) \quad (4)$$

where,

$Qliver_t$ = perchlorate liver burden at time t, mg • L $^{-1}$
$Qliver_{t+1}$ = perchlorate liver burden at time $t+1$, mg • L $^{-1}$
F = Blood flow rate into the liver, L/hour
v = Volume of liver, L
P = Compartmental partitioning coefficient for perchlorate,
B = Coefficient for the loss of perchlorate through biliary secretion
FG = Blood flow rate for the gut wall, L/hour
$Qplasma_t$ = perchlorate plasma burned at time t, mg • L $^{-1}$
$Qgutwall_t$ = AP gut wall burden at time t, mg • L $^{-1}$

The perchlorate gut wall burden at time t can be described as (5):

$$Qgutwall_{t+1} = Qgutwall_t - \left(\frac{FG \bullet (Qgutwall_t / v)}{P}\right) - D \bullet Qgutwall_t \ldots + \alpha \bullet Qgutcontent_t + \left(\frac{FG \bullet (Qplasma_t / v)}{P}\right) \quad (5)$$

where,

$Qgutwall_t$ = perchlorate gut wall burden at time t, mg • L $^{-1}$
$Qgutwall_{t+1}$ = perchlorate gut wall burden at time $t+1$, mg • L $^{-1}$
F = Blood flow rate of the gut wall, L/hour
v = Volume of gut wall, L
P = Compartmental partitioning coefficient for perchlorate
D = Coefficient for secretion of perchlorate out of the gut wall
FG = Blood flow rate for the gut wall, L/hour
$Qplasma_t$ = perchlorate plasma burned at time t, mg • L $^{-1}$
$Qgucontent_t$ = perchlorate gut content burden at time t, mg • L $^{-1}$
α = absorption coefficient for perchlorate contaminated gut contents

The burden of perchlorate in the gut contents may be determined as follows (6):

$$Qgutcontent_{t+1} = Qgutcontent_t - \beta \bullet Qgutcontent_t - \alpha \bullet Qgutcontent_t \ldots + B \bullet Qliver_t + D \bullet Qgutwall_t + If_t + Iw_t$$

where,

$Qgutcontent_t$ = perchlorate gut content burden at time t, mg • L $^{-1}$
$Qgutcontent_{t+1}$ = perchlorate gut contnet burden at time $t+1$, mg • L $^{-1}$
D = Coefficient for secretion of perchlorate out of the gut wall
B = Coefficient for the loss of perchlorate through biliary secretion
α = Absorption coefficient for perchlorate contaminated gut contents
β = Coefficient for the loss of perchlorate through defecation
If_t = Ingestion rate of perchlorate in food items (mg•h $^{-1}$)
Iw_t = Ingestion of perchlorate in water (mg•h $^{-1}$)

The ingestion rate of perchlorate in food, If_t, (mg•h $^{-1}$) may be written as (7):

$$If_t = \sum_{i=1}^{m} p_i \times Cf_i \times \upsilon_i$$

where

p_i = proportion of total diet contributed by item i at time t
Cf_i = consumption rate of food item i , mg•h $^{-1}$
v_i = perchlorate concentration in food item i , mg•kg $^{-1}$

Similarly, for ingestion of perchlorate in water, Iw_t (mg•h $^{-1}$) is (8):

$$Iw_t = Cw \times \upsilon$$

where

Cw_i = consumption rate of water, L•h $^{-1}$
v_i = perchlorate concentration in food item i , mg•L $^{-1}$

Parameters for the PBTK model were obtained from various sources. Quail organ blood flow rates, weights, and volumes were scaled to represent steady-state quail values (Strukie, 1986). Blood flow rates, weights, and volumes for the egg compartment were also scaled to represent reproductive processes in quail (Freeman, 1984).

The thyroid hormone sub-model was adapted from a model developed for the human thyroid system by DiStefano et al. (1975), DiStefano and Fisher (1976), Saratchandran et al. (1976), and DiStefano and Mori (1977). The number of parameters in the model developed by these authors were reduced or simplified in order to better represent the thyroid hormone system of wildlife species. The model is currently running with

parameter values calibrated to generate output that falls within two percent of the steady-state values as reported by Saratchandran et al. (1976). **Figure 5-177** depicts the flow diagram for the thyroid hormone system sub-model.

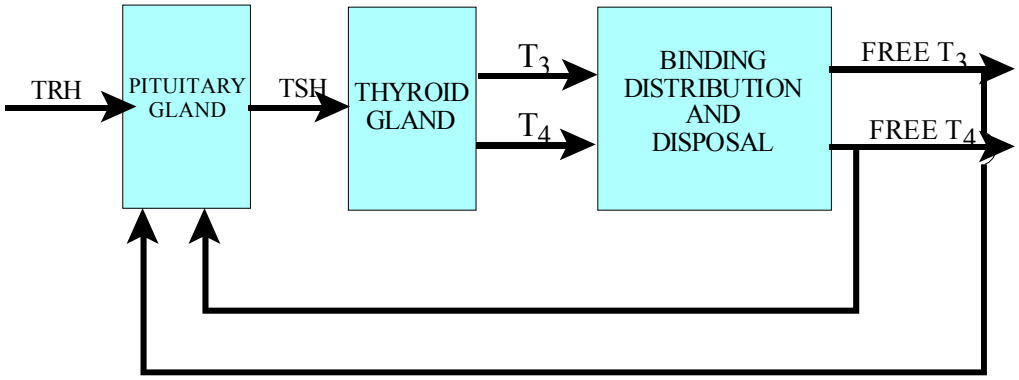

Figure 5-177
Flow Diagram of the Thyroid Hormone System Sub-Model

The modeling objectives were to (1) predict the uptake and distribution of perchlorate in mammal and avian body tissues, (2) predict maternal transfer of perchlorate to eggs, and (3) predict the effects of ammonium perchlorate on the thyroid hormone system (**Figure 5-178**). Internal dose and maternal transfer to egg were related to concentrations of perchlorate in the components of the diets of the avian species feeding in different drainages of the Bosque and Leon watersheds. The model was used to predict the dynamics of perchlorate uptake and distribution, based on stochastic feeding rates, elimination rates, and the effect of perchlorate on thyroid function. Several individuals were simulated to develop a mean distribution of perchlorate in the various tissues at the population level.

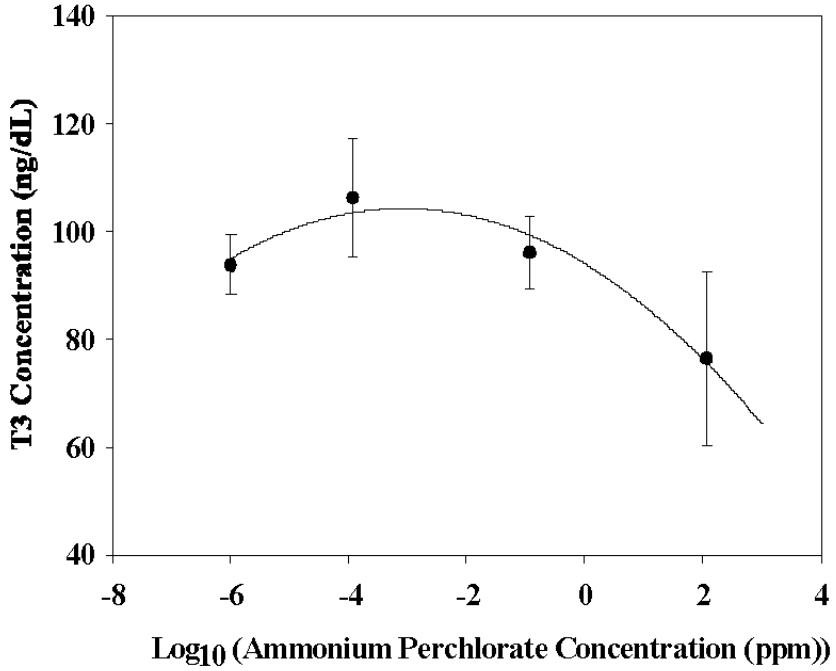

Figure 5-178
Relationship Between T_3 Concentration and Perchlorate Concentration

5.5.1.3.3 Data

5.5.1.3.3.1 Small Mammal Model

Simulations were run for small mammal populations feeding in two drainages of Bosque and Leon River Watersheds representing average and maximum exposures (Station Creek at Highway 107 (T23) and S Creek at Highway 317 (T15), respectively). The highest perchlorate concentrations (range = 67 - 540 ppb) were consistently observed at S Creek at Highway 317 (T15). For comparison purposes, we assumed that small mammals obtained their food and drinking water solely from a single area. Perchlorate concentrations for each area are presented in **Table 5-60**.

5.5.1.3.3.2 Station Creek at Highway 107 (T23)

Simulation output included graphs of perchlorate concentrations in small mammal organs and tissues (**Figure 5-179** and **Figure 5-180**), the secretion rates of T_3 and T_4 (**Figure 5-181**), and the concentration of T_3 and T_4 in plasma and slow and fast pools (**Figure 5-182**).

Table 5-60
Perchlorate Concentrations in Food Items and Drinking Water Used in Model Simulations

	Sampling Areas			
	Station Creek at Highway 107 (T23)		S Creek at Highway 317 (T15)	
	Mean	**S.D.[a]**	**Mean**	**S.D.[a]**
Food (ppm)				
Insects[b]	0.0015	0.0023	1.534	0.725
Plants[c]	30.0	48.24	150.0	26.0
Water (ppb)	24.78	34.20	270.48	157.56

[a] Standard deviation estimate based sample data
[b] LHAAP data
[c] Simulated data using plant model

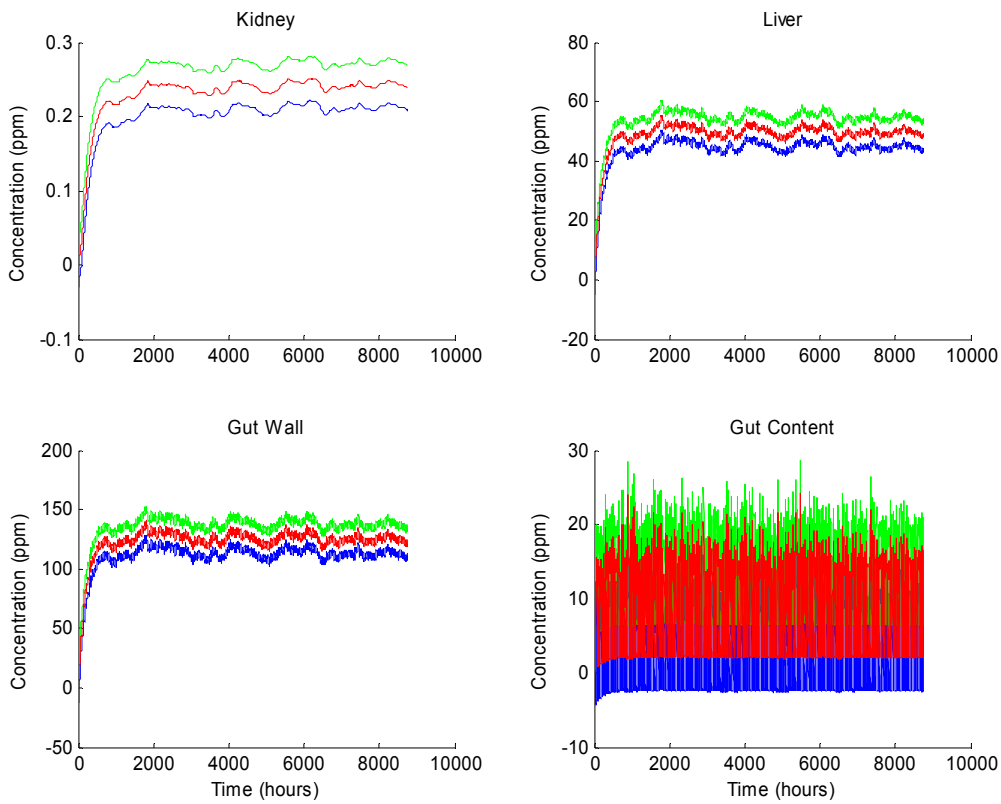

Figure 5-179
Mean Simulated Perchlorate Concentrations (Red) in Small Mammal Kidney, Liver, Gut Wall, and Gut Contents from Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

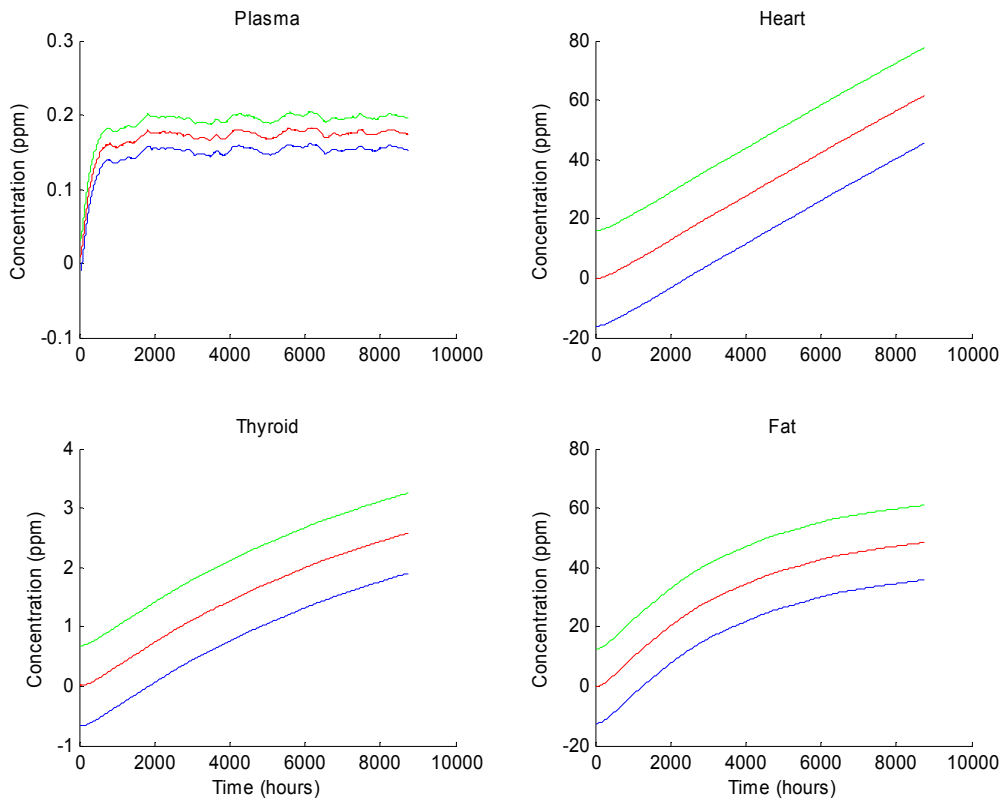

Figure 5-180
Mean Simulated Perchlorate Concentrations (Red) in Plasma, Heart, Thyroid, and Fat Compartments in Small Mammals from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

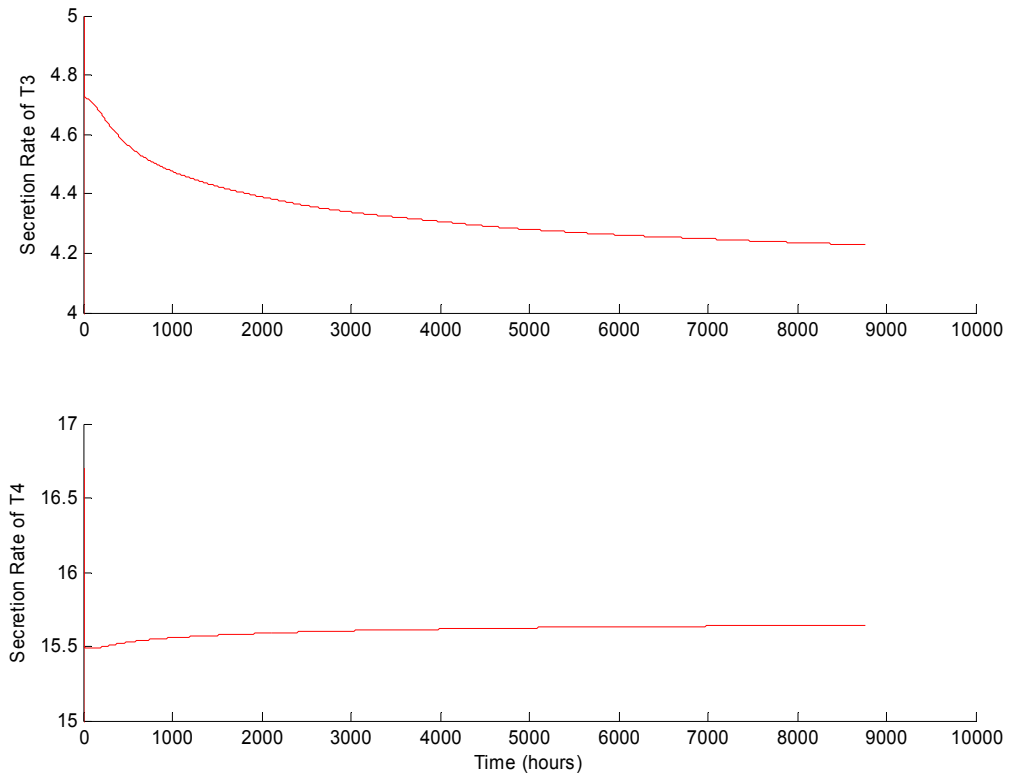

Figure 5-181
Simulated Secretion Rates of T_3 and T_4 Hormones in Small Mammals from Station Creek Drainage

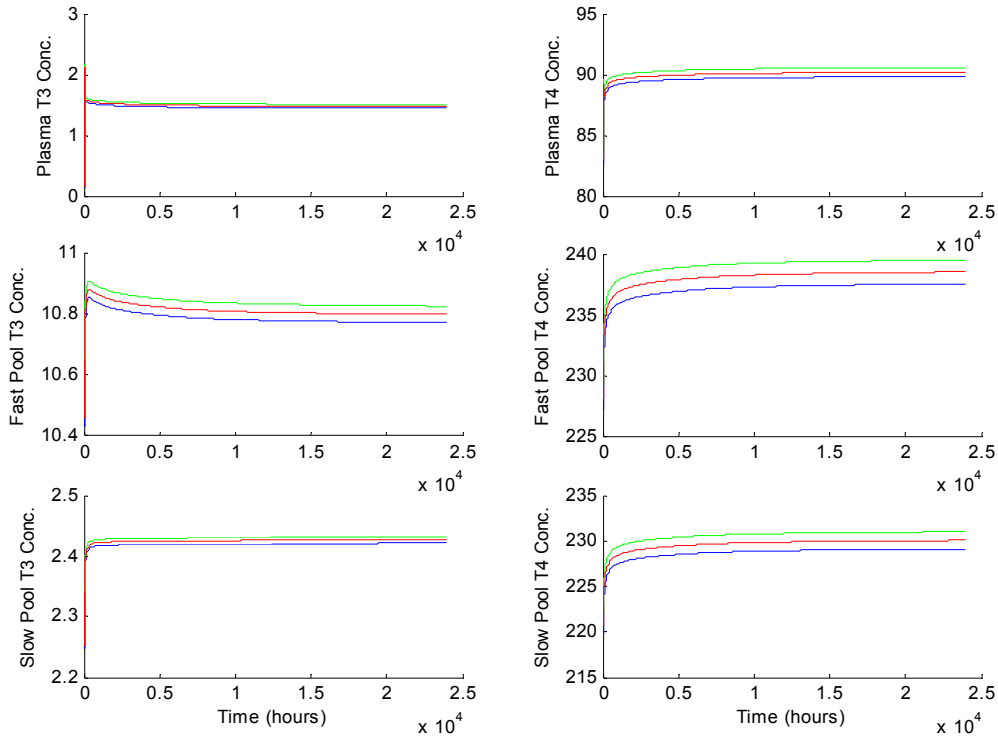

Figure 5-182
Mean Simulated Output of T_3 and T_4 Hormones (Red) in Slow and Fast Pools in Small Mammals in Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

5.5.1.3.3.3 S Creek at Highway 317 (T15)

Simulation output included graphs of perchlorate concentrations in small mammal organs and tissues (**Figure 5-183** and **Figure 5-184**), the secretion rates of T_3 and T_4 (**Figure 5-185**), and the concentration of T_3 and T_4 in plasma and slow and fast pools (**Figure 5-186**).

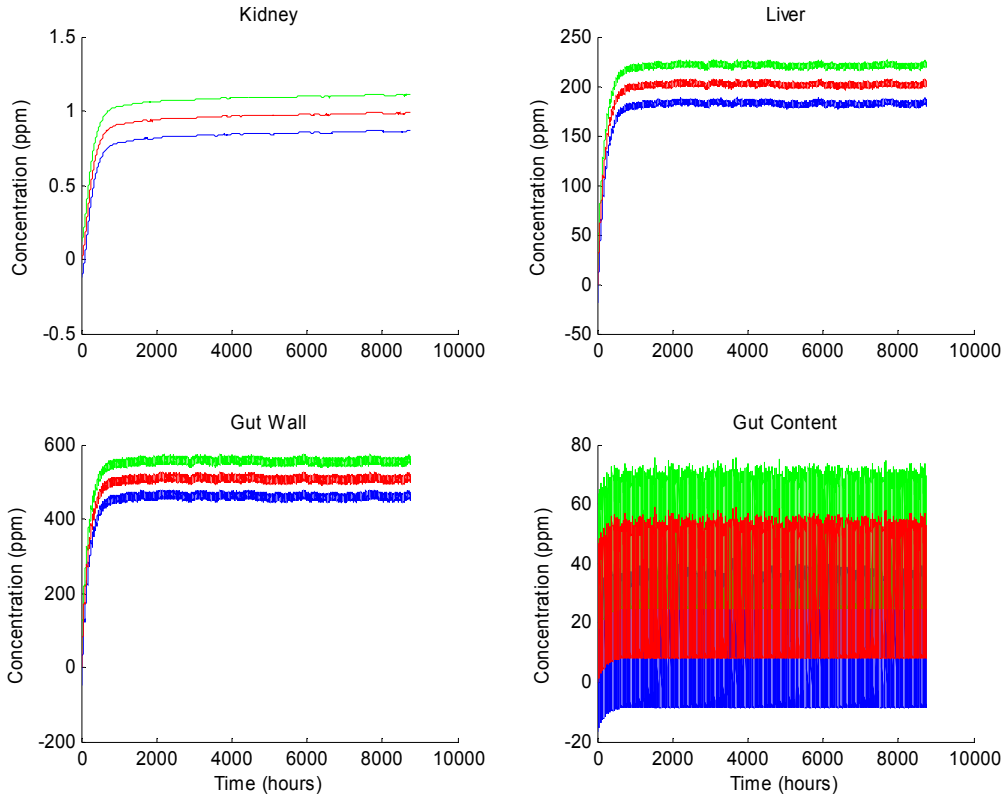

Figure 5-183
Mean Simulated Perchlorate Concentrations (Red) in Small Mammal Kidney, Liver, Gut Wall, and Gut Content Compartments in Small Mammals from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

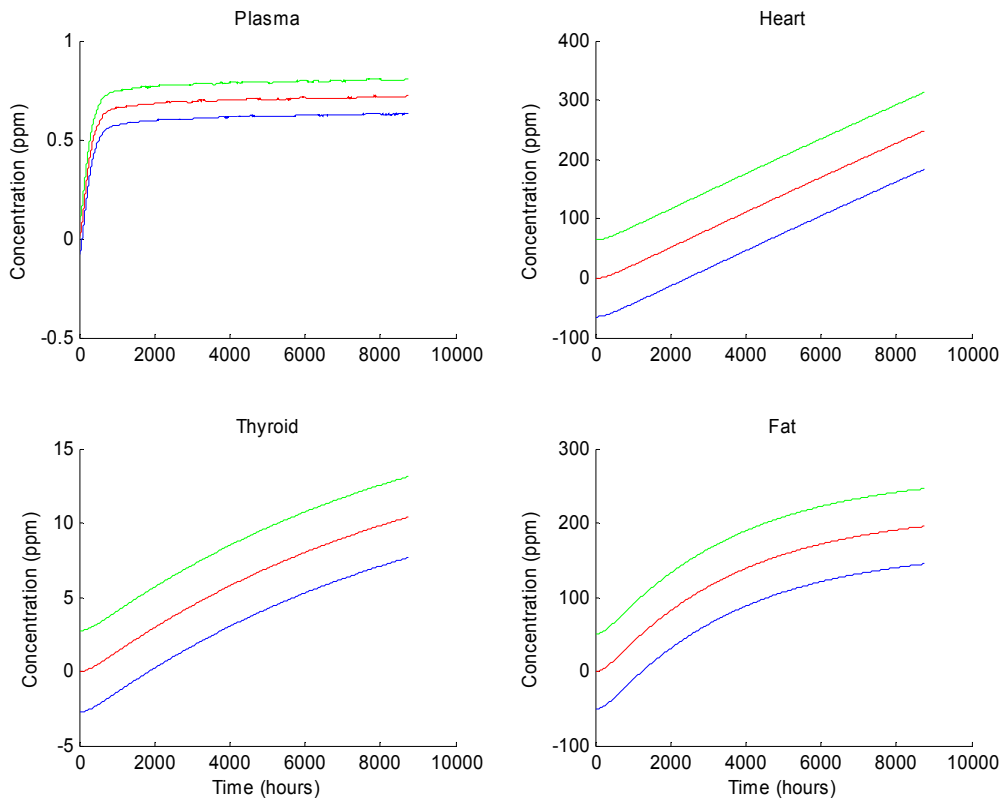

Figure 5-184
Mean Simulated Perchlorate Concentrations (Red) in Plasma, Heart, Thyroid, and Fat Compartments in Small Mammals from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

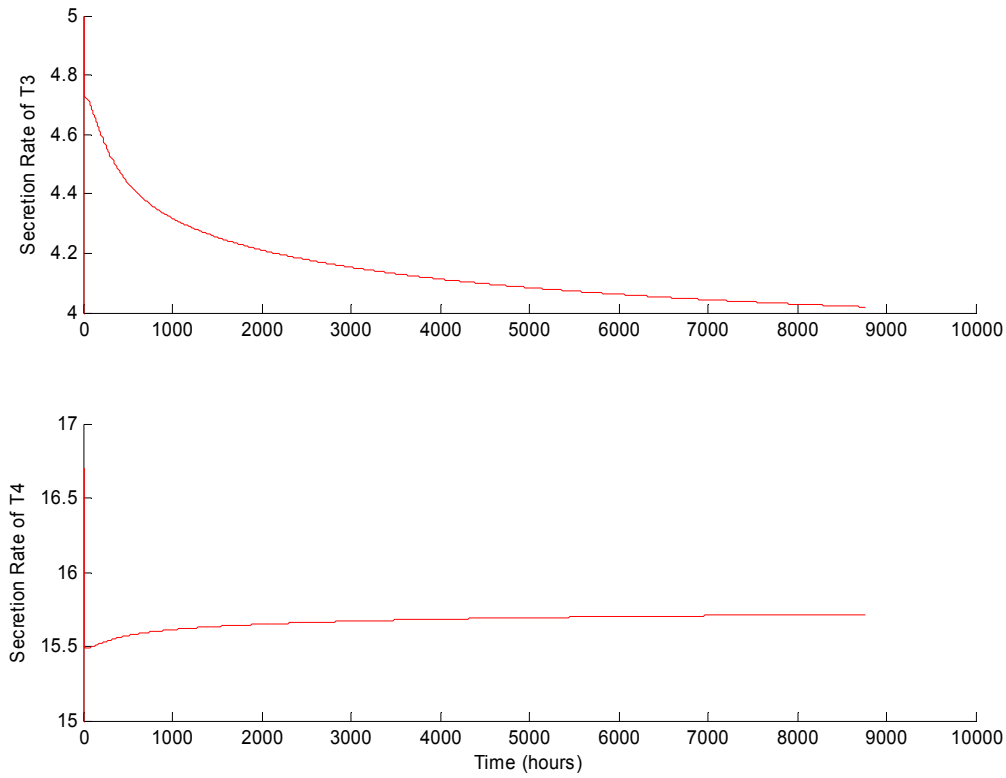

Figure 5-185
Simulated Secretion Rates of T_3 and T_4 Hormones in Small Mammals from S Creek Drainage

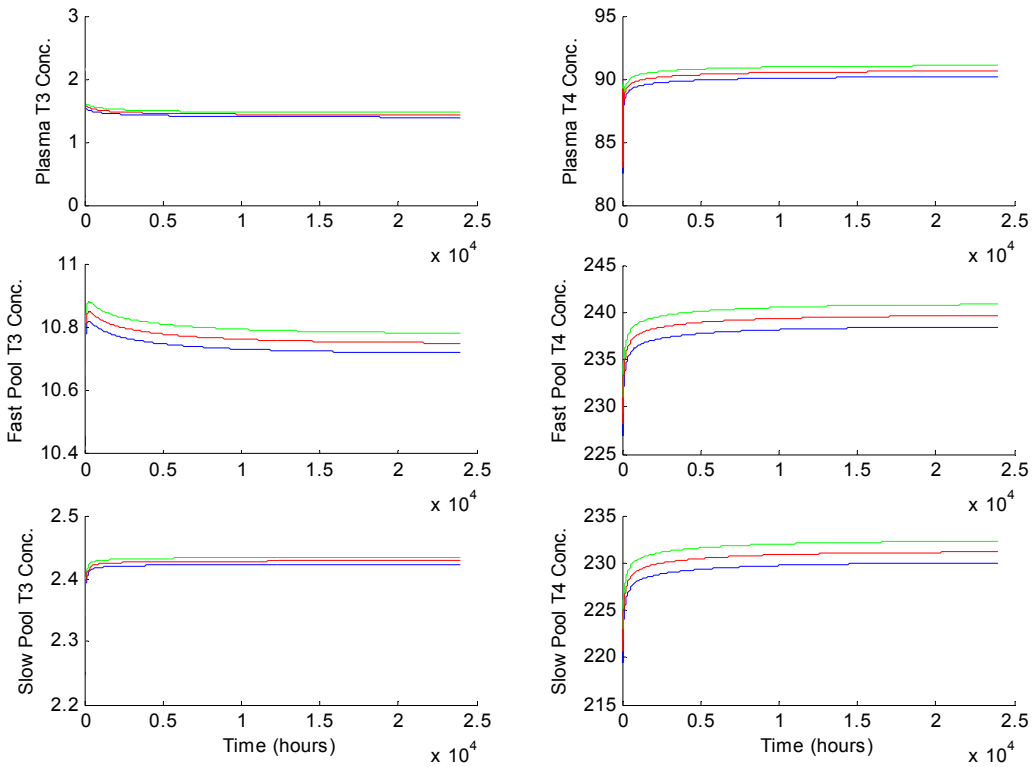

Figure 5-186
Mean Simulated Output of T_3 and T_4 Hormones (Red) in Slow and Fast Pools in Small Mammals in S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

5.5.1.3.3.4 Bird Model

Simulations were run for quail populations feeding in two drainages of the Bosque and Leon River Watersheds (Station Creek at Highway 107 (T23) and S Creek at Highway 317 (T15)). Each quail population consisted of 100 individuals with a mean body weight of 181g. For comparison purposes, we assumed that the quail obtained their food and drinking water solely from a single area. Internal dose and maternal transfer to egg were related to concentrations of perchlorate in the components of the diets of the avian species feeding in the two different drainages. The model was used to predict the dynamics of perchlorate uptake and distribution, based on stochastic feeding rates, elimination rates, and the effect of perchlorate on thyroid function. The 100 individual birds were simulated to develop a mean distribution of perchlorate in the various tissues. Food item perchlorate concentrations for each area are presented in **Table 5-60**.

5.5.1.3.3.5 Station Creek at Highway 107 (T23)

Simulation output for Station Creek included predicted perchlorate concentrations in quail organs and tissues (**Figure 5-187** and **Figure 5-188**), quail eggs (**Figure 5-189**), the concentrations of TSH in the plasma (**Figure 5-190**), the secretion rates of T_3 and T_4

(**Figure 5-191**), and the concentration of T_3 and T_4 in plasma, and the slow and fast pool compartments (**Figure 5-192**). Examples of slow and fast pool compartments include muscle and liver, respectively. The designations slow and fast pool, were derived from physiological blood flow to various organs. Those organs that were poorly perfused and demonstrated slow blood-flow rates were grouped as slow pool, while those that were richly perfused and demonstrated fast blood-flow rates were grouped as fast pool (Saratchandran et al., 1976). The majority of the simulation output is represented by three different colors. Where applicable, the red trend line represents the mean value of the variable under examination. The green, and blue lines represent the upper-95% and the lower-95% confidence interval for the simulation mean, respectively.

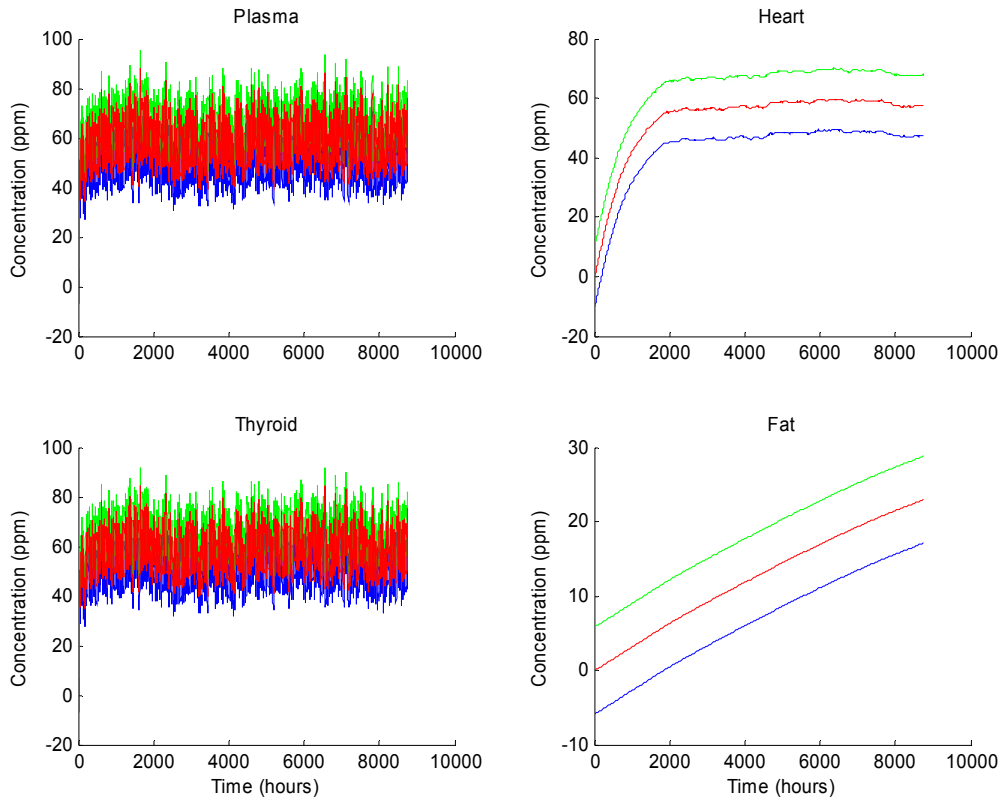

Figure 5-187
Mean Simulated Perchlorate Concentrations (Red) in Quail Plasma, Heart, Thyroid, and Fat from Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

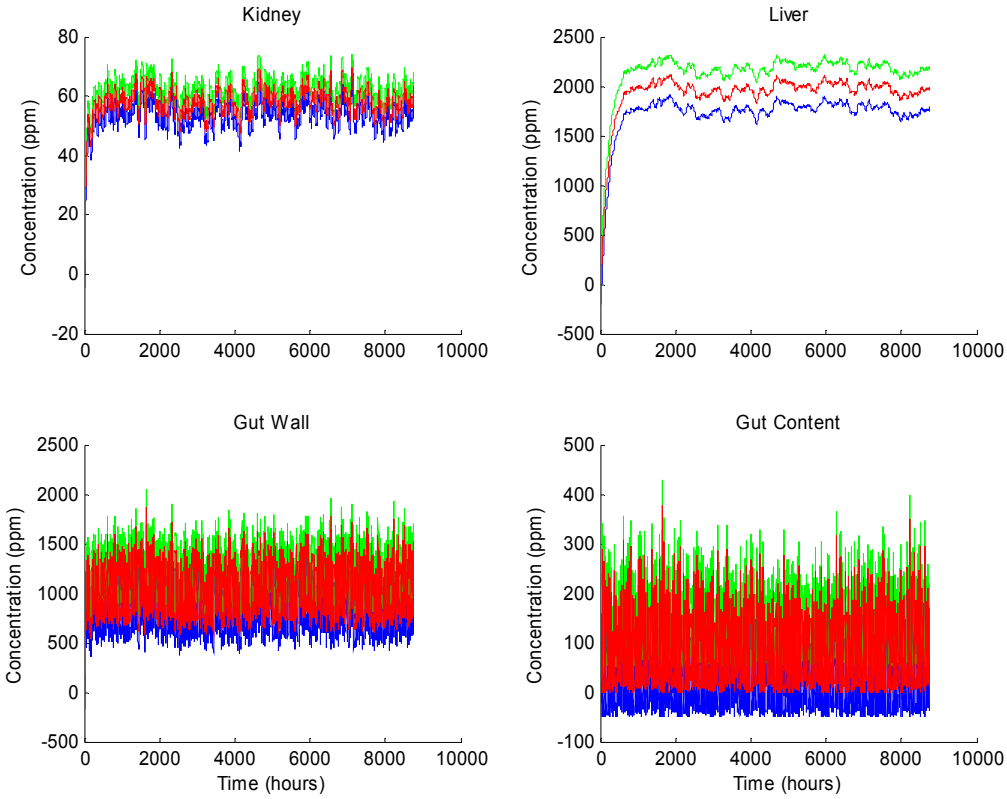

Figure 5-188
Mean Simulated Perchlorate Concentrations (Red) in Quail Kidney, Liver, Gut Wall, and Gut Contents from Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

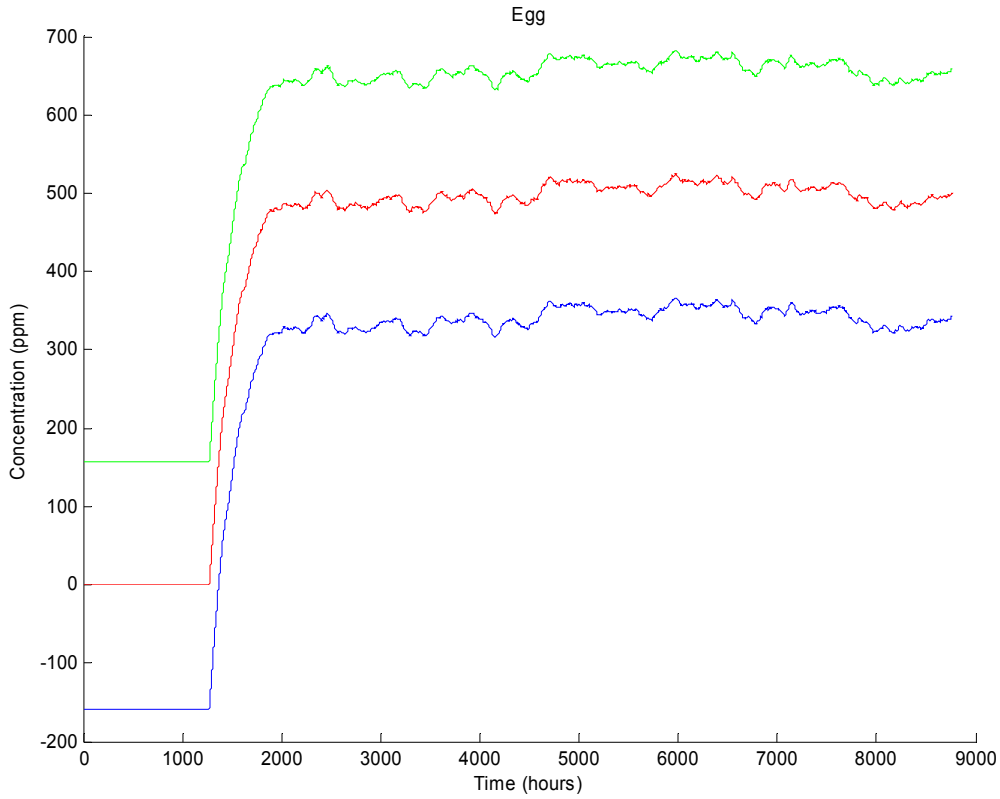

Figure 5-189
Mean Simulated Perchlorate Concentrations (Red) in Quail Eggs from Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

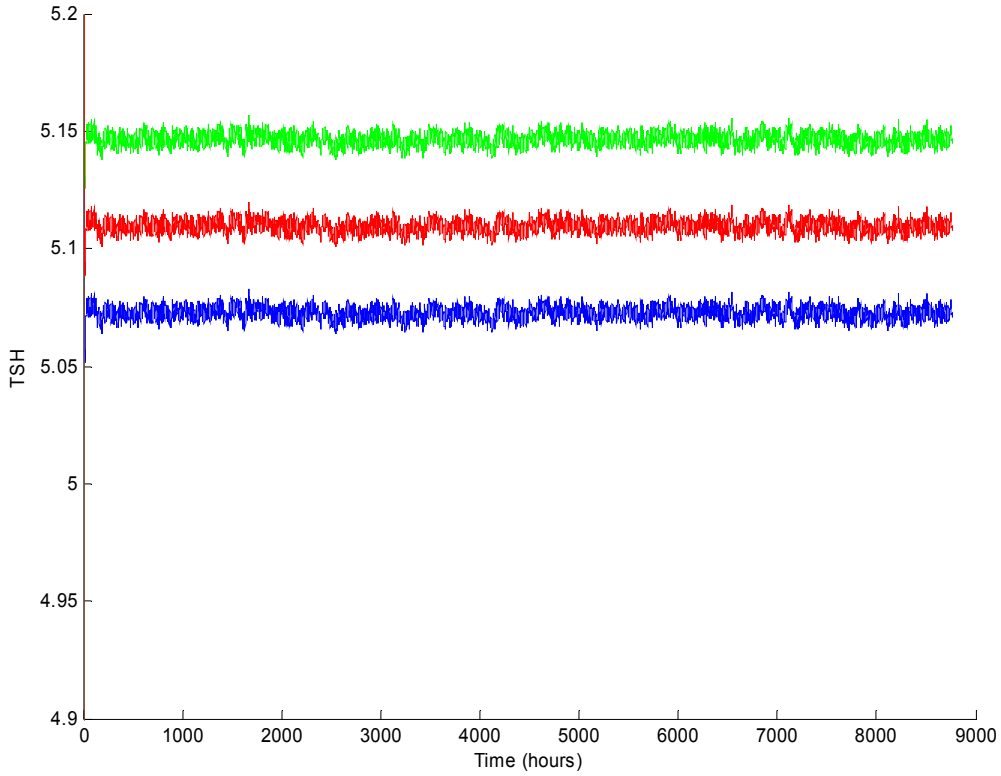

Figure 5-190
Mean Simulated Plasma Concentrations of TSH (Red) for Quail in the Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

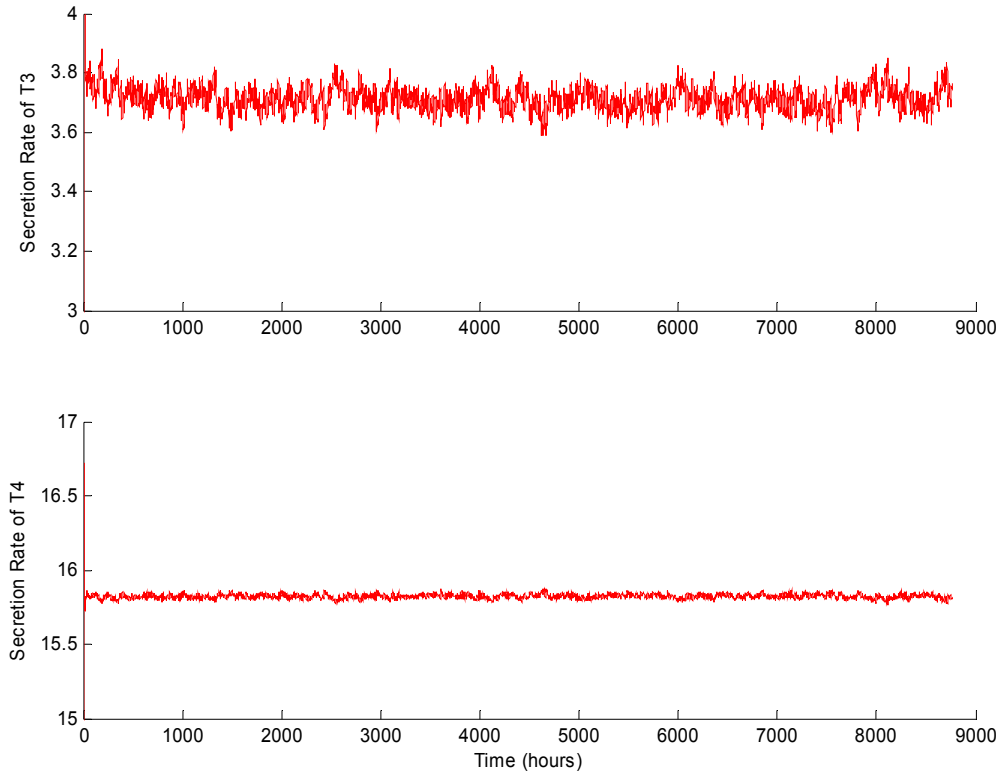

Figure 5-191
Simulated T_3 and T_4 Secretion Rates in Quail from the Station Creek Drainage

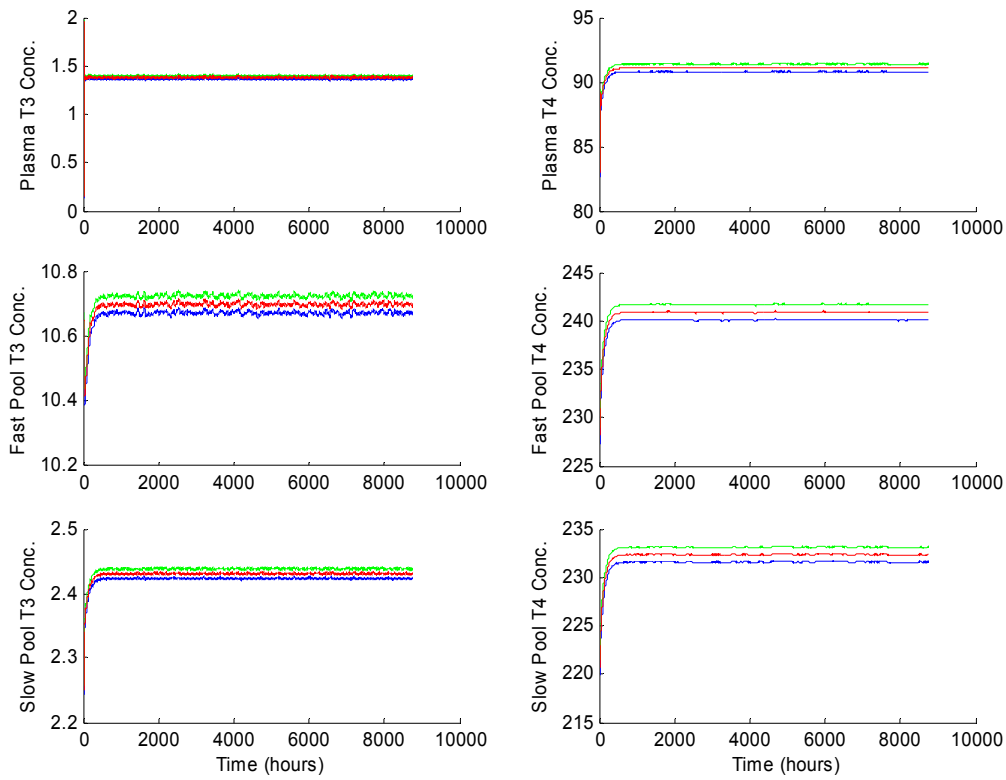

Figure 5-192
Mean Simulated T_3 and T_4 Concentrations (Red) in Quail from the Station Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

5.5.1.3.3.6 S Creek at Highway 317 (T15)

Similarly, simulation output for South Bosque drainage also included predicted perchlorate concentrations in quail organs and tissues (**Figure 5-193** and **Figure 5-194**), quail eggs (**Figure 5-195**), the concentrations of TSH in the plasma (**Figure 5-196**), the secretion rates of T_3 and T_4 (**Figure 5-197**), and the concentration of T_3 and T_4 in plasma and slow and fast pools (**Figure 5-198**).

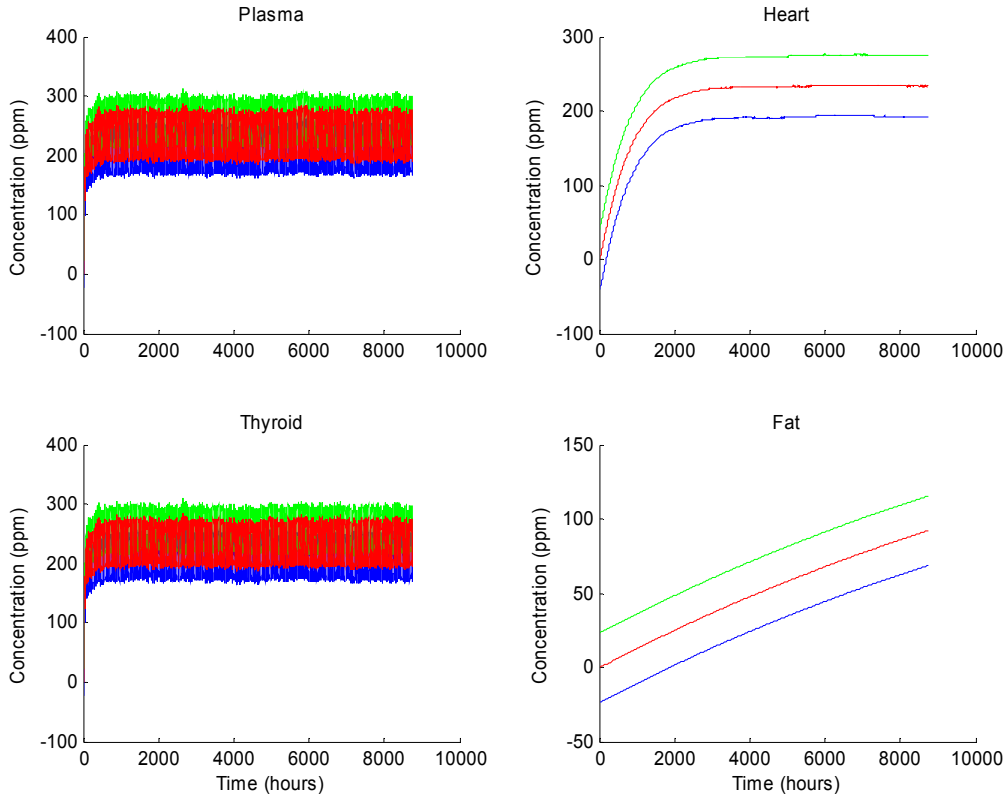

Figure 5-193
Mean Simulated Perchlorate Concentrations (Red) in Quail Plasma, Heart, Thyroid, and Fat from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

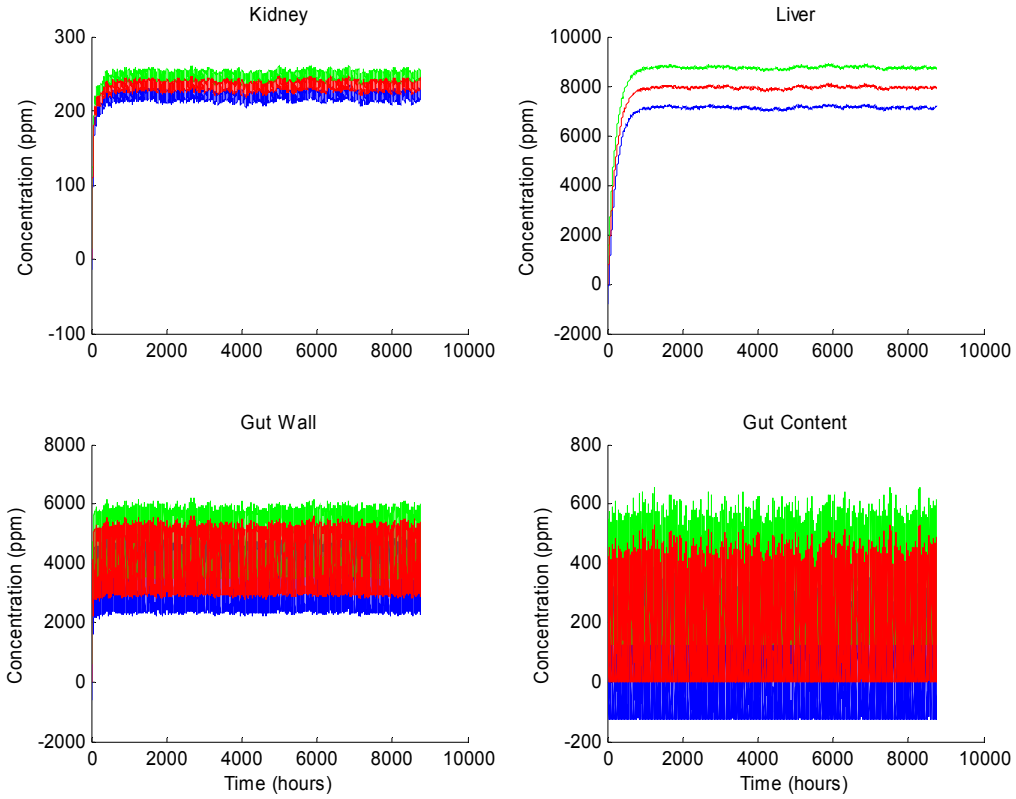

Figure 5-194
Mean Simulated Perchlorate Concentrations (Red) in Quail Kidney, Liver, Gut Wall, and Gut Contents from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

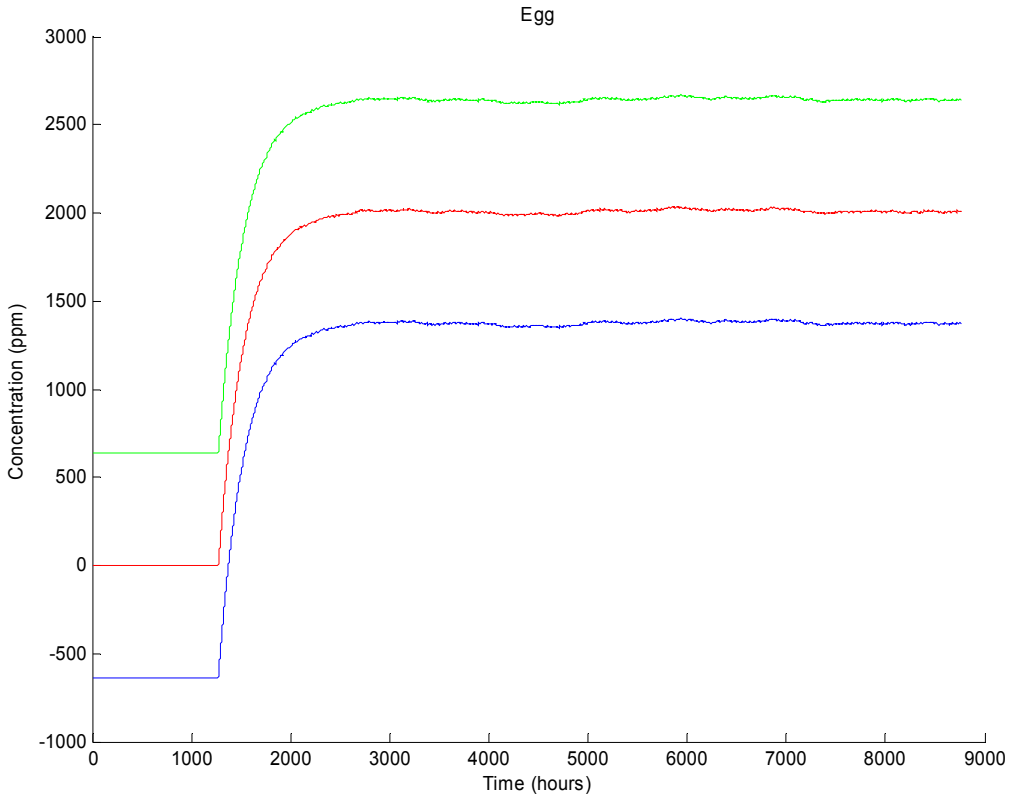

Figure 5-195
Mean Simulated Perchlorate Concentrations (Red) in Quail Eggs from S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

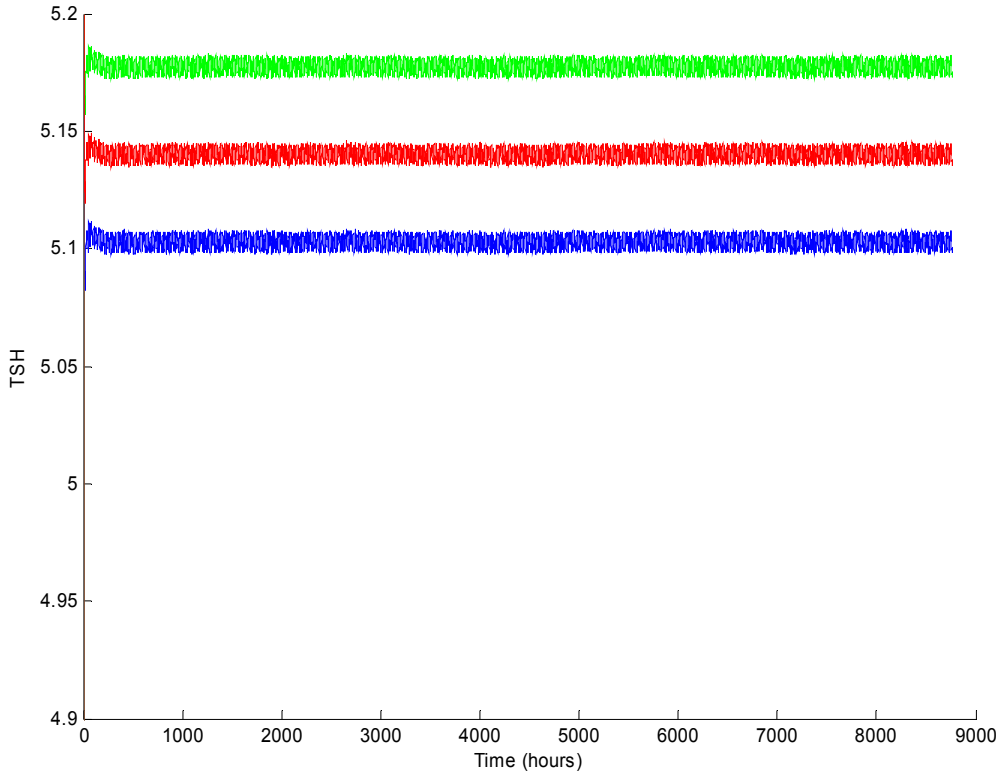

Figure 5-196
Mean Simulated Plasma Concentrations of TSH (Red) for Quail in the S Creek Drainage

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

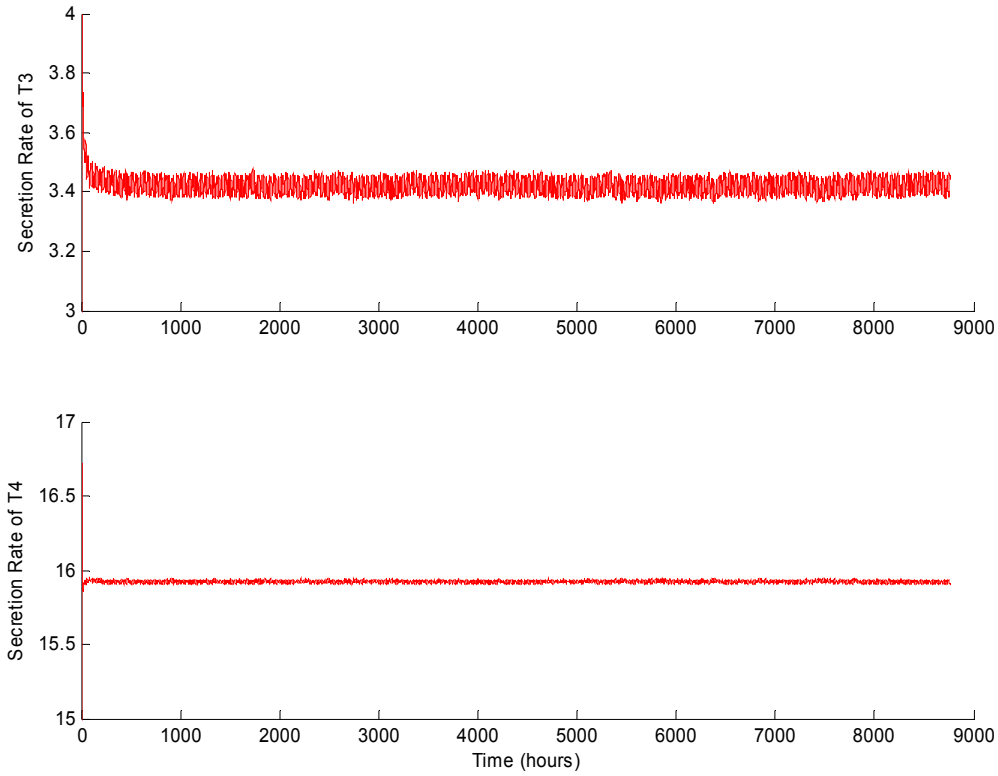

Figure 5-197
Simulated T_3 and T_4 Secretion Rates in Quail from the S Creek Drainage

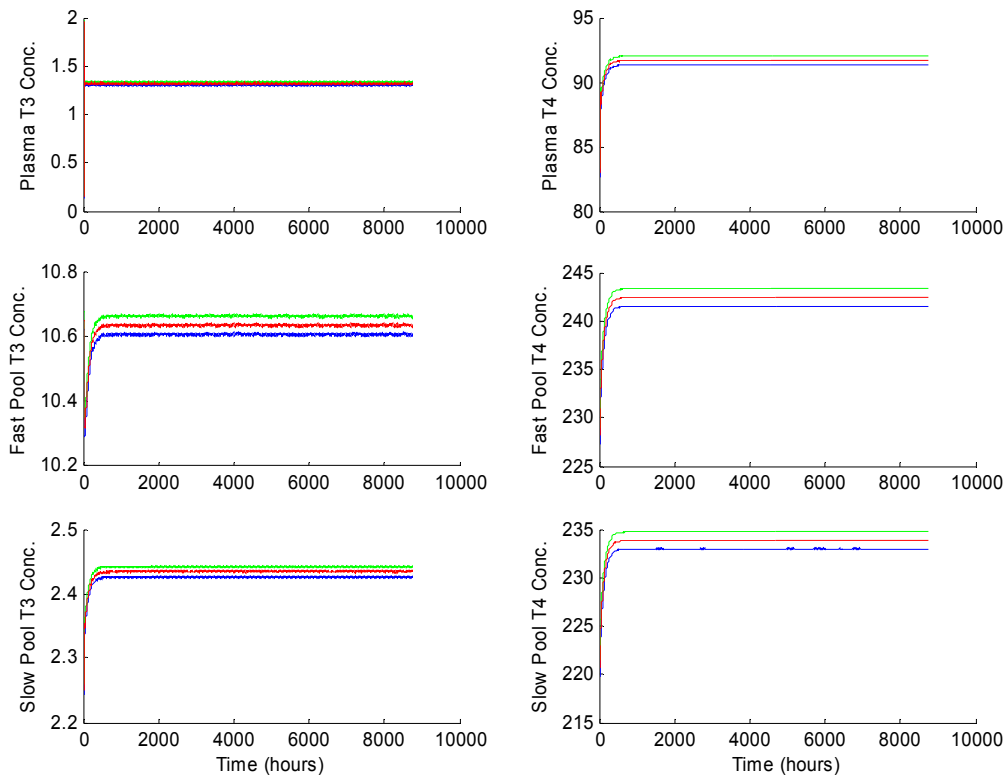

Figure 5-198
Mean Simulated T_3 and T_4 Concentrations (Red) in Quail from S Creek

(Green and blue lines represent the upper- and lower-95% confidence limit, respectively.)

The simulation output is similar in shape for the corresponding variables of both the S Creek and Station Creek Drainages. As the population of birds began to ingest perchlorate-contaminated dietary items, a corresponding perchlorate concentration increase was seen in each compartment. After a given amount of time, the concentration reached equilibrium as the result of a balance between ingested and excreted perchlorate. The process of ingesting and excreting perchlorate also explains the oscillation in each tissue compartment. The up-slope of the oscillation was simply due to ingestion of contaminated food items during the day, while the down-slope is explained by excretion through urine and feces. The model also predicted that higher dietary concentrations of perchlorate resulted in higher tissue concentrations. In addition, it was predicted that perchlorate could be transferred from a female to her eggs.

5.5.1.3.4 Discussion

There were significantly higher perchlorate concentrations predicted in all model small mammal organ and tissue compartments for the South Bosque area, compared with the Station Creek drainage. These differences did not, however, translate to reduced T_3 and T_4 secretion rates or lower T_3 and T_4 concentrations in model compartments. Although parameter estimates are based on literature values, conservative assumptions were made

in their estimation: 1) high transport rates from the liver to the blood, and 2) low elimination rates from the gut. These simulations are only preliminary, but with the current parameter set, additional simulation experiments showed reduced thyroid activity only with order-of-magnitude greater perchlorate concentrations than those in **Table 5-60**.

The model predicts significantly higher perchlorate concentrations in all modeled organ and tissue compartments for the S Creek drainage area compared with the Station Creek drainage area. In attempting to determine if there was a dose-dependent response in thyroid function as it relates to perchlorate concentrations in the thyroid gland, the simulations produced varying results. The plasma TSH level was only marginally reduced, while the various T_3 and T_4 concentrations displayed divergent results. The T_3 secretion rate increased significantly in birds residing in both drainages as compared to the baseline values. Subsequently, the plasma and fast pool T_3 concentrations also increased for both populations, while no significant change became evident for the slow pool T_3 concentration. The resultant T_4 secretion rate decreased for both populations when compared to the baseline values. Additionally, the plasma, fast pool, and slow pool concentrations of T_4 declined as well. Throughout the simulation process, it became apparent that fluctuating perchlorate concentrations at the thyroid did result in fluctuating TSH blood concentrations, T_3 and T_4 secretion rates, as well as T_3 and T_4 concentrations in model compartments. Although the model's output is not necessarily representative of actual hormone levels, the model does suggest that there is a dose-dependent response in thyroid function that is related to perchlorate concentrations at the thyroid gland. It is important to reiterate that the thyroid hormone submodel was based on mammalian studies and the assumption was that the avian thyroid system functioned similarly. Due to the high level of uncertainty in many of the parameters, it is important that these results not be interpreted as definitive. By incorporating more laboratory data into the calibration process, as the data become available, the predictive power of the model could be enhanced.

The predicted concentrations as a result of maternal transfer to eggs are within the same range as the predicted concentrations in the adult thyroid compartment. While the actual effects are unknown at this time, it is reasonable to assume that there is risk for altered thyroid function in the developing embryo resulting from the presence of perchlorate in the egg. This altered thyroid function could result in decreased egg viability or possible teratogenic effects. The egg compartment provides output closer to expected real values, but caution must be utilized here as well. Monitoring data from other sites indicate that perchlorate is rarely detected in eggs.

5.5.2 Medium Mammals

Perchlorate can be detected in soils at many contaminated sites, but it is most often associated with ground and surface water bodies. Therefore aquatic organisms including fish and amphibians are considered to be the most likely ecological receptors. However, perchlorate has been detected in terrestrial organisms as well (Smith et al., 2001), indicating that exposure pathways to terrestrial organisms exist. Since raccoons (*Procyon lotor*) consume many of these organisms and forage along water bodies, they were

chosen as a potential biomonitoring species to assess perchlorate exposure among medium-sized mammals in the Bosque/Leon River watershed areas that have been impacted by the NWIRP facility. Raccoons are closely associated with the terrestrial/aquatic interface and are common throughout much of Texas. Perchlorate exposure among raccoons may result from consumption of contaminated water or food items. Raccoons consume approximately 0.083 g water/ g body weight/ day along with a diet principally composed of plant and animal matter (insects, amphibians, crayfish, fish, rodents; USEPA, 1993). Previous research conducted at another contaminated site (Longhorn Army Ammunition Plant, Karnack, Texas) indicated that perchlorate can be detected in several of these food sources at various levels (Smith et al., 2001). Diets of raccoons may also contain a significant amount of soil (as much as 9.4%) from feeding and grooming (Beyer et al., 1994). Opossums (*Didelphis virginianus*) are a non-target species that is often captured when trapping raccoons. Since they inhabit much of the same habitat and consume many similar types of food items, they too may be suitable indicators of perchlorate contamination and subsequent exposure.

Based on the potential for exposure through water, food item, and soil ingestion, raccoons were selected as a biomonitoring species for this study. We hypothesized that raccoons (and opossums) would be exposed to perchlorate through consumption of food, water, and soil along impacted water bodies in the Bosque/Leon River watershed study area and that thyroid hormone concentrations would be reflect perchlorate exposure.

5.5.2.1 Perchlorate Residue in Native Medium Mammals

5.5.2.1.1 Methodology

Raccoons and opossums were trapped from January 9-10, 2003 using live-traps. Traps were set in areas with some degree of perchlorate contamination as determined by previous water and vegetation monitoring. Areas trapped included Station Creek (T23), the unnamed tributary near the wastewater treatment plant at Highway 317 (T14), the South Bosque at Highway 317 (T16), S Creek at Highway 317 (T15), and Harris Creek (T19). These areas are shown on **Figure 5-199**. Traps were baited with sardines, covered with vegetation, and placed at locations frequented by raccoons (as noted by tracks). Traps were set in the evening (ca. 1700 hrs) and checked the following morning (ca. 0600-0900 hrs).

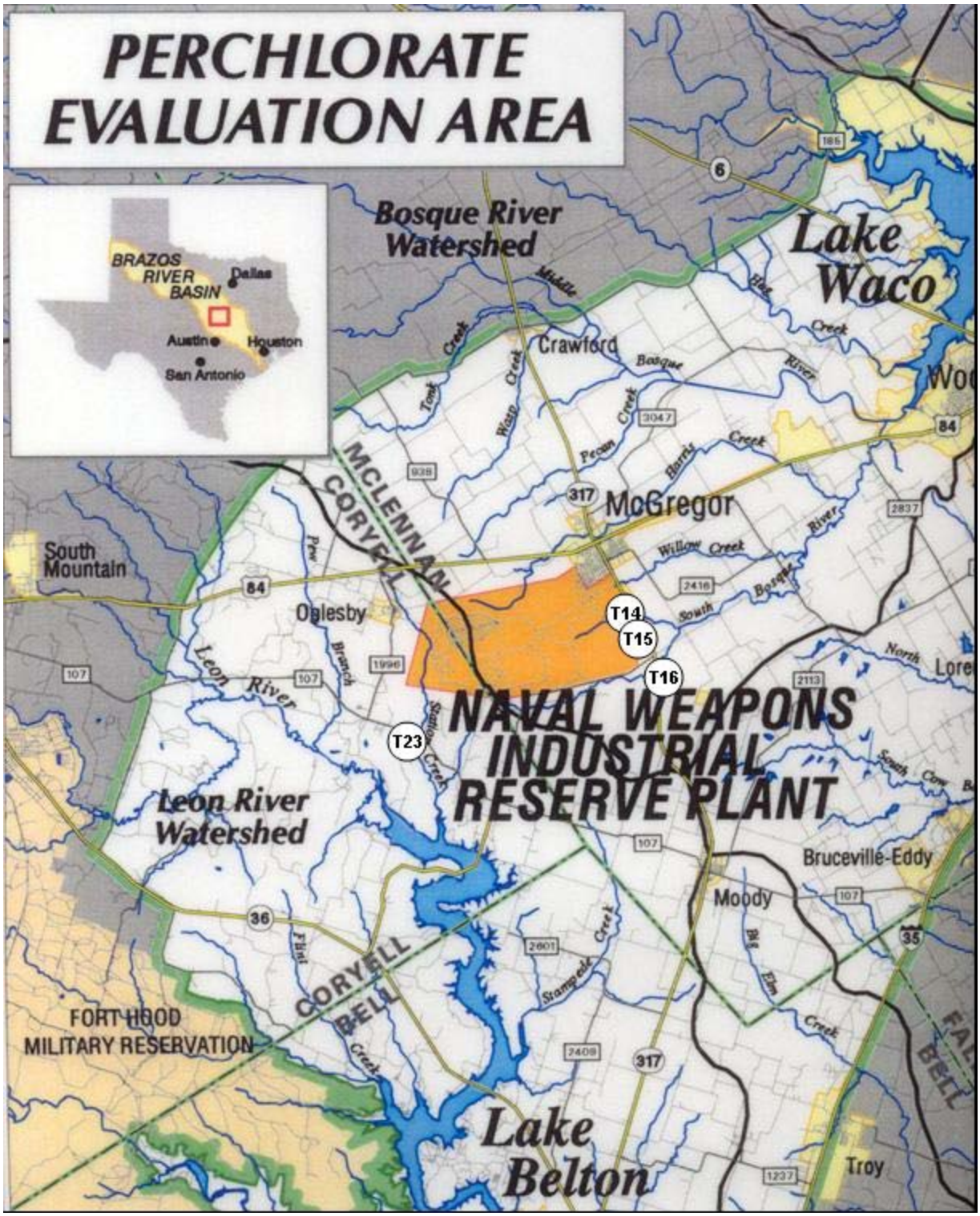

Figure 5-199
Map of Study Area Illustrating the Approximate Locations where Medium Mammal Samples Were Collected

Upon capture, raccoons and opossums were transferred to a squeeze cage and weighed using a Pesola scale calibrated to the nearest 0.5 kg. Weight of the animal was recorded and used to calculate the appropriate dose of ketamine hydrochloride (8-10 mg/kg) and xylazine hydrochloride (2 mg/kg) needed to sedate the animal. The sedative was injected intramuscularly into the left or right hip while animals were constrained in squeeze cages. Ophthalmic ointment was applied to the animal's eyes to keep them moist. Approximately 3 to 8 mL of blood, depending on body size, was taken from the jugular vein, placed in plasma tubes (containing EDTA), and centrifuged for 12 minutes. Plasma samples were frozen (-80 °C) until hormone and residue analysis could be performed. Animals were euthanized via overdose of sodium pentathol and necropsied. Thyroids were removed, placed in a triple aldehyde electron microscopy fixative (Tandler, 1990) for 10 minutes and stored in formalin until histopathology could be performed.

Plasma was analyzed to determine the presence of perchlorate using ion chromatography (EPA Method 314.0; Hautman et al., 1999) with slight modifications. Briefly, analytical samples were prepared for analysis by combining 5 mL ethanol and 1 mL raccoon plasma in a glass tube, mixing the solution for 5 seconds on a vortex and centrifuging for 10 minutes to isolate the supernatant. Then supernatants were decanted into new test tubes and vortex-evaporated until the level of supernatant was approximately 0.5 mL. The supernatant was then brought to a volume of 5 ml with Milli-Q water and filtered (0.45 µm). The final sample was then analyzed for perchlorate using ion chromatography (Anderson and Wu, 2002) (**Appendix X**).

5.5.2.1.2 Data

Medium mammal sampling efforts focused on areas where perchlorate had been detected in surface water and vegetation. The results of residue analyses of raccoon and opossum blood plasma indicate that despite the presence of perchlorate in drinking water at these sites, there was no quantifiable exposure measured in blood (**Table 5-61**).

Table 5-61
Perchlorate Residues in Plasma Samples from Raccoons and Opossum Collected from Areas near NWIRP

Species	Capture Location: UTM: ID[a]	Perchlorate (ng/mL)
Raccoon	Unnamed tributary near wastewater treatment plant at Highway 317: 652853E 3476589N: T14	ND
	S Creek at Highway 317: 653646E 3474993N: T15	ND
	Station Creek at Highway 107: 642988E 3471304N: T23	ND
	Unnamed tributary near wastewater treatment plant at Highway 317: 652853E 3476589N: T14	ND
	S Creek at Highway 317: 653646E 3474993N: T15	ND
Opossum	Station Creek at Highway 107: 642988E 3471304N: T23	ND
	South Bosque at Highway 317: 653810E 3473908N: T16	ND
	S Creek at Highway 317: 653646E 3474993N: T15	ND
	S Creek at Highway 317: 653646E 3474993N: T15	ND

[a]see **Figure 5-199** for approximate capture locations.

5.5.2.1.3 Discussion

Despite the likely occurrence of perchlorate exposure in raccoons and opossums, perchlorate residues in blood plasma (if present) were below the limit of detection. As the surface to volume ratio of an animal decreases, its relative exposure decreases. In other words, larger animals do not receive as large of an exposure (on a surface to volume ratio basis) as small animals. This toxicological concept likely contributed to the results observed for medium mammals. Additionally, perchlorate is excreted rapidly after exposure, and thus may have been eliminated from captured animals prior to collection of blood samples.

5.5.2.2 Thyroid Hormones and Histology in Native Medium Mammals

5.5.2.2.1 Hormone Methodology

Plasma samples were analyzed for T_3 and T_4, subject to the availability of plasma from individual animals. Coat - A- Count Total kits (TKT31 and TKT41; Diagnostic Products, Los Angeles, CA, USA) were used to measure T_3 and T_4 levels. In order to conserve plasma, all assay optimization (Newell et al., 1982) was completed using raccoon serum collected during a previous study.

The basic protocol of the T_3 kit was followed with slight modifications. The volume of plasma was optimized by increasing the amount used in each kit to achieve 40% binding (Chard, 1990). The optimal and most efficient use of plasma was determined to be 150 µL for T_3. Calibration points for the T_3 standard curve were 0, 20, 50, 100, 100, 200, and 600 ng/mL. Optimization of the T_4 kit was conducted in the same manner, and the optimal plasma volume was determined to be 100 µL. Calibration points for the T_4 standard curve were 0, 1, 4, 10, 16, and 24 µg/dL. Since raccoon plasma volumes used in each assay were greater than those specified by the kits, actual hormone concentrations would be lower than the values reported herein. Therefore, relative values are reported and should not be construed as actual raccoon thyroid hormone concentrations.

5.5.2.2.2 Histology Methodology

A Tissue-Tek V.I.P. 2000 Processor was used to process the thyroid tissues, which were then embedded in paraffin (Miles Scientific). Blocks containing the thyroid tissues were then cut into sections at 7 µm with a microtome (Cut 4055, Olympus America Inc.). Tissue sections were then mounted on glass microscope slides and stained following basic hematoxylin and EosinY staining technique (Ross, 1995).

Slides were examined with an Olympus BX52 compound light microscope with attached Olympus DP1-L digital camera and control pad (Olympus Optical Co., Ltd). Photographs of each specimen were taken at 40X magnification. Sections were selected from the slides containing thyroid tissue from each raccoon. Thyroid tissue was examined and scored for the amount of hyperplasia, hypertrophy and colloid depletion present based upon the guidelines established by the Pathology Working Group (Mann, 2000). Each trait was given a score of 0 for no indication of thyroid damage, 1 for slight damage, or 2 for severely damaged (Mann, 2000). An average score for all three traits was then

determined for individual voles and each of the three treatment groups by generation and sex. Additionally, the number of microfollicles present was recorded.

5.5.2.2.3 Data

Five raccoons and four opossums were collected from four areas around the NWIRP that had previously produced water or other environmental samples containing perchlorate in January, 2002. All raccoons and opossums appeared to be in good general condition, and no abnormalities were noted. Upon inspection, all thyroid glands appeared to be of normal color, size, and shape.

Raccoon plasma triiodothyronine (T_3) concentrations ranged from 49.85 ng/dL to 101.97 ng/dL, and thyroxine (T_4) concentrations ranged from 4.25 µg/dL to 7.20 µg/dL (**Table 5-62**). Mean raccoon T_3 and T_4 concentrations were 73.9 ± 8.57 ng/dL and 6.3 ± 0.56 µg/dL. Thyroid hormone concentrations among raccoons inhabiting the perchlorate study near the NWIRP site are higher than the mean thyroid hormone concentrations in raccoons (56.53 ± 3.1 ng/dL and 3.57 ± 0.25 µg/dL, respectively) inhabiting the Longhorn Army Ammunition Plant (LHAAP) in Karnack, Texas. Mean T_3 and T_4 concentrations for opossums were 45.13 ± 4.70 ng/dL and 1.73 ± 0.22 µg/dL, respectively.

Table 5-62
Raccoon and Opossum Triiodothyronine (T_3) and Thyroxine (T_4) Concentrations

Species	Capture Location: UTM: ID[a]	T3 (ng/dL)	T4 (µg/dL)
Raccoon	Unnamed tributary near wastewater treatment plant at Highway 317: 652853E 3476589N: T14	75.17	7.20
	S Creek at Highway 317: 653646E 3474993N: T15	64.75	4.25
	Station Creek at Highway 107: 642988E 3471304N: T23	49.85	6.13
	Unnamed tributary near wastewater treatment plant at Highway 317: 652853E 3476589N: T14	78.10	7.20
	S Creek at Highway 317: 653646E 3474993N: T15	101.97	6.90
Opossum	Station Creek at Highway 107: 642988E 3471304N: T23	39.54	1.22
	South Bosque at Highway 317: 653810E 3473908N: T16	55.18	1.71
	S Creek at Highway 317: 653646E 3474993N: T15	50.73	1.69
	S Creek at Highway 317: 653646E 3474993N: T15	35.05	2.30

[a]see **Figure 5-199** for approximate capture locations.

In addition to thyroid hormone analysis, thyroid glands from all raccoons and opossums were examined histologically. Thyroid gland sections were examined for the presence of hyperplasia, hypertrophy and colloid depletion. No histological abnormalities were detected in any of the raccoon or opossum tissues.

5.5.2.2.4 Discussion

These data suggest that medium-sized mammals inhabiting areas adjacent to the NWIRP and affected streams are not exposed to perchlorate at concentrations high enough to result in physiological effects. This study examined medium-sized mammals during the winter. Food item selection and water consumption differ seasonally among these organisms (USEPA, 1993), therefore alternative exposure rates may occur at other times of the year. In the spring and summer, raccoons and opossums rely more heavily on vegetation and fruits. Given the potential for perchlorate uptake into vegetation, these animals may be exposed to higher concentrations of perchlorate in spring and summer. However, medium-sized mammals do not appear to be at risk outside the NWIRP facility due to their extensive foraging ranges and limited spatial distribution of perchlorate contamination.

5.5.3 Large Mammals

Cattle inhabiting pastures near perchlorate-contaminated water bodies may be at risk for exposure through drinking water or consumption of plant matter grown in the presence of perchlorate. Cattle are obligate herbivores, and as such may be at risk for perchlorate exposure and subsequent effects since perchlorate can accumulate to high concentrations in plants. Wild birds and rodents collected from contaminated terrestrial environs have been found to contain elevated concentrations of perchlorate in tissues (Smith et al., 2001).

Beef and dairy cattle may represent a direct exposure pathway to humans. Therefore, we evaluated perchlorate exposure among cattle inhabiting a pasture in the study area that had a constant influx of perchlorate. We examined exposure via plasma and tissue (consumable beef samples) concentrations and thyroid hormone responses. The objective of this study was to assess potential human exposure to perchlorate through beef cattle inhabiting the Bosque/Leon River watershed study area.

5.5.3.1 Perchlorate Residue in Large Mammals

5.5.3.1.1 Methodology

5.5.3.1.1.1 Study Description and Sample Collection

The study was conducted over 14 weeks during the spring of 2003. Heifer calves (~ 535 lbs. each) were purchased commercially and held for 1 week prior to being placed on two sites (2 heifers per site) near McGregor, McLennan County, Texas. The reference site was a pasture a sufficient distance from the facility such that perchlorate was not expected to be present in surface or groundwater. The treatment site was a pasture near the facility boundary with a spring-fed stream bisecting the property (between locations T18 and T19). Previous monitoring data for the spring indicated that perchlorate was present (20-60 ng/mL). Both sites are shown on **Figure 5-200**.

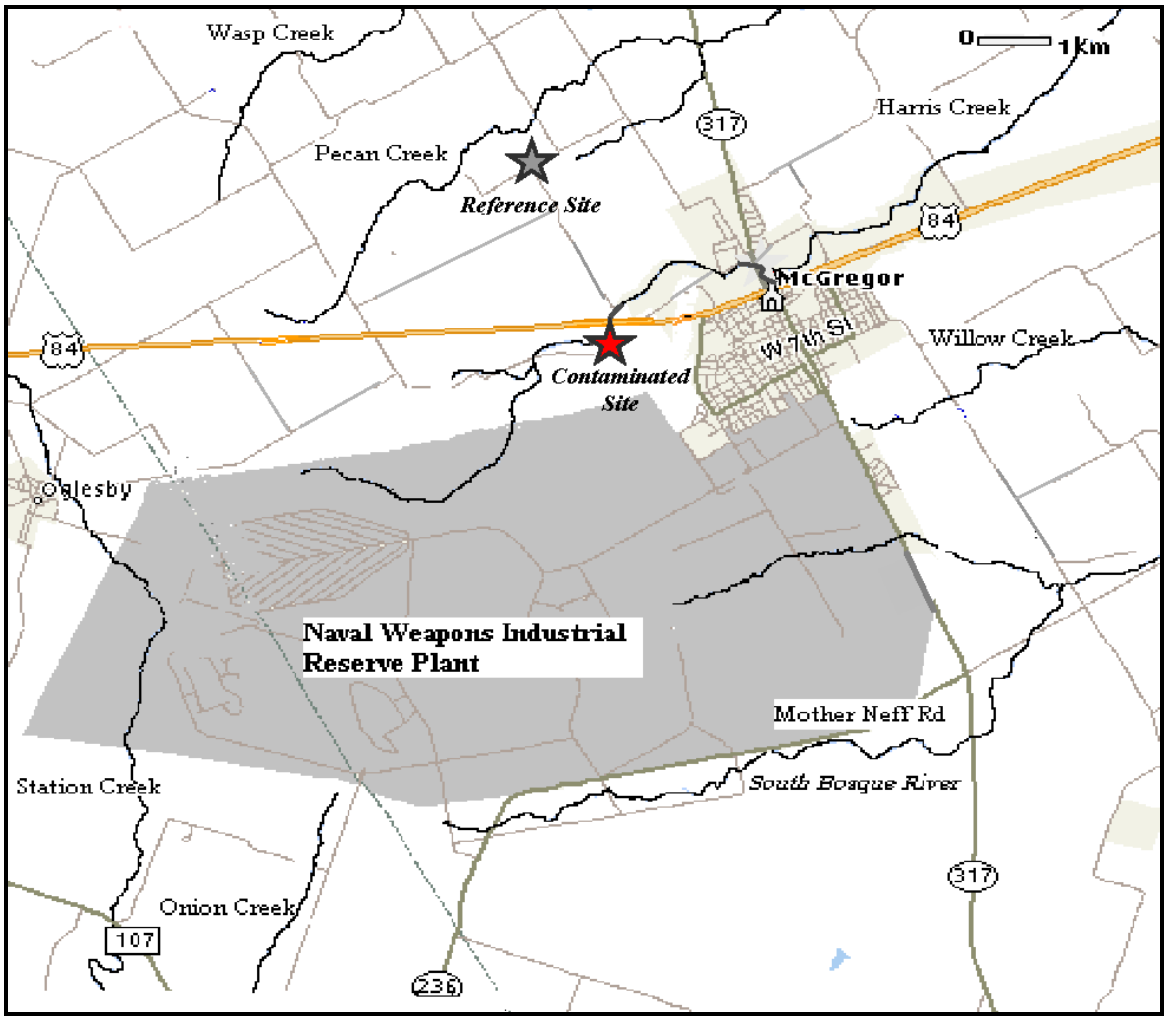

Figure 5-200
Locations of the Reference Site and the Contaminated Site for the Cattle Study

Heifers on both sites were visually monitored on a daily basis and blood was collected from each animal every 2 weeks. Blood samples were always collected in the morning (before 9:00 am). At the time of blood collection, drinking water that the animals had access to was also collected. Plasma and serum from the blood were isolated in the field via centrifugation. Samples were stored on dry ice during transport back to the laboratory, and stored frozen (-80 °C) until analysis.

At the conclusion of the study, the heifers described above and two older cows that had resided on the contaminated property for several years, were processed in a manner identical to a commercial beef processing operation. The following tissue samples were obtained from each heifer for residue analysis: liver and various meat cuts (sirloin steak, round steak, t-bone steak, and roast). Liver, ground beef, and "chili meat" from the two older cows were also examined for perchlorate residues.

5.5.3.1.1.2 Sample Preparation and Perchlorate Analyses

A potassium perchlorate ($KClO_4$) standard solution was obtained as a custom standard from AccuStandard, Inc. (New Haven, CT). Sodium hydroxide (NaOH), 50% (w/w) aqueous solution was purchased from Fisher Scientific. All solutions were prepared in 18.2 MΩ Milli-Q water. Ethanol was purchased from Fisher Scientific.

Plasma samples were processed using methods similar to those described previously (Fisher et al., 2000; Anderson and Wu, 2002; Narayanan et al., 2003) prior to analysis. First, 1 mL of plasma was precipitated with 4 mL of ethanol (ice-cold) and then centrifuged (4 °C) at 3750 rpm for 5 min. The supernatant was removed, evaporated to dryness under nitrogen, and reconstituted in 5 mL Milli-Q water (Fisher et al., 2000; Narayanan et al., 2003). Samples were then cleaned up using Alumina and C_{18} solid phase extraction (SPE) cartridges, and filtered (0.45 µm) prior to IC analysis.

Tissue samples were also processed prior to IC analysis. Samples (10- 20 g) were air dried and then extracted with Milli-Q water using Accelerated Solvent Extraction (ASE; Dionex Corp.) Extraction conditions were as follows: pressure = 1500 psi, temperature = 100 ºC, extraction time = 15 min. Sample extract volumes were measured, diluted (5x), and cleaned up with Alumina and C_{18} SPE cartridges. Eluates were filtered (0.45 µm) prior to IC analysis.

Surface water samples collected from the two sites during the course of the study were filtered (0.45 µm) prior to analysis.

All samples (plasma extracts, tissue extracts, and surface water) were analyzed by ion chromatography similar to EPA Method 314 (See **Appendix X** for specific procedures).

5.5.3.1.2 Data

Perchlorate was not detected in water samples collected from the reference site. In contrast, perchlorate was detected throughout the study in water samples from the treatment site (25.4 ± 5.8 ng/mL; n = 8). The levels of perchlorate in water from the treatment site are consistent with more than two years of monitoring data from that location.

Perchlorate was not detected in blood plasma samples from either cow occupying the reference site (**Figure 5-201**). Similar results were obtained for the two cows on the treatment site with one exception; perchlorate was detected (15 ng/mL and 22 ng/mL) in one of the cows on consecutive sampling periods four and six weeks after the beginning of perchlorate exposure.

Perchlorate was not detected in any of the tissue samples collected from heifers occupying the reference site. In addition, perchlorate was not detected in any of the tissue samples collected from heifer cows occupying the treatment site.

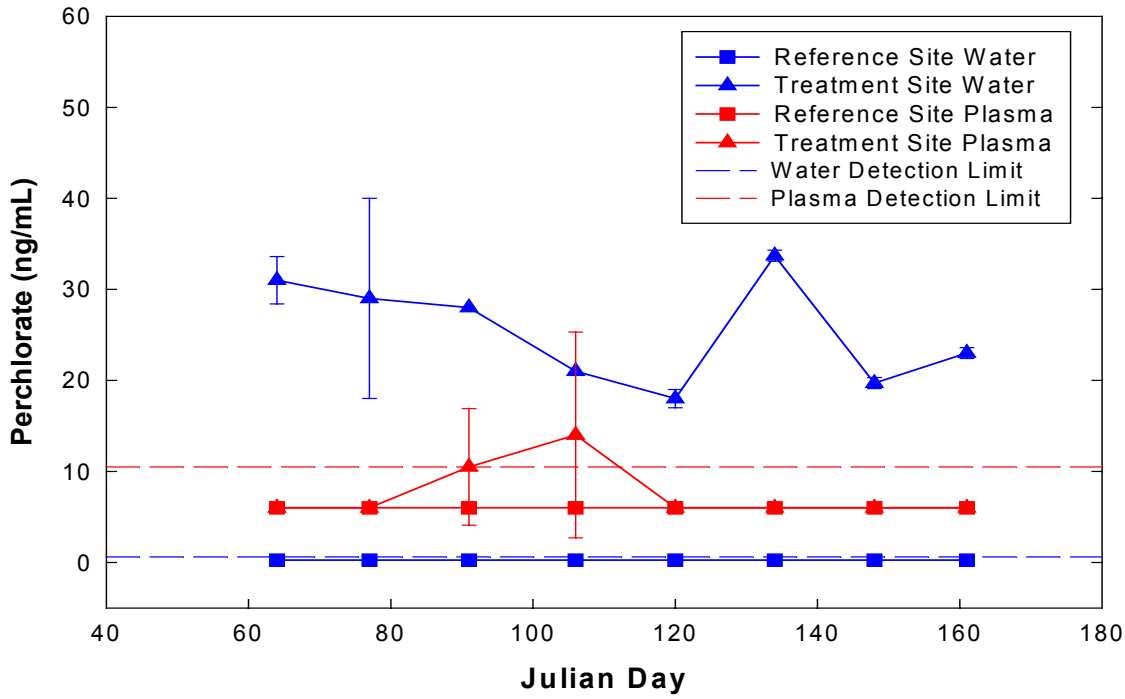

Figure 5-201
The Relationship Between Perchlorate Exposure in Drinking Water and Perchlorate in Cow Plasma

All reference cattle plasma data were below the analytical limit of detection (11 ppb) and were therefore assigned a value of one half the detection (limit 5.5 ppb) for data presentation. Similarly, all reference water data were below detection limit and are presented as one half the detection limit (0.5 ppb).

5.5.3.1.3 Discussion

Our results indicate that despite the presence of perchlorate in drinking water at the treatment site, there was little quantifiable exposure measured in blood plasma from the cattle. In other words, constant exposure to 25 ppb perchlorate in water over 14 weeks did not result in measurable residues in blood plasma. Water intake in cattle is reasonably well understood. An adult cow takes in around 40 L of water per day and excretes around 30 L per day (20 L in feces, 10 L in urine). Based on this assumption and the measured drinking water concentrations, the cows on the Treatment Site were ingesting 250 µg perchlorate per day. If one makes the typical assumption that a cow has 60 mL of blood per kg body weight, the expected perchlorate concentration in plasma should be around 18 ppb. This value is consistent with the 2 detections that were observed (15 ppb and 22 ppb).

Perchlorate was detected with greater frequency and at higher concentrations in cattle from two Kansas farms adjacent to facilities that used or handled perchlorate. Cattle on these farms were not restricted to water supplies containing perchlorate as were those on the McGregor treatment site. Nonetheless, perchlorate was detected in 4/33 and 17/26

cattle at the two Kansas farms. The highest plasma perchlorate concentrations observed in the Kansas cattle were 43 and 32 ppb, respectively.

Perchlorate can be rapidly excreted in urine, with reported urinary excretion half-lives ranging from 8 to 20 hours in rats (Wolff, 1998; Fisher et al., 2000). Perchlorate accumulation was not consistently observed in plasma from cows on the McGregor treatment site, most likely due to rapid excretion rates. Batjoens et al. (1993) reported that prolonged perchlorate administration (4 g/day for 10 days) in cows resulted in a relatively longer excretion period in urine which may have been more indicative of exposure than plasma as was examined in our study.

Considerable effort was made to develop a method for perchlorate analysis in plasma (See **Appendix X**). The method can reduce the typical background interference significantly, and perchlorate recovery of spiked samples is consistent and reproducible (85.3 ± 0.5%). In addition, the detection limit for perchlorate in blood plasma (S/N = 3) was low enough (11 ppb) to detect perchlorate if present.

5.5.3.2 Thyroid Hormones in Large Mammals

5.5.3.2.1 Methodology

Plasma was analyzed for total T_3 and total T_4 using Coat-A-Count radioimmunoassay (RIA) kits (Diagnostic Products Corporation DPC, Los Angeles, CA). The assay was performed according to manufacturer's instructions. Plasma samples were assayed on a Packard Cobra E5005 gamma counter. Samples were assayed in triplicate and standards (six points) that came with the kit were run at the beginning of each assay and intermittently throughout the assay.

5.5.3.2.2 Data

Plasma samples were collected from individual heifers approximately every two weeks from March 5, 2003 to June 10, 2003. Individual heifer thyroid hormone concentrations varied considerably from week to week (**Figure 5-202** and **Figure 5-204**). T_3 concentrations ranged from 0.83 to 1.75 ng/mL with a mean concentration of 1.26 ± 0.07 ng/mL standard error (SE) in the perchlorate-exposed heifers. Reference heifer T_3 concentrations ranged from 1.24 to 2.11 ng/mL with a mean concentration of 1.61 ± 0.07 ng/ml SE. Mean T_3 concentrations ranked from highest to lowest were: 152 control = 1.73 ± 0.08 ng/mL, 153 control = 1.50 ± 0.11 ng/mL, 154 treated = 1.36 ± 0.09 ng/mL, and 151 treated = 1.16 ± 0.11 ng/mL.

T_4 concentrations ranged from 39.47 to 80.80 ng/mL with a mean concentration of 55.58 ± 3.36 ng/ml SE in the perchlorate-exposed heifers. Reference heifer T_4 concentrations ranged from 41.38 to 88.19 ng/mL with a mean concentration of 64.38 ± 3.38 ng/mL SE. Mean T_4 concentrations ranked from highest to lowest were: 152 control = 70.73 ± 2.57 ng/mL SE, 151 treated = 65.80 ± 3.72 ng/mL, 153 control = 58.03 ± 5.55 ng/mL SE, and 154 treated = 45.37 ± 2.15 ng/mL. There was a noticeable decrease in T_4 concentrations among all cattle on the April 16, 2003 sampling period (**Figure 5-204**) that may have been due to assay-related error. Overall, there appeared to be a general trend of

decreasing thyroid hormones in both the reference and the exposed animals (**Figure 5-203** and **Figure 5-205**).

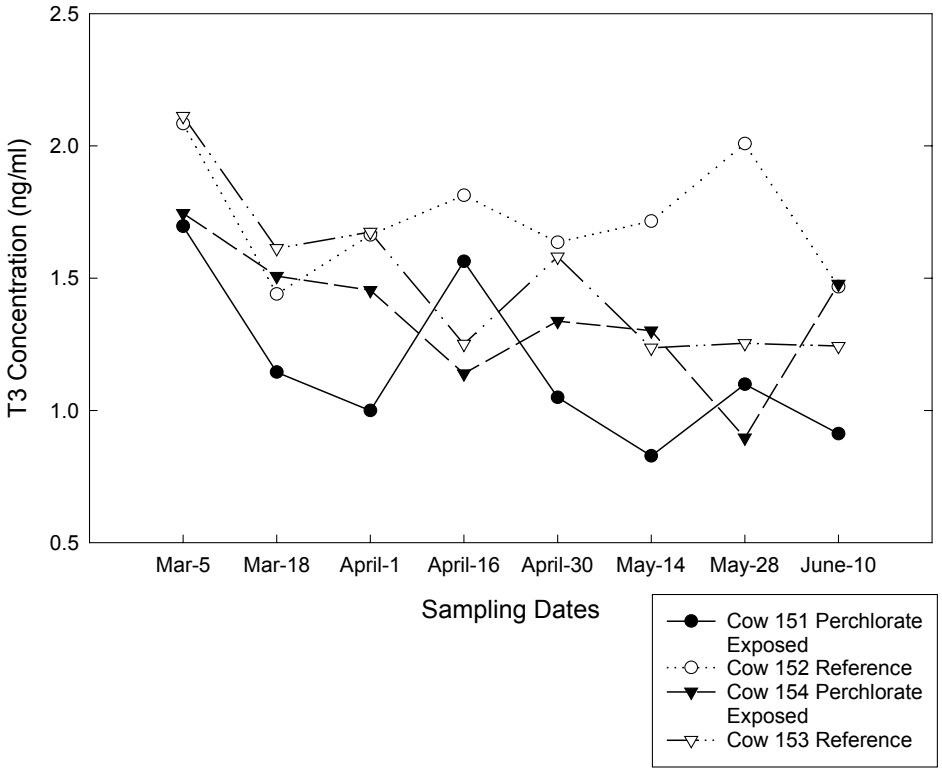

Figure 5-202
Total T_3 Concentrations in Plasma from Four Heifers on Either Perchlorate-Contaminated (151, 154) or Reference Areas (152, 153) near McGregor, TX 2003

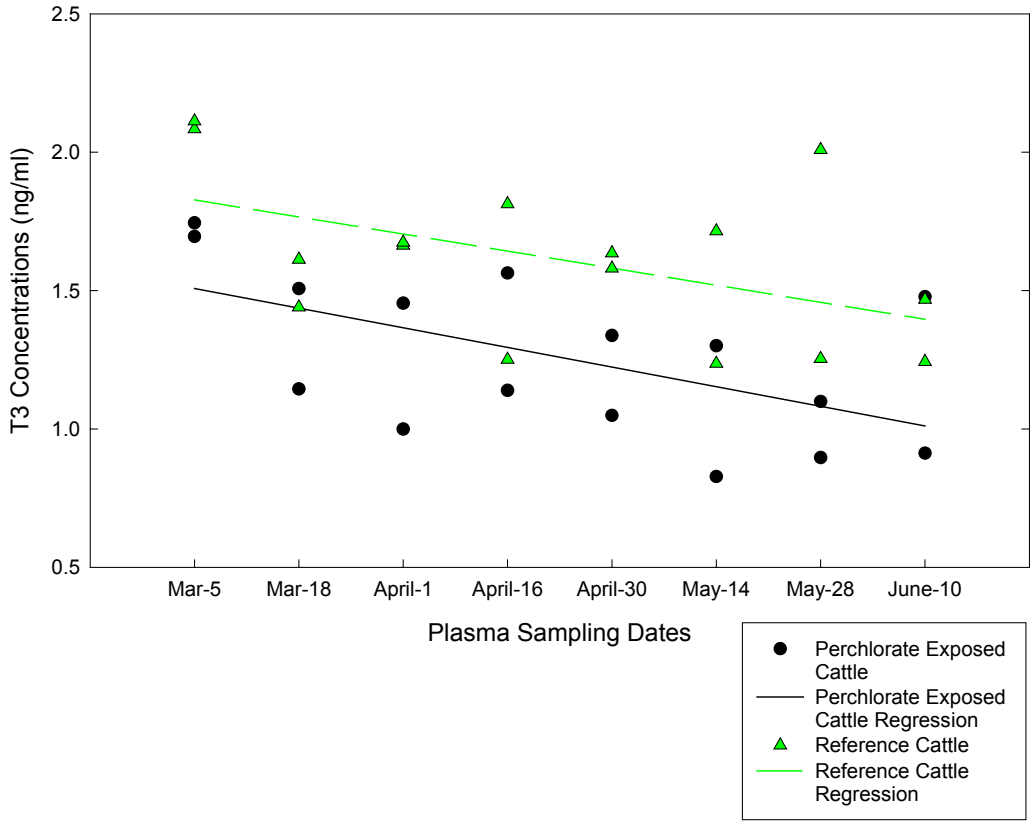

Figure 5-203
Linear Regression of Plasma T_3 Concentrations in Heifers Maintained on a Perchlorate-Contaminated, or a Reference Pasture near McGregor, TX, 2003

(The slope for the reference heifer regression line is -0.06 and the r^2 is 0.25. The slope for the perchlorate-exposed heifer regression line is -0.07 and the r^2 is 0.33.)

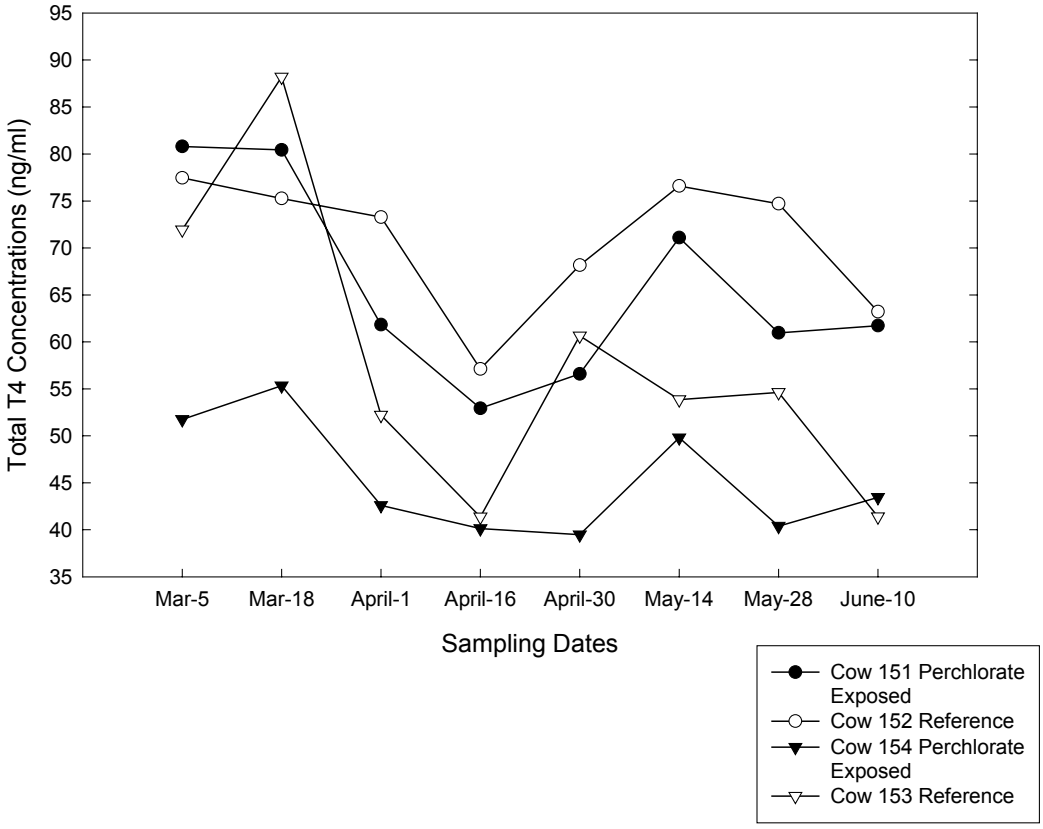

Figure 5-204
Total T_4 Concentrations in Plasma from Four Heifers on Either Perchlorate-Contaminated (151, 154) or Reference Pastures (152, 153) near McGregor, TX 2003

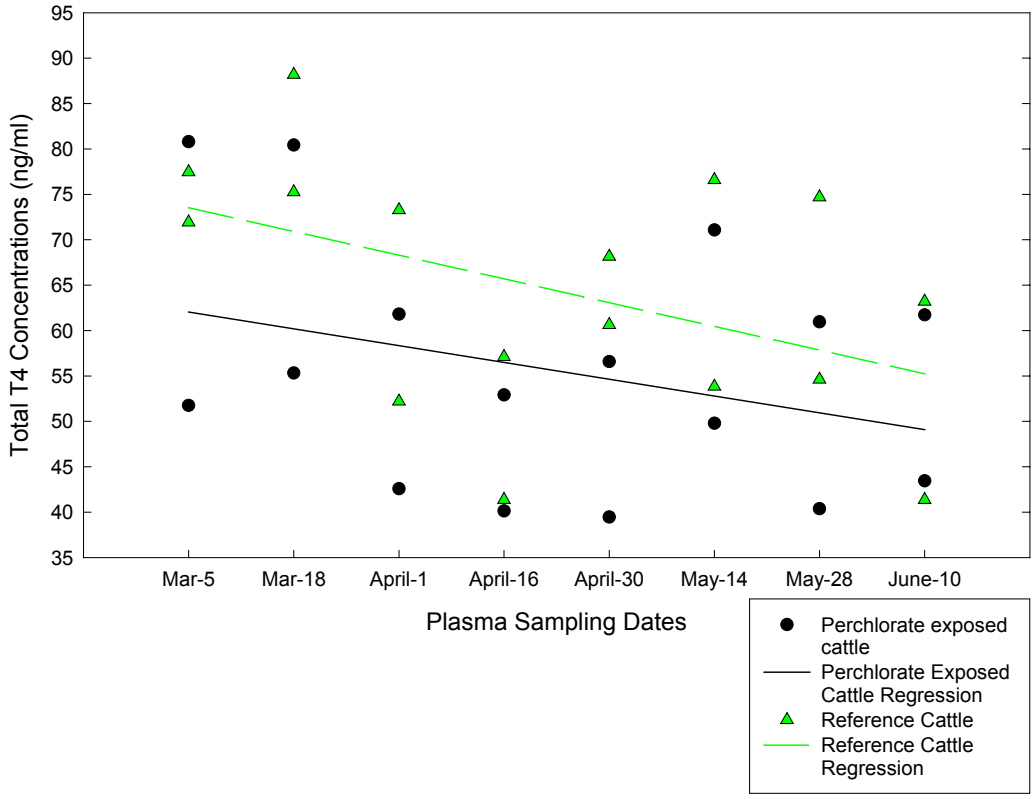

Figure 5-205
Linear Regression of T_4 Plasma Concentrations in Heifers Maintained on a Perchlorate-Contaminated or a Reference Pasture near McGregor, TX, 2003

(The slope for the reference heifer regression line is -2.61 and the r^2 is 0.21. The slope for the perchlorate-exposed heifer regression line is -1.85 and the r^2 is 0.11.)

5.5.3.2.3 Discussion

There was considerable variability in thyroid hormone concentrations in both perchlorate-exposed and reference heifers, and there did not appear to be any perchlorate-related reductions in thyroid hormone concentrations. Perchlorate is known to cause hypothyroidism; characterized by a decrease in thyroid hormone levels, an increase in weight and lethargy (Mannisto et al., 1979). De Moraes et al. (1998) reported average blood serum concentrations for T_3 at 1.6 ng/mL and for T_4 at 88.5 ng/mL collected over a five week period from Brahman cattle. Grigsby and Trenkle (1986) reported average blood plasma T3 concentrations of 1.75, 1.22, 1.52 ng/mL and for T_4 concentrations of 72.5, 70.5, 81.0 ng/mL in Angus, Limousin, and Simmental steers, respectively. These studies illustrate the variability in thyroid hormone concentrations among cattle breeds. Our results are comparable to those of Grigsby and Trenkle (1986). The cattle used for this study were mixed breed which may have contributed to the variability observed in thyroid hormone concentrations. The perchlorate-exposed cattle mean thyroid hormone concentrations were lower than those of the reference heifers throughout the study, and

the rate of decrease in thyroid hormones throughout the study periods were similar between the two treatment groups (**Figure 5-203** and **Figure 5-205**). In fact, the rate of decline in T_4 concentrations among the reference heifers was slightly greater than that of the perchlorate-exposed heifers. Kansas cattle potentially exposed to perchlorate exhibited a negative relationship between thyroid hormone concentrations and age, but no noticeable alterations in thyroid hormone status that could be linked to perchlorate exposure (TIEHH data on file). Therefore, it is doubtful that perchlorate inhibited thyroid hormone production in the McGregor treatment site (or reference) heifers. Therefore, we conclude that low concentrations of perchlorate in forage and water do not significantly affect sub-adult cattle thyroid hormone concentrations. However, additional studies are needed to assess developmental effects in perchlorate-exposed calves, and the effects of longer-term exposures.

6.0 UPDATED HYDROLOGIC CONCEPTUAL SITE MODEL

As an initial part of this study, a hydrologic conceptual site model was developed to assess current knowledge of the hydrology of the study area. In order to more fully understand the area's hydrology and the resulting effects on the transport of perchlorate through the environment, several data gaps were identified and prioritized as previously discussed in Chapter 3. Study data collected to fill many of these data gaps were presented in Chapter 5. This chapter summarizes key points from the original conceptual site model and expands the conceptual model based on the new data collected.

6.1 OVERVIEW

NWIRP McGregor straddles the boundary between the Bosque River and Leon River watersheds, which drain into Lake Waco and Lake Belton, respectively. The southerly flows from NWIRP McGregor drain into the Leon River via Station Creek and Onion Creek. The northerly surface water flows originating from the site drain to Lake Waco via several larger streams, including the South Bosque River, Willow Creek, and Harris Creek. Groundwater movement also connects the McGregor site to Lake Waco and Lake Belton.

6.2 HYDROGEOLOGY

Although both shallow and deep aquifers exist in the NWIRP McGregor region, there is no evidence for interaction between them. The deep aquifers in this region are separated by over 900 feet of shale and limestone from the shallow aquifers. Furthermore, the documented recharge zone for the deep aquifers is 70 miles to the northwest. There are no known local faults that would provide conduits for shallow water recharge to the deep aquifers. No perchlorate has been detected in water samples from deep production and private drinking water wells, which suggests there is minimal potential for cross-connection between the shallow water-bearing zone and the deep aquifers (EnSafe, 1999a). Therefore, the deep aquifers will not be discussed further in this report. All further discussions of aquifers in this chapter refer to the shallow aquifer.

6.2.1 Aquifer Properties and Groundwater Chemistry

The shallow, unconfined system is located in fractured limestone and is both heterogeneous and anisotropic on a local level due to weathering, tectonics, changes in lithology, and variations in fracture density. This means that the ability for groundwater to flow can vary both with location in the aquifer and with direction of flow. Therefore, there could be a preferential direction of water movement when considering a limited area of the aquifer. However, because the direction of local anisotropy and heterogeneity can vary significantly over the region, the shallow aquifers can be considered homogeneous on a larger scale, with flow generally following the topography. In other words, contaminated groundwater will tend to flow toward streams under natural conditions much as that described by Cannata (1988). Several values for the properties of

the shallow aquifer as determined by previous studies were given in the *Final Conceptual Site Model* (MWH 2002a). These values are summarized in **Table 6-1**.

Table 6-1
Shallow Aquifer Properties

Property	Range	Comments
Matrix Porosity	8%	Howell (1972)
Fracture Porosity	1-2%	Myrick (1989)
Hydraulic Conductivity	10^{-3} m/s to 10^{-10} m/s	Clark (2000) and EnSafe (1999b). Greater near the surface and perhaps near faults and along streams
Lateral Anisotropy	1:1.1 at 10 feet depth, 1:1.73 at 20 feet depth	Increases with depth (Edwards 1991).
Flow Rate	Up to 100 feet per year, possibly higher during strong recharge events.	Yelderman (2002)

The groundwater chemistry is fairly consistent and represents a diffuse flow system. Ionic concentrations in the aquifer fluctuate slightly in response to seasonal changes in the water table. The aquifer properties and groundwater chemistry are described in detail in the *Final Conceptual Site Model* (MWH, 2002a). No additional data were collected during this study to alter the conceptual model of the aquifer properties and chemistry.

6.2.2 Interactions with Surface Flow and Response to Precipitation

Several hydrogeologic data needs were presented in the *Final Conceptual Site Model* (MWH, 2002a). Of these data needs, a better understanding of year-round groundwater/stream interactions and rainfall-runoff relationships were subjected to further study. Two different views regarding the extent of surface-aquifer flow interactions were presented in the *Final Conceptual Site Model* (MWH, 2002a). According to Ensafe, the water table is inconsistent. Flows are altered seasonally, with groundwater contributing to streamflow during the wet season and streams contributing to the groundwater system during drier periods (EnSafe, 1999b). However, according to Dr. Yelderman, there are few times during the year that the streams lose water to the aquifer. The losses that do occur are limited to the uppermost reaches of the streams (Dr. Yelderman, personal communication, February 2002). (Nawrocki, 1996; Myrick, 1989).

Most aquifer recharge was stated to occur from precipitation events during winter and spring when soil moisture is high. Spring is also a likely time for aquifer discharge, as the aquifer becomes saturated due to precipitation. Collins comments that "during the late spring months, the aquifer is characterized by high amplitude water table fluctuations following high intensity, short duration precipitation events" (1989). At this time, water-table levels rise to within a few feet of the surface throughout the drainage basins and the aquifer is described as flooded. Aquifer discharge occurs from numerous small episodic overflow springs and seeps in addition to perennial springs. The upper basins receive more baseflow per basin area because the streams are shallow and the stream dissection is

in the most fractured portion of the aquifer material. Groundwater is also lost to evapotranspiration, and much of the evapotranspiration occurs along streams. There is not much evapotranspiration during the winter months while the late summer is usually the driest and hottest time of the year.

As presented in Section 5.1.3, extensive rainfall, stream and groundwater level data were collected to further evaluate the potential for contaminant migration between streams and groundwater. Conclusions from these data were presented for each station in detail in Section 5.1.3.3. The resulting overall conclusions based on these data are presented here, grouped by watershed.

6.2.2.1 Leon River Watershed

The portion of the Leon River watershed potentially affected by runoff from NWIRP McGregor is characterized by monitoring stations on Station Creek, Tributary M, Onion Creek, and the Leon River downstream of Station Creek. Stream level and groundwater level data collected over one year are presented in Section 5.1.3.3. These data allow each station to be characterized as a gaining or losing stream location based on a comparison of the stream level and groundwater level data. With the exception of monitoring station OC1, level data collected generally show the streams to trend from gaining streams in the upper reaches of the watershed to losing streams in the lower reaches. **Table 6-2** summarizes the findings presented in Section 5.1.3.

Streamflows estimated and presented in Section 5.1.4.1 can also be used to characterize the reaches between stations as gaining or losing streams. In the Leon River watershed, calculated flows typically supported the findings based on relative stream and groundwater levels. Based on comparisons in flow between stations, the stream reach from the confluence of Station Creek and Tributary M to monitoring station SC3 may typically be a gaining stream. Flows calculated at monitoring station SC3 were typically higher than the sum of flows at upstream stations SC1 and TRM1. There do not appear to be any other significant tributaries draining to monitoring station SC3. The stream seems to be gaining the most in winter and early spring. However, by late summer all three of these stream locations are dry. The stream reach between upstream stations SC3 and OC1 and downstream station SC5 appears to be a losing stream based on the flow data, except during significant rainfall events. Flows at monitoring station SC5 were lower than those at monitoring stations SC3 or OC1, except during peaks in flow following rainfall events. Monitoring station SC5 was frequently dry, even when streams at upstream stations were flowing.

Table 6-2
Stream Classifications in the Leon River Watershed, Based on Level Data

Monitoring Station	Stream Classification	Comments
SC1	**Gaining** (Intermittent)	GW levels were always higher than stream levels during the data collection period. This is an intermittent stream, indicating that GW levels are not high enough during dry periods to contribute to stream flow.
TRM1	Typically **gaining**, but losing during NWIRP discharge events. Intermittent stream.	GW level was higher than stream level, except during stream level peaks attributed to NWIRP discharges. Peaks in GW level were evident at MW-SC3 during these discharge events, demonstrating a connection between surface water in Tributary M and groundwater during these events.
SC3	**Transitional**; typically gaining during wet weather, losing during late summer and other dry periods	GW level varies from above to below stream level, depending on frequency and duration of rainfall events
OC1	**Losing**	GW levels consistently lower than stream levels.
SC5	**Transitional**; gaining during winter/spring (intermittent), losing during late summer.	GW levels higher than stream levels until late summer, when they fall below the stream level.
LR1	**Losing**, but weak connection.	GW levels always below stream levels. However, the connection between stream and groundwater systems appears weak, as GW levels do not seem to respond appreciably to changes in stream level.

Stream level data, groundwater level data and estimated streamflows suggest a combination of the views presented in the *Final Conceptual Site Model* (MWH, 2002a) existed in this portion of the Leon River watershed during the data collection period. Most upstream stations in this watershed were typically gaining streams, although intermittent. Groundwater may not be high enough at the stream bed during dry periods to contribute to stream flow. Stations farther downstream were transitional depending on the season. Onion Creek at monitoring station OC1 also appears to be a losing stream. The furthest downstream stations were predominantly losing. These data indicate that contaminated groundwater could potentially enter streams in upstream areas of this watershed and move between the streams and groundwater in central regions on Station Creek. However, contaminated water is unlikely to move between the stream and groundwater around monitoring station LR1.

6.2.2.2 Bosque River Watershed

The portion of the Bosque River watershed potentially affected by runoff from NWIRP McGregor is characterized by monitoring stations on Harris Creek, the South Bosque River, and the Middle Bosque River downstream of the South Bosque River. Stream level and groundwater level data collected over one year are presented in Section 5.1.3.3. These data allow each station to be characterized as a gaining or losing stream location based on a comparison of the stream level and groundwater level data. Due to lack of groundwater data at some stations in this watershed, the Bosque River watershed stations can not be as thoroughly characterized as the Leon River watershed stations were. Monitoring stations in this watershed did not exhibit a trend from gaining to transitional to losing streams as clearly as the Leon River watershed stations. **Table 6-3** summarizes the findings presented in Section 5.1.3.

Table 6-3
Stream Classifications in the Bosque River Watershed, Based on Level Data

Monitoring Station	Stream Classification	Comments
HC1	Transitional	GW levels variable relative to stream level, with GW levels typically higher than stream level in winter/early spring, and stream level higher than GW level in late spring and summer.
HC2	Unknown	Stream level higher than GW level, but the monitoring well is in a bad location and may not be representative (as discussed in Section 5.1.3)
SBR3	Gaining	GW levels consistently higher than stream levels.
SBR1	Gaining	GW levels consistently higher than stream levels.
SBR2	Losing	GW levels typically lower or only slightly higher than stream levels
SBR4	Unknown	No monitoring well at this location.
SBR5	Gaining	GW levels typically higher than stream levels.
MBR1	Unknown	No monitoring well at this location.

Streamflows estimated and presented in Section 5.1.4.1 may also be used to some extent to characterize the reaches between stations. Additional tributaries exist between each monitoring station on Harris Creek and the South Bosque River. Therefore, an increase in streamflow from upstream to downstream does not necessarily mean the stream is a gaining stream in the sense used during this study (i.e., gaining water from groundwater). However, if streamflow decreases from upstream to downstream, that does indicate that the stream reach may be a losing stream. Streamflow calculations for stations on the Harris Creek branch (HC1, HC2, and SBR3) indicate that the stream reach between monitoring stations HC1 and HC2 was a losing stream during the summer months.

During other times of the year, streamflows increased somewhat from upstream stations to downstream stations. Stations on the South Bosque branch (SBR1, SBR2, and SBR4) likewise suggested that the reach between SBR1 and SBR2 could be a losing stream during the summer months.

Stream level data, groundwater level data and estimated streamflows suggest that there is the potential in the Bosque River watershed for contaminated groundwater to enter streams and vice versa. Despite the characterization of these streams as losing streams in some areas, observations do not contradict Yelderman's estimate that 40-60% of the baseflow in the upper portions of this watershed is from groundwater (Dr. Joe Yelderman, personal communication, August 1999). Unlike the upper portions of the Leon River watershed, streams in the Bosque watershed never went dry even during extended periods with limited rainfall.

6.3 LAKE ATTRIBUTES

This summary of the attributes of Lake Waco of the Bosque River watershed and Lake Belton of the Leon River watershed is based on published and technical report data and on the personal observations of Dr. Lind. The principal attributes of Lakes Waco and Belton that are relevant to the fate and transport of river-borne materials are discussed briefly in the subsections below. More detailed information can be found in the *Final Conceptual Site Model* (MWH, 2002a). This section also expands the conceptual model of Lake Belton based on data collected during the ADCP study discussed in Chapter 5.

6.3.1 Lake Waco

The Bosque River watershed drains to Lake Waco, with potentially contaminated surface drainage from NWIRP McGregor travelling via Harris Creek and the South Bosque River to the Middle Bosque River and finally into Lake Waco. Other streams draining into Lake Waco include Hog Creek and the North Bosque River. The North Bosque River, which does not receive any runoff from NWIRP, contributes the majority of inflow to Lake Waco, approximately 80% on average (Dr. Owen Lind, personal communication). The principal attributes of Lake Waco relevant to the fate and transport of river-borne materials include: mixing patterns, flushing rate, water transparency, lake morphometry, and trophic state.

Lake Waco is a moderately eutrophic lake with a reservoir volume of 144,830 AF. During development of the CSM, the normal pool elevation of Lake Waco was 455.0 feet above mean sea level (TWDB, 1994). At this pool elevation, Lake Waco did not develop persistent density (thermal) stratification (Kimmel and Lind, 1972); wind mixing assured both horizontal and vertical mixing of dissolved and suspended materials. However, the USACE and the City of Waco recently completed a pool raising project in Lake Waco that increased the normal pool elevation by seven (7) feet. Although there is no evidence so far of significant changes to this lake as a result of the pool raising project, there is a possibility that the greater depth could allow Lake Waco to stratify. The City of Waco has been conducting an on-going limnological study of this lake, and more data will become available as a result of their work.

Strong wave action is common in Lake Waco and causes shoreline erosion. This wave action re-suspends clays and contributes to turbidity. There is also an aeration system located near the dam that further contributes to vertical mixing in the lake.

Mean multi-year flushing time is one year. (Kimmel and Lind, 1972; Rendon-Lopez, 1997). However, because of the great climatic variability of the region, water retention time is highly variable among years with a range of approximately 0.1 to 5 years. (McFarland et al., 2001). The longer the water retention time, the more opportunity for biological uptake of contaminants. Thus, under high flushing conditions any material entering the reservoir has much less probability of biological uptake than under normal flushing rates.

The warm temperature of the bottom of Lake Waco results in high deep-water bacteria metabolism. Assuming traditional temperature-metabolism coefficients, the metabolic rate would be approximately double that of Lake Belton's deep bacteria (Atlas and Bartha, 1998). Dissolved oxygen solubility in water is a function of temperature: the higher the temperature, the lower the solubility. At 25 degrees Celsius the maximum oxygen is approximately 8 mg/L (ppm) whereas at 15 degrees Celsius it is almost 10 mg/L (Lind, 1985). Because of this low initial solubility and the relatively high content of dissolved organic matter, dissolved oxygen diminishes with depth, even though the Lake is well-mixed. The concentration rarely reaches zero, but does so briefly in scattered depressions (McFarland et al., 2001).

6.3.2 Lake Belton

The Leon River watershed drains to Lake Belton, with potentially contaminated surface drainage from NWIRP McGregor travelling via Station Creek to the Leon River and finally into Lake Belton. Cowhouse Creek also drains into Lake Belton, but the Leon River is Lake Belton's principal source of water.

Lake Belton lies in a long (approximately 21-mi.), narrow and tortuous valley in a generally southerly flowing segment of the Leon River (Lind, 1976), has a volume of 434,500 acre-feet (AF) and has a normal pool elevation of 594.0 feet above mean sea level (TWDB, 1994). The trophic state of Lake Belton may be classified variously because of its length. It experiences one period of top to bottom mixing and one period of density stratification each year (Hutchinson, 1957). The reservoir's morphometry and alignment to the prevailing winds enable the long period of stratification (Lind, 1982). The down-reservoir deeper portion is aligned perpendicular to the prevailing westerly winds. It is situated in a relatively deep valley that provides shelter. The maximum fetch to northwesterly winds is only about 3 mi. The top of the hypolimnion for much of the stratification period is approximately 18-m. The lower 8 to 10 miles of reservoir are of sufficient depth to stratify. Significant portions of the lake of lesser depth are not stratified (Cowhouse arm and upper Leon River arm).

Mean multi-year water retention time is 1.9 years. However, because of the great climatic variability of the region, water retention time is highly variable among years with a range of approximately 0.5 to 6.3 years. The longer the water retention time the more

opportunity for biological uptake of contaminants. Thus, under high flushing conditions, any material entering the reservoir has much less probability of biological uptake than under normal flushing rates.

The dissolved oxygen concentration diminishes rapidly with depth (Lind et al., 2002), reaches zero throughout the lowest layer of the lake by the first part of July and remains so until autumnal mixing, typically during mid November. This stratification is significant, as anaerobic bacteria present in Lake Belton were demonstrated during this study to have the ability to reduce perchlorate to more innocuous species (see Section 5.2.2).

There is evidence of unusual properties of the hypolimnion anaerobic bacterial community of Lake Belton (Rutherford, 1998; Lind et al, 2002; Christian et al., 2002). For most lakes, the abundance and individual cell volumes of bacteria are inversely correlated with oxygen; i.e., anoxia results in more and larger bacteria. For Lake Belton, this is not so. For the hypolimnion near the dam, there was no correlation of either abundance or volume with oxygen concentration. For the hypolimnion near the upper reservoir limits of stratification, the correlation was strongly direct. It has been postulated that this may be the result of different types of organic matter to promote bacterial production.

One data gap identified in the *Final Conceptual Site Model* (MWH, 2002a) was whether preferential flow paths exist in Lake Belton. Based on seasonal ADCP surveys conducted during this study (Section 5.1.4.2), consistent preferential flow and current profiles were not identified. Therefore, thalweg flow does not appear to be consistently present. There also appeared to be no preferential flow toward water intakes.

6.4 WATER BUDGETS

In order to assess the fate and transport of perchlorate in the study area, the volumes of water moving through the surface water and groundwater system from NWIRP McGregor to the lakes needs to be better understood. A water budget, a quantification of inflows and outflows in a watershed, was presented for both Lake Belton and for Lake Waco in the *Final Conceptual Site Model* (MWH, 2002a). These water budgets were described from the perspective of the lakes as inflows, outflows, and changes in lake storage based on data collected by the USACE. Inflows consist of stream inflow, groundwater inflow via seeps and springs, precipitation falling directly on the lake, and discharges of treated effluent into the lakes. Outflows include municipal pumping, dam releases, evapotranspiration and groundwater outflow. If the amount of water stored in each lake does not change (as indicated by the water elevation), inflows should be approximately equal to outflows. If lake storage changes, inflows would not equal outflows for that period of record. This section compares streamflows estimated during this study, as presented in Section 5.1.4.1, to estimated inflows into the lakes to assess the potential volume of water reaching the lakes from NWIRP McGregor.

6.4.1 Leon River Watershed

Runoff from NWIRP McGregor could reach Lake Belton via flows from Tributary M, Onion Creek, and Station Creek discharging into the Leon River, which supplies most of the inflow into Lake Belton.

An analysis of many years of USACE data was presented in the *Final Conceptual Site Model* (MWH, 2002a). These data indicated typically higher inflows from early spring until mid-summer and lower flows during the rest of the year, with extended periods of higher inflows during years with higher than normal rainfall. The average daily inflow (including inflow from all sources) into Lake Belton was calculated to be approximately 690 cfs, with a peak of 81,300 cfs and minimums near zero cfs.

The *Final Conceptual Site Model* (MWH, 2002a) also presented annual water budgets from 1994 to 1999, calculated based on the USACE data. The minimum inflow into Lake Belton during that period was approximately 148,100 AF, and the maximum inflow was estimated to be 1,649,600 AF. Inflow into Lake Belton from October 17, 2002 through October 2, 2003 based on preliminary USACE data was estimated to be approximately 248,000 AF, closer to the minimum inflow observed between 1994 and 1999 than to the maximum inflow. **Table 6-4** presents total flow estimated at each of the Leon River watershed stations during this same period.

Table 6-4
Total Estimated Streamflow at Leon River Watershed Stations
October 17, 2002 through October 2, 2003

Monitoring Station	Total flow (Acre-Feet)	Peak Daily Flow (cfs)	Percentage of Total Inflow into Lake Belton	Percentage of Peak Daily Inflow into Lake Belton
SC1	1,260	30	0.5 %	0.5%
TRM1	1,370	10	0.6%	0.2%
SC3	7,490	60	3.0%	0.9%
OC1	4,370	55	1.8%	0.8%
SC5	1,220	100	0.5%	1.5%
LR1	224,000	1,780	90%	27%
(Lake Belton)	248,000	6,500	100%	100%

The total and peak daily flows at each of the stations upstream of LR1 demonstrate that drainage from NWIRP McGregor makes up a very small percentage of inflow into Lake Belton. The Leon River, however, seems to make up most of the inflow into Lake Belton under average conditions, as 90% of the total inflow into Lake Belton during this period was estimated to come from the Leon River. This observation supports the assumption stated previously in Section 6.3.2 that the Leon River provides most of the inflow into Lake Belton. Despite the high percentage of total flow entering Lake Belton from the Leon River, monitoring station LR1 showed a much lower peak daily flow than the peak flow reported entering Lake Belton. This difference between the high percentage of total

inflow versus the moderate percentage of peak daily inflow suggests that there may be several intermittent streams draining to Lake Belton.

6.4.2 Bosque River Watershed

Runoff from NWIRP McGregor could reach Lake Waco via flows from Harris Creek and the South Bosque River, which discharge into the Middle Bosque River. The Middle Bosque River supplies a portion of the inflow into Lake Waco.

An analysis of many years of USACE data was presented in the *Final Conceptual Site Model* (MWH, 2002a). These data indicated typically higher inflows from early spring until mid-summer and lower flows during the rest of the year, with extended periods of higher inflows during years with higher than normal rainfall. The average daily inflow (including inflow from all sources) into Lake Waco was calculated to be approximately 490 cfs, with a peak daily inflow of 134,400 cfs and minimums near zero cfs.

The *Final Conceptual Site Model* (MWH, 2002a) also presented annual water budgets from 1994 to 1999, calculated based on the USACE data. The minimum annual inflow into Lake Waco during that period was approximately 71,700 AF, and the maximum annual inflow was estimated to be 961,000 AF. Inflow into Lake Waco from October 17, 2002 through October 2, 2003 based on preliminary USACE data was estimated to be approximately 227,000 AF, closer to the minimum inflow observed between 1994 and 1999 than to the maximum inflow. **Table 6-5** presents total flow estimated at each of the Bosque River watershed stations between October 17, 2002 and May 20, 2003, because the data sets at monitoring SBR3 and SBR4 were incomplete. Flow for the entire period from October 17, 2002 through October 2, 2003 are included in parentheses, where available.

Table 6-5
Total Estimated Streamflow at Bosque River Watershed Stations
October 17, 2002 through May 20, 2003

Monitoring Station	Total flow (Acre-Feet)*	Peak Daily Flow (cfs)	Percentage of Total Inflow into Lake Waco*	Percentage of Peak Daily Inflow into Lake Waco
HC1	4,540 (6,190)	65	2.5% (2.7%)	1.9%
HC2	6,280 (6,890)	75	3.5% (3.0%)	2.2%
SBR3	9,960	160	5.5%	4.8%
SBR1	3,460 (4,470)	550	1.9% (2.0%)	17%
SBR2	23,400 (23,500)	450	13% (10%)	14%
SBR4	19,500	920	11%	28%
(Lake Waco)	182,000 (227,000)	3,340	100% (100%)	100% (100%)

* Values in parentheses are for the period from October 17, 2002 through October 2, 2003

Based on these data, drainage from Harris Creek and the South Bosque River could make up approximately 15% of the inflow into Lake Waco on average and approximately 35% of the daily inflow during peak flow conditions. The majority of inflow (approximately 80%) into Lake Waco is thought to come from the North Bosque River, which does not receive any surface runoff from NWIRP.

6.5 MIGRATION PATHWAY ANALYSIS

The hydrologic model and water budget results suggest that significant dilution should occur before perchlorate contamination reaches the lakes. With the exception of a single surface water detection in Lake Waco, two detections in Lake Belton, and two detections downstream of Lake Belton, historical perchlorate data collected by the U.S. Navy support this supposition.

This section adds to information presented in the *Final Conceptual Site Model* (MWH, 2002a) to discuss results from the extensive perchlorate sampling completed during this study. This new information is integrated with the updated hydrological conceptual model and perchlorate fate and transport characteristics to assess potential migration pathways between perchlorate sources and Lakes Belton and Waco.

6.5.1 Nature and Extent of Contamination

Extensive stream water, lake water, and pore water sampling was completed during this study, as described in Chapter 5. Sampling from the lakes included the following:

- Delta area grid sampling of surface water (22 samples from each lake)
- Delta area grid sampling of pore water (63 samples from Lake Belton, 65 samples from Lake Waco)
- Potable water intake sampling (77 samples)
- Irrigation water intake sampling (41 samples)
- Sampling on each ADCP transect, above and below the thermocline (83 samples total)
- Sampling on ADCP transects for preferential flows (22 samples total)

Analytical results for all of the lake samples listed above were below the MDL (1 μg/L) for perchlorate.

Sampling from streams that potentially contain runoff from the NWIRP property included the following:

- Grab sampling from various points on the streams
- Bi-weekly automated sampling from the 15 monitoring stations (typically 35-55 samples per station)
- Automated sampling during two storm events (typically around 100 samples per station per storm)
- Stream pore water sampling

Table 6-6 lists summary statistics for each site from the bi-weekly automated sampling results.

Table 6-6
Summary Results for Automated Stream Sampling

Monitoring Station	Average Perchlorate Concentration* (µg/L)	Maximum Perchlorate Concentration (µg/L)	Maximum Perchlorate Concentration During a Storm Event (µg/L)
Leon River Watershed:			
TRM1	10.1	111	3.0
SC1	ND	ND	ND
SC3	5.11	38	11
OC1	0.56	3.0	ND
SC5	2.73	7.6	No samples collected; site dry
LR1	ND	ND	ND
Bosque River Watershed:			
HC1	1.81	6.5	2.0
HC2	1.25	4.8	2.0
SBR3	0.88	2.0	ND
SBR1	2.73	4.7	4.0
SBR2	1.51	3.0	2.0
SBR4	1.10	3.0	1.0
SBR5	0.81	2.0	2.0
MBR1	ND	ND	ND
Cowhouse Creek:			
CHC1	ND	ND	ND

* Averages from bi-weekly automated sampling. These averages do not include storm samples.
ND = < 1µg/L

As seen on **Table 6-6**, perchlorate concentrations decreased moving from upstream at monitoring station TRM1 on Tributary M downstream to monitoring station LR1 on the Leon River. Likewise, perchlorate concentrations detected along Harris Creek and the South Bosque River also decreased moving downstream. Neither monitoring station LR1 on the Leon River nor monitoring station MBR1 on the Middle Bosque River, both of which discharge directly into Lake Belton and Lake Waco, respectively, had any samples with detectable perchlorate concentrations.

Prior to sampling, storm events were thought to potentially cause more perchlorate contamination to reach the lakes, due to first flush effects. However, the stream sampling conducted during two storm events discounted this theory at most stations. As seen on the data plots from the first storm event, included in **Appendix F**, monitoring stations SBR1 and SC3 were the only two stations where a first-flushing effect was observed, evidenced

by a small spike in perchlorate concentrations following the storm. However, perchlorate concentrations were then diluted rapidly due to increased flow. No other monitoring stations showed a first flush effect, but some uncertainty on possible first-flush effects remains. The two storm events sampled during the study were not the largest storms that occurred during the study period. Monitoring station SC3 was the only station with a perchlorate concentration above the RL (4 µg/L) during a storm event.

6.5.2 Primary Migration Pathways

Because NWIRP McGregor is located on a ridge separating the Bosque and Leon River watersheds, releases of perchlorate from the facility disperse in two main directions, primarily in surface water and groundwater. Harris Creek and the South Bosque River transfer water northeast to the Middle Bosque River and finally to Lake Waco. Station Creek transports water south to the Leon River and finally to Lake Belton. The major inflow source of water to the watersheds is precipitation.

As discussed in Section 6.2.2**,** several of the streams studied apparently transition between gaining and losing streams, allowing the chance for contaminant migration from groundwater to surface water and vice versa. Additionally, groundwater is thought to make up a significant percentage of the baseflow in the upper portions of the streams in the Bosque River watershed.

Initial analysis presented in the *Final Conceptual Site Model* (MWH, 2002a) suggested that significant dilution should occur before perchlorate contamination reaches the lakes. The extensive stream and lake sampling conducted during this study supported this initial assessment and further demonstrated that significant dilution occurs by the time perchlorate contamination reaches the Leon and the Middle Bosque Rivers. Estimates of the perchlorate concentration necessary to potentially cause a detection at or above the RL (4 µg/L) in one of the lakes are presented in **Table 6-7**.

These estimates are based on the percentage of total lake inflow at each of the monitoring stations presented in Section 6.4. This approach assumes that all of the flow at any particular station eventually reaches the lake and assumes that the lake inflow is well-mixed.

The concentrations listed for average lake inflow are the concentrations required at that station to result in a concentration in the lake inflow at the RL, based on nearly one year of station flow data and lake inflow data. To achieve a sustained perchlorate concentration at the RL in the lake during this year, the concentration at a particular station would need to average the value presented in this table. A spike in concentration to these levels may cause a spike up to the RL in the lake inflow, but would quickly be diluted by less contaminated water. Actual average perchlorate concentrations detected at the Leon River watershed stations during this period ranged from 0.1% to approximately 11% of these values. The 11% is based on assuming a value of 0.5 µg/L, half of the MDL (1 µg/L), at station LR1, as there were no detections at this station. Not including this station, percentages ranged up to approximately 2% of these values. Actual average

perchlorate concentrations detected at the Bosque River watershed stations ranged from approximately 0.8% to 4% of these values.

Concentrations listed under peak day inflow are the concentrations that would have been required under peak flow conditions (storm events) to result in a peak concentration in the lake inflow at the RL during the monitoring period. Again, a spike in the perchlorate concentration to these levels would have resulted in a spike in the lake inflow concentration to the RL, but if station concentrations subsequently decreased, that spike in the inflow concentration would quickly be diluted. Actual concentrations measured during storm events ranged from 0.1% to approximately 17% of these values.

Table 6-7
Estimated Perchlorate Concentrations Necessary to Cause a Detection at the RL (4 µg/L) in the Lakes

Monitoring Station	Percentage of Total Lake Inflow	Percentage of Peak Daily Lake Inflow	Perchlorate Conc. To Reach RL in Lake Inflow (Average Inflow) µg/L	Perchlorate Conc. To Reach RL in Lake Inflow (Peak Day Inflow) µg/L
Leon River Watershed				
SC1	0.5%	0.5%	800	800
TRM1	0.6%	0.2%	670	2000
SC3	3.0%	0.9%	130	440
OC1	1.8%	0.8%	220	500
SC5	0.5%	1.5%	800	270
LR1	90%	27%	4.4	15
Bosque River Watershed				
HC1	2.7%	1.9%	150	210
HC2	3.5%	2.2%	110	180
SBR3	5.5%	4.8%	73	83
SBR1	2.0%	17%	200	24
SBR2	13%	14%	31	29
SBR4	11%	28%	36	14
SBR3 + SBR4	17%	33%	24	12

7.0 EXPOSURE ASSESSMENT

Exposure assessment is an analysis of the potential exposure pathways between the source of a chemical or physical contaminant and human or ecological receptors. A preliminary exposure assessment was presented in the CSM report prepared by the project team (MWH, 2002a). This section of the report presents a summary of the preliminary information contained in the CSM report and updates the exposure assessment based on the extensive field and laboratory work conducted since the initial assessment.

Previous sections of this report have described the current understanding of the potential for perchlorate releases from former NWIRP McGregor to impact various environmental media (i.e., surface water, groundwater, soil, sediment, or biota) within the Bosque and Leon River watersheds. The results of extensive investigations into the effects of perchlorate on various receptors were also presented. Based on these data, the updated exposure analyses presented in this section describe potential exposures of human or ecological receptors to perchlorate at the point of contact with these known or potentially impacted media. To the extent possible, the effects of these exposures are also described, either from the results of field investigations, laboratory studies, or modeling.

Information on potential exposure pathways is presented in Section 7.1. A human health exposure analysis is presented in Section 7.2. An analysis of potential exposures for ecological receptors is presented in Section 7.3.

7.1 DESCRIPTION OF POTENTIAL EXPOSURE PATHWAYS

For human or ecological exposure to a chemical contaminant from a site to occur, a complete exposure pathway between the source of the contaminant and a receptor must exist. A complete exposure pathway as defined by Risk Assessment Guidance for Superfund (RAGS) (USEPA, 1998a) consists of four essential elements, as follows:

- Source: A source of contamination and a mechanism of release.
- Medium: A receiving or transport medium (soil, sediment, groundwater, surface water, air, or food). Once released to the receiving or transport medium, transformations may occur (for example, biodegradation) that can change the chemical.
- Exposure Point: A point of potential human or ecological contact with the receiving or transport medium.
- Exposure Route: A route for the contaminant to enter the organsim, such as eating and drinking (ingestion), skin (dermal) contact, or breathing (inhalation).

If one or more of the above elements is absent, then the exposure pathway is incomplete, and no exposure can occur.

Source and Transport Media. NWIRP McGregor is a known source of perchlorate contamination. From this source, perchlorate could be released to various receiving and transport media such as surface water, groundwater, sediment, soil, or food. Erosion and runoff from former NWIRP McGregor soils that are contaminated with perchlorate, direct deposition of perchlorate-containing wastes, or interaction between surface water and contaminated shallow groundwater could potentially contaminate surface water and sediment within the Bosque and Leon River watersheds. Perchlorate can enter groundwater through migration from surface and subsurface soils, surface water, or through direct deposition of wastes into water-bearing soils. Data from this study document extensive interactions between surface water and shallow groundwater along the stream systems that originate at NWIRP McGregor (Section 5.1.3, Chapter 6), providing a pathway for any perchlorate contamination present to migrate between the surface water system and groundwater system. Irrigation of commercial and residential lawns, other garden/landscape areas, and stock ponds with perchlorate-contaminated water may transport perchlorate to soil. Additionally, this study has documented that perchlorate can be released back into the soil from plant leaves after they fall (Section 5.3.3).

Plants and animals exposed to perchlorate contamination directly from any of the transport media discussed above could themselves become transport media to higher trophic level species (including humans) via the food chain. Uptake of perchlorate by aquatic producers (e.g., phytoplankton and algae) was demonstrated to occur within surface water bodies of the Bosque and Leon River watersheds. Once absorbed by producer level receptors, there is a potential for transfer of perchlorate to consumer level receptors including aquatic invertebrates, planktivorous fish, and herbivorous aquatic birds. Additionally, leaf litter data collected from terrestrial plants during this study suggest that perchlorate could be released from fallen tree leaves. This may be viewed as an additional mechanism for perchlorate exposure to lower trophic systems. Consumer species in turn provide a means of perchlorate transfer to higher trophic level receptors including omnivorous or carnivorous fish, amphibians, mammals, and birds that may prey on the contaminated herbivores. For receptors that are located closest to an area of perchlorate contamination, those species with small foraging ranges (e.g., bullhead minnow, yellow mud turtle, red-winged blackbird) would be expected to have higher exposures to perchlorate than those species with large foraging areas (e.g., white bass, armadillo, great blue heron) because the latter tend to forage over areas of varying degrees of contamination.

Exposure Points. Exposure points exist wherever humans or ecological organisms could come into contact with potentially contaminated receiving or transport media. Examples of potential exposure points include a contaminated body of water in which a person or organism swims, a contaminated spring where people or animals drink water, or the root zone of a plant in contact with contaminated soil and groundwater. Potential exposure points vary depending on the human or ecological receptor group considered. These groups and their likely exposure points are discussed in more detail in Section 7.2 Human Exposure Analysis and Section 7.3 Ecological Exposure Analysis.

Exposure Routes. Of the three potential exposure routes presented above (ingestion, inhalation, or dermal contact), ingestion (including uptake by plants) is considered to be by far the most significant route of perchlorate exposure for both human and ecological receptors. Exposure to perchlorate via dermal contact is deemed insignificant because the high ionic charge on perchlorate inhibits transfer through the skin (Scheuplein and Bronaugh, 1983). Inhalation of gaseous phase perchlorate is considered insignificant because the vapor pressure of perchlorate salts and acid solutions is low (Allred, 1998). Although inhalation of particulate contaminants in ambient air or contaminated water as an aerosol could potentially result in minor exposure, incidental ingestion of larger particles or droplet-sized water is considered to be a much more significant exposure route. Therefore, in discussion of potential human and ecological exposure pathways to perchlorate, ingestion is considered the only realistic exposure route.

Potentially complete and incomplete exposure pathways for various human and ecological receptors are described in Section 7.2 and Section 7.3.

7.2 HUMAN EXPOSURE ANALYSIS

The updated human exposure analysis described in this section considers current and future land uses, human activities and receptors consistent with these land uses, and exposure pathways between human receptors and contaminated media based on investigations conducted during this study.

7.2.1 Land Uses

The study area includes portions of Bell, Coryell, and McClennan counties. There are rural or developed communities within each of these counties. Current land use indicates urban, pasture, range/forest, crops, and dairy waste fields (EnSafe, 1999a). Land and properties surrounding NWIRP McGregor are primarily agricultural, but little to no farming occurs in the Washita Prairie west of the South Bosque River due to calciferous soils. East of the South Bosque River are fertile, black soils that support non-irrigated row-crop farming. The City of McGregor, which adjoins the facility at the northeast corner, has a population of approximately 4,700. Land bordering the east side of NWIRP McGregor is zoned residential and land near the southern boundary supports commercial and light manufacturing operations and a university research center. Just south of the residential area is the McGregor High School. The remainder of the area is sparsely populated open farming and grazing land (EnSafe, 1999a).

Lake Waco and Lake Belton provide drinking water for nearly 500,000 citizens (Brazos River Authority, 2001b). Seeps, dug wells, and private wells supply additional sources of drinking water and/or water for irrigation or stock ponds.

Lake Waco and Lake Belton also support a variety of recreational activities including boating, swimming, and fishing. The Bosque River supports a significant recreational fishery.

7.2.2 Human Receptors

Based on the land uses described above, several potential human receptors were identified. These potential human receptors are listed in **Table 7-1**.

Table 7-1
Potential Human Receptors

Potential Human Receptor	Description
Public Water Supply Users	Individuals who use water from the public water supply. This category makes up the vast majority of potential human receptors.
Residential Users of Local Surface Water and/or Shallow Groundwater	Individuals who rely on water directly from springs, streams, or shallow groundwater within the study area instead of on the public water supply for their potable water source. This category includes a limited number of potential receptors.
Commercial/Industrial Workers	Commercial and/or industrial workers on lands formerly comprising NWIRP McGregor or in the vicinity of NWIRP McGregor within the study area. This group does not include NWIRP site workers.
Agricultural Workers	Agricultural workers within the study area
Recreational Users	Hunters, hikers, campers, fisherman, swimmers, water skiers and other recreational users within the study area.
NWIRP Vicinity Residents	Individuals residing in proximity to NWIRP McGregor and/or areas where surface water, groundwater, soil or sediment media are impacted by perchlorate.

The potential exposure media and possible exposure routes associated with each medium are described in the following subsections. **Table 7-2** lists potential human receptors and the exposure pathway elements for each receptor. The sub-sections following this table present more detailed findings for each of these receptor groups.

Table 7-2
Potential Human Receptors and Exposure Pathways

Receptor	Exposure Pathway Elements (+ means present)					Comments
	Perchlorate Source and Release	Receiving or Transport Medium	Exposure Point	Exposure Route	Complete Exposure Pathway	
Public Water Supply Users	+	+	-	+	No	Perchlorate is not detectable in the public water supply, water supply intakes, or the Lake Belton and Lake Waco intake source waters.
Residential Users of Local Surface Water and/or Shallow Groundwater	+	+	+	+	Yes	Surface water and shallow groundwater in the vicinity of NWIRP are impacted by perchlorate, but most residents within the study area are thought to be in the Public Water Supply Users category.
Commercial/Industrial Workers	+	+	-	-	No	Exposure of commercial /industrial workers is not anticipated due to the absence of points of exposure and exposure routes.
Agricultural Workers	+	+	+	+	Yes	Soil and sediment pathways are considered to be theoretically complete via incidental ingestion, but the likelihood of such an exposure occurring is thought to be extremely small.
Recreational Users	+	+	+	+	Yes	Complete pathways include the food chain and incidental ingestion of surface water, soil and sediment.
NWIRP Vicinity Residents	+	+	+	+	Yes	Complete pathways include the food chain and incidental ingestion of soil and sediment.

7.2.2.1 Public Water Supply Users

The public water supply users receptor group includes approximately 500,000 people who rely on public water from the Bosque and Leon River watersheds. This group makes up the vast majority of potential human receptors. The primary transport medium of

concern for this group is surface water, specifically water from Lake Belton and Lake Waco.

Public water supply in the study area is a mix of surface water from Lakes Waco and Belton as well as limited local groundwater from city wells (Study Area Stakeholders Alliance, 1998). The City of Waco operates the Lake Waco intake structure located adjacent to the dam and serves the City of Waco and several adjacent communities (Study Area Stakeholders Alliance, 1998).

Three intake structures draw water from Lake Belton:

- City of Gatesville Intake – supplies water to the Cities of Gatesville, North Fort Hood, Coryell, Grove, Flat, Bound, Pancake, Mountain Community, and Fort Gates (City of Gatesville, personal communication, August 1999);.
- Blue Bonnet Water Supply Corporation – supplies water to the Cities of McGregor, Moody, Bruceville-Eddy, Woodway, Moffat, Pendleton, Elm Creek, and Spring Valley (Bluebonnet Water Supply Corporation, personal communication, August 1999); and
- Bell County WCID #1 – supplies water to the Cities of Belton, Nolanville, Fort Hood, Killeen, Harker Heights, and Copperas Cove (Bell County WCID, personal communication, August 1999).

Downstream of the Lake Belton Dam, the City of Temple operates a diversion and accompanying intake structure that supplies water to the Cities of Temple, Morgan's Point, Troy, and Little-River Academy (Jerry Kean, City of Temple, personal communication, August 1999).

Although the public water supplies for the Cities of Oglesby and Robinson are obtained from city wells screened in the Trinity Aquifer (Study Area Stakeholders Alliance, 1998), these water supply sources are not anticipated to be a potential source of exposure. This aquifer is a deep aquifer and is not connected to the shallow aquifer affected by contamination at NWIRP (refer to Section 6.2).

Extensive sampling during previous studies (approximately 1200 samples) have resulted in only five perchlorate detections. Sampling throughout the lakes and at the water intakes during this study demonstrated that perchlorate is not present at detectable concentrations in any of these potable water sources. Therefore, based on data collected during this study, direct human consumption of the public water supply is considered to be an incomplete pathway, and public water supply users are at no risk of perchlorate exposure from this source.

7.2.2.2 Residential Users of Local Surface Water and/or Shallow Groundwater

As discussed in **Table 7-1**, the residential users receptor group includes people who rely on water from streams, springs, or dug wells for their potable water source. The transport media of concern for this receptor group are surface water and groundwater.

Surface water potentially used for human consumption includes springs at a few residential locations and streams. Some dug wells have also been historical sources of drinking water (EnSafe, 1999a). Due to the extensive connection between the surface water and groundwater systems documented during this study, residential users of local surface water and/or shallow groundwater in the vicinity of NWIRP could potentially be exposed to perchlorate from such a water source anywhere surface water or groundwater have detectable concentrations of perchlorate. The U.S. Navy has documented the locations of perchlorate-contaminated groundwater plumes in the vicinity of NWIRP McGregor. The extent of these plumes is shown on **Plate 6**. Surface water areas that tested positive for perchlorate at any time during auto-sampling (see Section 5.1.1.2) during this study are also shown on **Plate 6**. The likelihood of potential exposure from consumption of surface water or groundwater decreases with increasing distance from NWIRP. Perchlorate was not detected (detection limit of 1 µg/L) in the Leon River or in the Middle Bosque River.

7.2.2.3 Commercial/Industrial Workers

This human receptor group includes commercial and/or industrial workers on lands formerly comprising NWIRP McGregor or in the vicinity of NWIRP McGregor within the study area (not including NWIRP site workers). This group has no known potential for contact with contaminated media, for several reasons. These reasons include the following: (1) many source areas have been remediated in some way, thereby limiting direct contact with source areas, (2) commercial and industrial workers do not work in areas with residual contamination, (3) other workers, such as construction workers, would work under an approved health and safety plan, and (4) deed restrictions exist on the use of certain properties formerly comprising NWIRP to ensure that activities are limited to those that would not result in exposure to perchlorate.

7.2.2.4 Agricultural Workers

Agricultural workers in the NWIRP area could potentially be exposure to perchlorate via incidental ingestion of soil particulates. Although the exposure pathway is technically complete for this group, the likelihood of such an exposure is thought to be extremely small. A limited amount of agricultural work is done near NWIRP and there is very little possibility that an agricultural worker could ingest enough particulates to constitute a significant exposure.

7.2.2.5 Recreational Users

Hunters, hikers, campers, fishermen, swimmers, water skiers, and other recreational users within the study area make up the recreational users receptor group. Transport media of potential concern to this group include surface water, soil, sediment, and food items such as plants or animals obtained from contaminated areas.

<u>Surface Water.</u> Because detectable concentrations of perchlorate were not found in either Lake Waco or Lake Belton during this study, recreational users of Lake Waco and Lake Belton are not considered to be susceptible to exposure via incidental ingestion while swimming in the lakes. However, incidental ingestion during swimming or wading could occur elsewhere in the watersheds where perchlorate is present at detectable

concentrations. Additionally, as with the Residential Users of Local Surface Water and/or Shallow Groundwater group, exposure could occur if recreational users consume water from perchlorate-contaminated springs or streams. **Plate 6** shows areas where perchlorate was detected in one or more surface water auto-samples (see Section 5.1.1.2) during this study.

Soil and Sediment. Any outdoor activities that involve digging into or contacting soil or sediment may result in potential incidental ingestion of particulates. This pathway is therefore technically complete for recreational users in portions of the study area contaminated by perchlorate. However, the likelihood of direct exposure from these media is extremely small. Locations that had detectable perchlorate concentrations in sediment pore water are indicated on **Plate 6**.

Food. Human receptors may also be exposed to perchlorate indirectly via consumption of plants or animals that have taken up this contaminant through ingestion of contaminated water, sediment, soil, or food sources. Laboratory studies and field sampling within the study area indicated that perchlorate is readily accumulated in plants exposed to water containing perchlorate. Therefore, recreational users who gather and consume wild vegetation near perchlorate-contaminated springs or streams could potentially be exposed to perchlorate. Based on field and lab data, the highest potential exposure to perchlorate from plants is most likely to come from leafy vegetation of plants, although modeling predicts that perchlorate should also accumulate in fruit and seeds (Section 5.3.5). This exposure source is limited to plants along streams where perchlorate is detected regularly in surface water. The likelihood that plants would contain perchlorate decreases with increasing distance from a contaminated water source. **Plate 6** indicates streams where perchlorate was detected at any time during this study, as well as locations where sampled vegetation had detectable levels of perchlorate.

Fishers and hunters who eat their game may also potentially be exposed to perchlorate through this source. Perchlorate was detected in some fish collected throughout the Bosque and Leon River watersheds during this study. Perchlorate detections in fish were sporadic, with perchlorate being detected in only some individuals at a particular location. This study found that, in fish, the greatest area of accumulation is the head, but perchlorate was also detected in some fillets. Since the fillet tissue is typically consumed by humans, consumption of fish from contaminated areas is a potential human exposure point.

Unlike plants, detections in fish were not restricted to areas with contaminated water, and there were detections in a few of the fish caught within both Lake Waco and Lake Belton. Perchlorate was detected in edible portions of fish fillets at levels as high as 1.4 ppm in sport fish (largemouth bass) and as high as 3.9 ppm in non-game fish (spotted gar). In Lake Waco there were a total of eight perchlorate detections in both fish fillets and heads (detection limit was approximately 50 ppb, higher for some species). Three of the detections were in fillets, out of 65 total fillets analyzed. The fish in which perchlorate was detected were large-mouth bass, channel catfish, and black crappie. All of these fish were of legal size and are typical sport fish. In Lake Belton there were a total of six

perchlorate detections in fish fillets out of 54 fillets analyzed (detection limit was approximately 50 ppb, higher in some species). The fish in which perchlorate was detected in Lake Belton were largemouth bass, channel catfish, spotted gar, and drum. All of these fish were of legal size, although spotted gar and drum are not typically desired species for human consumption. Most of the fish collected from streams in the study area (the most perchlorate-impacted areas) were not of legal size (> 8 inches), and the streams identified to have the greatest perchlorate concentrations are not likely large enough to support fish of legal catchable size.

Although human exposure to perchlorate via consumption of fish caught in the watershed is possible, both laboratory and field data indicate that human exposure to perchlorate through this pathway in the study watersheds is unlikely. This conclusion is based on (1) where perchlorate occurs in surface water and the size of fish supported by those streams, (2) the preferential accumulation of perchlorate in rarely consumed tissue (head) rather than the fillet, and (3) the low frequency of detections in catchable fish. The data indicate that people who consume fish from Lake Waco and Lake Belton may periodically be exposed to low levels of perchlorate, but because benchmark doses have not yet been established, it is not possible to determine if the potential exposure is within acceptable limits. Locations where perchlorate was detected in the head or fillet of at least one fish are shown on **Plate 6**. Note that not all of these detections occurred in fish typically consumed by humans or in fish of legal catchable size.

Medium and large animals typically hunted by people were not tested for perchlorate during this study. However, raccoons and opossum captured near the most contaminated stream in the study area (S Creek) did not contain perchlorate in blood (detection limit of approximately 14 ppb), nor did they show signs of thyroid histology abnormalities. This study also evaluated the potential for exposure to perchlorate from consumption of beef exposed to perchlorate in the study area. Perchlorate was seldom detected in the plasma of cattle exposed to perchlorate through water, and was not detected in any of the samples of beef typically consumed by humans. These results suggest that the potential for perchlorate exposure to hunters who consume game from the study area is small. Some uncertainty in this conclusion remains, however, as mammal species typically hunted and consumed by people were not directly tested during this study.

7.2.2.6 NWIRP Vicinity Residents

The NWIRP Vicinity Residents receptor group includes individuals residing in proximity to NWIRP McGregor and/or areas where surface water, groundwater, soil, or sediment are impacted by perchlorate. This group is not intended to include people who rely on springs and shallow groundwater for their potable water source, as these people were included in the Residential Users of Surface Water and/or Shallow Groundwater group discussed in Section 7.2.2.2, but it does include people who may use the water for other purposes, including garden irrigation. In addition, people who gather and consume wild vegetation or who fish or hunt in the area were included in the Recreational Users group discussed above in Section 7.2.2.5.

Although individuals in this group could potentially be exposed to perchlorate through incidental ingestion of contaminated soil or sediment, as discussed for other receptor groups, exposure through these media is extremely unlikely. Therefore, the primary medium presenting a potential exposure to this group is food. Research performed during this study analyzed the potential for perchlorate uptake in vegetable and animal products typically consumed by humans.

Vegetation. NWIRP vicinity residents who irrigate their gardens with perchlorate-contaminated stream or well water and consume vegetation from these gardens may be exposed to perchlorate. Laboratory studies and field sampling within the study area indicated that perchlorate is readily accumulated in plants. Cucumbers, soybeans, and lettuce were included in the perchlorate uptake experiments performed in the laboratory. All were found to take up perchlorate when exposed, with the highest perchlorate concentrations taken up in lettuce. The highest exposure is likely to come from green leafy vegetation, but modeling predicts that perchlorate should also accumulate in fruit and seeds (refer to Section 5.3.5). If plant species are irrigated naturally or artificially with water containing perchlorate, uptake will occur, including uptake into edible portions of the plant. During this study, however, very few vegetable/edible plant gardens were found near water sources with detectable levels of perchlorate. Surface water sources found to have at least occasional detectable perchlorate concentrations during the extensive sampling conducted during this study are shown on **Plate 6**. The plumes of contaminated groundwater as determined by the U.S. Navy are also shown on **Plate 6**.

Animal Products. Animals typically consumed by humans potentially could uptake perchlorate from a contaminated water or food supply (Smith et al., 2001). This study evaluated the potential for exposure to perchlorate from consumption of beef derived from cattle exposed to perchlorate in the study area. These cattle had constant exposure to perchlorate in water for 14 weeks. Perchlorate was detected in the plasma of one animal but was not detected in any of the edible tissues typically consumed by humans (detection limit of 23 ppb). Direct consumption of beef from the study area therefore does not appear to represent an exposure pathway, although some uncertainty regarding this remains due to limitations in the study.

Although perchlorate in the low ppb range has recently been reported in milk intended for human consumption (outside of the NWIRP area) (Kirk et al., 2003), no commercial dairies were identified within watershed areas where perchlorate impacts were identified. Non-commercial use of milk from local cattle or goats in the study area remains an area of uncertainty. Potential exposure to perchlorate through consumption of products from other livestock (chickens, pigs, etc.) also remains an area of uncertainty.

7.3 ECOLOGICAL EXPOSURE ANALYSIS

The updated ecological exposure analysis described in this section includes an assessment of the biological resources within the study area, and an evaluation of potential exposure pathways between these resources and contaminated media.

7.3.1 Study Area Biological Resources

Biological resources consist of vegetation and wildlife in addition to ecological processes and significant ecological features of the area. A description of biological resources within the study area was presented by the project team in the CSM report (MWH, 2002a), including comprehensive lists of the vegetation and wildlife potentially occurring in the study area. Only some of the classes and species of organisms potentially inhabiting the study area were directly tested for exposure to perchlorate. These specific ecological receptors are discussed in Section 7.3.2, below.

Within the study area, there also potentially exists species listed as state and/or federally endangered, threatened, candidate, sensitive, species of concern, proposed threatened, proposed endangered, and priority for conservation and management. There could also be species identified as rare without specific listing status. Texas' special species that occur within the counties of Bell, Coryell, Falls, and/or McLennan were listed in the CSM report (MWH, 2002a, Table 6-6). These species were not specifically evaluated in this study, and the potential impacts of perchlorate on threatened and endangered species is an area of uncertainty. Although studies conducted on avian and mammalian species indicate that species inhabiting the study area are at low risk for population level declines related to perchlorate exposure and/or toxicity, none of the species examined were listed as threatened or endangered by the US Fish and Wildlife Service. Therefore uncertainty remains as to potential individual injury or population declines among any of these species inhabiting or migrating through the study area which may be subsequently exposed to perchlorate. Due to uncertainty about the occurrence of threatened and/or endangered species within the study area and a lack of perchlorate-specific toxicity data pertaining to these species potentially occurring within the study area, no definitive assessment of the implications of perchlorate on threatened or endangered species can be derived from the results of this study.

7.3.2 Ecological Receptors

The CSM report (MWH, 2002a) indicated that potential for perchlorate exposure, however remote, exists for all species present within the riparian habitat. However, actual exposures will vary significantly between feeding guilds and individual species depending upon the manner in which they interact with individual components of the watersheds. This study focused on filling high priority data gaps necessary to provide a more detailed evaluation of the potentially complete and incomplete exposure pathways for broad categories of ecological receptors.

Ecological receptor groups tested for potential exposure to perchlorate included aquatic and terrestrial plants, fish, frogs, birds, and small, medium, and large mammals. For most of the potential receptors, those that are located nearest to areas impacted by perchlorate appear to have the highest exposure to perchlorate. Fish were demonstrated to be one exception to this rule, as detectable concentrations of perchlorate were found in some fish in areas with no detectable perchlorate in the water at the time of collection (detection limit in water of 1 ppb), and some fish that were collected from contaminated water had no detectable concentrations of perchlorate (detection limit in fish of approximately 50

ppb). Each of the ecological receptor groups studied are discussed in the following subsections.

7.3.2.1 Plants

Sampling of plants in and near perchlorate-contaminated streams in the study area indicate that plants near these streams may take up perchlorate. Findings pertaining to exposure of aquatic and terrestrial plants and results from modeling of perchlorate uptake in plants are discussed in the subsections below.

7.3.2.1.1 Aquatic Plants

This study found that in all streams in which perchlorate was detected, the two aquatic plant species (smartweed and water cress) present in the offsite area were correspondingly found to have detectable concentrations of perchlorate. In general, good linear correlation was found between water concentrations and plant concentrations, indicating that surface water provides a complete and significant pathway for exposure of aquatic plants to perchlorate. Concentration factors for perchlorate in aquatic plants were in the range of 100 to 300. Perchlorate concentrations in aquatic plant species appear to exist largely in a state of pseudo-equilibrium with perchlorate concentrations in the water. Plant concentrations will often lag changes in water concentrations and may represent a better indicator of long-term exposure than water, as water concentrations can be highly variable depending on source control efficiency and precipitation. Locations where perchlorate was detected in aquatic plant tissues are shown on **Plate 6**.

7.3.2.1.2 Terrestrial Plants

Terrestrial plants are capable of perchlorate uptake if exposed to perchlorate. Trees located near contaminated surface water or contaminated groundwater were found to have perchlorate residues in their leafy vegetation. Perchlorate concentrations in terrestrial plants were more variable than in aquatic plants, probably due to the more variable nature of the source water. Most tree species obtain their water from within the vadose zone of the soil profile and not from bulk free flowing surface water. As such, changes in biogeochemistry throughout the year coupled with fluctuating water tables and water availability equate to a highly variable source contribution. In addition, potential leaching of perchlorate from leafy vegetation during precipitation events may also contribute to the large variability found. Regardless, leaf concentrations of perchlorate generally increased throughout the growing season and in general represented a higher perchlorate burden by mass than source water. Vegetative uptake of perchlorate in terrestrial species appears to be a function of both exposure concentration and length of exposure, suggesting a larger potential exposure for terrestrial plants than aquatic plants. Uptake of perchlorate by terrestrial plant species cannot be viewed as permanent sequestration. Leaf litter data suggest that perchlorate could be released once plant leaves senesce in the fall. This process may be viewed both as a mechanism of perchlorate exposure to lower trophic systems and a means of contaminant migration.

7.3.2.1.3 Plant Modeling

Simulations representing “worst-case” scenarios were run for terrestrial plants and aquatic macrophytes using perchlorate concentrations (270 ppb ± 157 ppb) measured at S

Creek. It was assumed that both terrestrial and aquatic plants were exposed to the same concentrations of perchlorate. The modeling indicates that dynamic patterns of perchlorate concentration are quite similar in terrestrial plants and aquatic macrophytes. The simulation results showed an initial increase in perchlorate concentration at the start of the growing season. As plant biomass increased, relative to perchlorate uptake, there was a small decrease in concentration. When the plants reached maximum growth, biomass remained relatively constant, uptake of perchlorate continued, and concentration also increased. At the end of the growing season, both biomass and perchlorate decreased as a result of senescence and mortality, resulting in a decrease in perchlorate concentrations within the plants.

The predicted concentrations in both the leaves and fruits increased significantly more than in the roots and stems. This was a result of the water being translocated from the root and stem compartments and sequestration of perchlorate in the leaves and fruits. The model predicted that the concentrations in both leaves and fruits continued to increase after the end of the growing season. Although there is no perchlorate accumulating in the leaves during this time of year, the amount of perchlorate decreases slightly less than the leaf biomass decreases, resulting in a higher concentration.

The model predicts that perchlorate is capable of bioaccumulation in the leaves and fruits of exposed plants. The model results are consistent with lab and field observations and suggest that there is potential for perchlorate exposure to wildlife and humans that consume exposed plants. Although parameter estimates were based on calibration with lab experimental data, direct measurements of unknown or assumed model parameters would improve the accuracy of model predictions.

7.3.2.2 Aquatic Animals

Field and laboratory studies were conducted on fish and frogs in the study area to evaluate the potential for these organisms to be exposed to perchlorate. The extent of perchlorate exposure determined by these studies is discussed in the following subsections.

7.3.2.2.1 Fish

Fish Field Survey. Perchlorate was detected sporadically in fish tissue samples from fish collected from streams and lakes in the study area, but it was detected in only some individuals and some species. Perchlorate was found more often in small insectivorous species (e.g., mosquitofish), sunfish, largemouth bass, and catfish, and often was found in fish tissues at concentrations higher than those found in the water. This difference in concentrations could be due to pathways of exposure other than the water column (e.g., food sources such as invertebrates, algae, etc), or to temporal variability in perchlorate exposure, followed by a slower decrease in fish tissues than in the water column. Plants may represent a larger exposure pathway to fish than direct water, as algae are a good food source for aquatic organisms, and bioconcentration factors in algae were determined to range from 100 to 300. Nonetheless, these findings do indicate that fish are being exposed to perchlorate and taking it up from the environment. Locations where perchlorate was detected in fish are shown on **Plate 6**.

The thyroid histopathology of fish collected from sites contaminated with perchlorate suggests that these fish were exposed to perchlorate found in these streams. The level of exposure was associated with monitoring data indicating the presence of perchlorate, although perchlorate is not the only environmental contaminant that could influence changes in thyroid histology. Because of the involvement of thyroid hormones in reproduction and development, it is possible that the ecological fitness of these fish may be affected. However, because population-level and community-level endpoints were not investigated, the relationship to higher-level effects is unclear.

Fish Laboratory Studies. Laboratory studies showed that perchlorate uptake from water was rapid in the fish species studied, reaching steady state within a matter of days. Perchlorate elimination was also relatively rapid, but there was a large amount of inter-individual variation in elimination rates. In particular, after 20 days of depuration, perchlorate was still detected in some individuals, but not in others. If perchlorate exposure in the field is highly variable, with perchlorate spikes being followed by rapid drops in water concentrations, this high inter-individual variability in elimination may contribute to the findings from field-collected fish (i.e., perchlorate found in only some individuals, and at concentrations greater than in the water). Laboratory studies also indicated that perchlorate did not bioconcentrate in mosquitofish or channel catfish. However, these laboratory studies were conducted at extreme perchlorate concentrations (100 ppm). These laboratory studies indicate that if inter-individual variation in perchlorate elimination and temporal variation in perchlorate water concentrations are the basis for the patterns seen in the field, then the spikes in concentrations of perchlorate in the water would have to be at least 10 times the concentrations seen in the fish tissues. Finally, perchlorate uptake and elimination rates vary with species and tissue examined. The greatest concentrations of perchlorate are typically found in the head.

Fish Modeling. A "worst-case" scenario was simulated, in which individual catfish were exposed to the highest measured field perchlorate water concentrations (540 ppb in S Creek) until their tissues reached equilibrium. Most tissue compartments took approximately 120 hours to equilibrate, with the gill and thyroid compartment taking 250 and 500 hours, respectively. If we assume that 540 ppb is the highest level any fish is exposed to, then all measured field tissue concentrations should be less than or equal to those simulated. The highest measured muscle concentration, based on tissue wet weight, was 60 ppb. Simulated muscle concentrations were ~150 ppb. Since thyroid tissue cannot be collected in fish, the head was used as an approximate measure. Field measurements had a high wet weight concentration of 850 ppb compared to a mean simulated thyroid concentration of 880 ± 120 ppb (mean ± 95% CI) after 500 hours. The thyroid concentration data were then used in the hormone secretion model to determine the level of hormone inhibition. Perchlorate inhibition was initiated after 1000 hours to allow the hormone compartments to reach steady-state prior to exposure. T3 hormone levels decreased 18.7-22% and T4 levels decreased 56.5%.

The rapid and substantial decrease in hormone levels should be viewed cautiously. We cannot fully characterize the system without the data necessary to determine a T_3

inhibition term. We took a conservative approach in assuming the inhibition term for T_3. It may be that T_3 levels do not decrease that much (behave similarly to T_4), but that is a source of uncertainty. At present, the simulated results are only a "best guess" and should not be viewed as definitive. It is also important to note that the stream (S Creek) used in this "worst case" simulation is not large enough to support fish of legal catchable size (fish that could be consumed by humans).

7.3.2.2.2 Frogs

Studies on the thyroid histology of native frogs indicate that there is a statistically significant positive relationship between thyroid follicle cell height in adult frogs and perchlorate content of surface water collected from six sites in the Lake Waco watershed. However, the relationship explains less than 40% of the variation in thyroid follicle cell height across the 86 animals examined. Although the relationship suggests that perchlorate might contribute to the statistically greater follicle cell height in frogs from the South Bosque and Harris Creek, we did not find evidence for colloid depletion in frogs from these sites. Under laboratory conditions, perchlorate exposure results in marked follicle cell hypertrophy and colloid depletion. Recent evidence suggests that similar histological changes occur in developing frogs exposed to relatively high concentrations of perchlorate (10 mg/L) in East Texas (Carr et al., 2003). However, such high part-per-million perchlorate concentrations as observed in East Texas were not observed at any of the NWIRP-McGregor sites studied. Furthermore, changes in follicle cell height may be caused by any environmental condition that a) stimulates TSH secretion, or b) inhibits thyroid hormone secretion and thereby results indirectly in elevated TSH secretion. Because the statistically significant increase in follicle cell height was not observed with a coincident depletion of colloid, we cannot say with certainty that the changes resulted from perchlorate exposure. Data from Goleman et al. (2003) also indicate that adult frogs are much less sensitive to perchlorate than are immature stages. Specifically, adult African clawed frogs, *Xenopus laevis*, show no alterations in thyroid histology after 10-week exposure to part-per-million concentrations of perchlorate. These data suggest a marked reduction in sensitivity of adult frogs to perchlorate compared to developing frogs. Taken together, the lack of evidence for colloid depletion and the observed lack of adult sensitivity to perchlorate suggest uncertainties regarding the precise causative agent(s) at Harris Creek and the South Bosque River that caused the observed changes in thyroid histology.

We found no evidence to suggest that surface water collected from any of the study sites contained sufficient perchlorate to alter thyroid function in the frog metamorphosis assay during the 38-day exposure of *X. laevis* tadpoles. If perchlorate levels were sufficient to alter thyroid activity, we would have expected that animals exposed would have exhibited either delayed metamorphosis or would not have completed metamorphosis, and would have mortality as low as that observed in the controls. Control animals in this study exhibited low mortality (3%), and the percent completing metamorphosis was 90%. The only water samples (sites along Harris Creek, the South Bosque, and Station Creek) in which metamorphosis was delayed also caused considerable mortality, suggesting that the slower rate of metamorphosis was the result of toxicity related to one or more water contaminants. None of the water samples contained perchlorate concentrations that would

have been expected to produce mortality. In *X. laevis*, the concentration of ammonium perchlorate lethal to 50% of test animals (LC_{50}) is 510 mg perchlorate/L (Goleman et al., 2002a). Thus, our data suggest that mortality and reduced metamorphosis in the sites along Harris Creek, the South Bosque River, and Station Creek are due to the toxic effects of an as yet unidentified factor(s) in these water samples, or to the synergistic effect of perchlorate and another contaminant(s).

In frogs, sex determination is generally believed to be genetic. Genetic sex generally determines the phenotype of the gonadal tissue, producing ovaries in genetic females and testes in genetic males, but there are many reports of frogs with mixed phenotype, i.e. exhibiting gonads with mixed female/male characteristics (intersex gonads). There is evidence that aquatic contaminants, including perchlorate, can alter the gonadal phenotype in *X. laevis*. Chronic exposures to perchlorate at 60 µg perchlorate/L and 14,000 µg perchlorate/L during larval development can result in altered phenotypic sex ratios (more tadpoles with phenotypic female gonads, Goleman et al., 2002b). We did not observe any evidence of intersexual gonads in any of the field-collected *Acris crepitans* or laboratory reared *X. laevis* exposed to surface water samples collected from the study area. Furthermore, male and female frogs were collected at most sites in the study area. The site with the greatest mean perchlorate concentration (S Creek) tended to have more females, but we were only able to collect eighteen animals total at this site. A chi-square analysis revealed that this ratio was not statistically different from 50:50, probably because of the small sample size. Although there was a trend toward more female X. laevis reared in water from Station Creek, mortality in this group was 55%, and the animals that died prematurely could not be identified with respect to gonadal sex as they had not yet reached stage 66, the earliest stage required for phenotypic sex identification.

7.3.2.2.3 Benthic Organisms

Benthic organisms were not directly tested for exposure to perchlorate during this study. However, sampling of sediment pore water constituents for perchlorate was conducted both in streams and in the delta areas of the lakes. Benthic species could potentially be exposed to perchlorate by direct ingestion of sediment. Benthic flora may accumulate perchlorate through root uptake and transfer contamination to consumer species.

The results of sediment pore water sampling indicate that there appears to be a low probability of exposure to these benthic species in the delta areas of Lake Belton and Lake Waco, as all perchlorate concentrations in sediment pore water samples collected in these areas were below the detection limit. However, sediment pore water farther upstream in the watershed did have detectable levels of perchlorate (refer to Section 5.2.1). Benthic species in these areas would be exposed to perchlorate.

7.3.2.3 Terrestrial Animals/Birds

Small and medium mammals and birds were collected from the study area near streams and springs known to be contaminated with perchlorate. Tissues from these animals were tested to determine whether they were exposed to perchlorate. Laboratory studies and modeling were also conducted to better understand the nature of perchlorate exposure in

these organisms. Potential exposures to these organisms are discussed in the following subsections.

7.3.2.3.1 Small Mammals and Birds

This study demonstrated that animals and birds in affected areas can be exposed to perchlorate either directly from drinking contaminated stream water or indirectly by consuming plants that have been exposed to perchlorate. Laboratory studies (Thuett et al., 2002) indicate the highest potential exposure levels near NWIRP may be sufficient to cause thyroid histopathological impacts in small mammals. These conclusions are based on information contained in Section 5.5.1. However, there is uncertainty regarding whether such histopathological impacts would have any direct relevance to adverse effects on individual organisms or populations of small mammals or birds present within the study area.

Field and Laboratory Studies. Small mammals and birds inhabiting portions of the study area are being exposed to perchlorate as indicated by tissue concentrations. It is likely that the exposure occurs through consumption of perchlorate-contaminated water and food. In general, the concentration of perchlorate in tissues of small mammals follows a perchlorate concentration gradient across the different sampling locations. Further evidence of variation in perchlorate exposure among sites was indicated by significant differences in quantifiable perchlorate concentrations in kidney and liver samples among small mammals. Exposure risk in small mammals appears to be at expected levels or greater at all sites except Harris Creek at Highway 84 (as measured by the number of quantifiable perchlorate hits in kidney and/or liver samples, see Section 5.5.1.1.3). Overall, the highest concentrations of perchlorate in small mammals were detected in animals collected from the following sites: Station Creek at Highway 107 (T23), the unnamed tributary near the wastewater treatment plant at Highway 317, the spring on Oglesby Road, and the spring-fed stream on the Hollan property. Note, however, that only a few animals were captured and analyzed from the spring on Oglesby Road and the spring-fed stream on the Hollan property. Small mammals at Harris Creek at Highway 84 generally had low perchlorate concentrations although their capture location was only about 200 to 400 meters from the spring on Oglesby Road and the spring-fed stream on the Hollan property. However, the exposure of Harris Creek animals may have been minimized by dilution of perchlorate from Harris Creek, the main tributary that received water flow from the spring on Oglesby Road, and the spring-fed stream on the Hollan property.

Exposure to birds was essentially measured at one site only (near the spring on Oglesby Road). Concentrations of perchlorate in these birds were consistently high and all birds had measurable concentrations of perchlorate in kidney samples. Migratory birds (doves) collected early-on in the study from a location near the spring on Oglesby Road had no perchlorate in their tissues. Uncertainty remains regarding the potential effect of perchlorate on piscivorous birds inhabiting or utilizing perchlorate-impacted sites within portions of the study area. While perchlorate was detected in numerous small fish species collected from several water bodies throughout the study area, most residues were detected in fish from tributaries rather than Lakes Waco and Belton. Also, piscivorous

birds were rarely observed in tributaries, and likely utilized lake and pond environments as their primary foraging areas. Although little information is available on trophic or food chain transfer of perchlorate, based on its water solubility, the relative lack of tissue bioaccumulation in the species studied, and its rapid excretion, perchlorate would not be expected to accumulate to toxic levels in piscivorous birds. However, no data were generated during this study to completely rule out exposure to elevated and potentially harmful concentrations of perchlorate among piscivores.

Assuming that a larger sample of small mammals would have shown similar results, the likelihood of exposure to perchlorate at the spring on Oglesby Road and the spring-fed stream on the Hollan property is considered high for wildlife in this area.

Given concentrations of perchlorate measured in birds and small mammals from different sampling locations within the study area and the observed effects of perchlorate in the laboratory dosing studies on deer mice and prairie voles, it appears that field exposures are occurring at levels sufficient to cause thyroid histology impacts and plasma hormone concentrations. This conclusion is especially true for animals inhabiting the spring on Oglesby Road, the spring-fed stream on the Hollan property, Station Creek at Highway 107 (T23), and the unnamed tributary near the wastewater treatment plant at Highway 317. To a lesser extent, the same holds true for Harris Creek at Highway 84, although overall concentrations of perchlorate are lower in those animals. However, there is uncertainty regarding whether such histopathological impacts would have any direct relevance to adverse effects on individual organisms or populations of small mammals or birds present within the study area.

Uncertainty in these results includes low sample numbers from many of the sites. This result stems largely from a general lack of habitat at many of the sites, resulting in low capture success of small mammals and birds during different times of the year. The best small mammal trapping success occurred at Harris Creek at Highway 84, in a small pocket of heavy grass that supported a very localized, but dense, population of rodents. This condition was quite rare, however, with most of the trapping sites consisting of over-grazed, sparsely vegetated areas along creeks and drainages. Therefore, it is somewhat difficult to make firm conclusions about perchlorate exposure across all sites. When taken as a whole, however, animals were collected from along drainages known to originate on NWIRP, or from along groundwater springs with known perchlorate contamination, and these animals are being exposed to perchlorate as indicated by tissue residues.

Small Mammals and Birds Modeling. Worst-case exposure simulations were run for small mammal and bird populations using data from S Creek as a means of establishing an upper bound for perchlorate exposure. We assumed that small mammals and birds obtained their food and drinking water solely from this single area.

The simulation output showed that the concentration reached equilibrium as the result of a balance between ingested and excreted perchlorate. The process of ingesting and excreting perchlorate also explains the oscillation in each tissue compartment. There was

an increase in tissue perchlorate levels caused by ingestion of contaminated food items during the day and a decrease resulting from excretion through urine and feces. In birds, it was predicted that perchlorate could be transferred from a female to her eggs.

The model predicts significant increases in perchlorate concentrations in all modeled organ and tissue compartments for the S Creek area. These increases did not, however, translate to reduced T_3 and T_4 secretion rates or lower T_3 and T_4 concentrations in model compartments. Although parameter estimates are based on literature values, conservative assumptions were made in their estimation: 1) high transport rates from the liver to the blood, and 2) low elimination rates from the gut. These simulations are only preliminary, but with the current parameter set, additional simulation experiments showed reduced thyroid activity only with order-of-magnitude greater perchlorate concentrations than those found in S Creek.

In birds, the predicted concentrations in eggs as a result of maternal transfer are within the same range as the predicted concentrations in the adult thyroid compartment, although the actual effects of this conclusion are unknown at this time.

7.3.2.3.2 Medium Mammals and Large Mammals

Perchlorate was not detected in medium-sized mammals. Raccoons and opossum captured near the most contaminated stream in the study area (S Creek) did not contain perchlorate in blood (detection limit around 14 ppb), nor did they show signs of thyroid histology abnormalities. Thyroid hormone levels in medium-sized mammals captured near contaminated streams in the study area appeared normal, consistent with residue and thyroid histology data. Perchlorate exposure (approximately 25 ppb) to large mammals (cattle) in the study area did not adversely affect thyroid hormones.

7.3.3 Limitations

Limited site access throughout the study may have prevented the determination and assessment of areas potentially containing significantly greater concentrations of and larger spatial distributions of perchlorate. Therefore, the possibility exists, although considered remote, that areas of greater perchlorate contamination or impacts than the studies conducted and presented in this report indicate exist within the study area. Furthermore, the degraded or diminished habitat (e.g. overgrazed pastures) available for investigation on many of the study sites may not accurately reflect the species composition or abundance in other areas which may also be contaminated with perchlorate. Therefore, uncertainty exists surrounding the potential for perchlorate to impact individuals, populations, or species inhabiting inaccessible locations.

8.0 STUDY CONCLUSIONS

Based on the historical data reviewed and the large amount of data collected as part of this study, the project team developed an extensive set of findings. These findings have been developed based on the most likely exposure routes within the study area and include potential exposure of plants, aquatic animals, terrestrial animals, and humans. Many of these findings on the occurrence of perchlorate in the study area and on potential human and ecological exposures to perchlorate are dependent upon continued remediation efforts by the U.S. Navy at the NWIRP site. A summary of findings is provided in the following sections, with references directing the reader to sections within the report that contain more detailed information. This conclusion section focuses on the presentation of findings related to major study objectives; it does not necessarily contain all individual conclusions identified during the study.

8.1 OCCURRENCE, FATE AND TRANSPORT OF PERCHLORATE IN WATERSHEDS

Before the exposure potential could be comprehensively evaluated and assessed by the project team, a better understanding of the occurrence, fate, and transport of perchlorate within local streams, Lake Belton, and Lake Waco was necessary. Sampling of stream water, lake water, intake water, and sediment pore water helped the study team better quantify perchlorate concentrations in affected media, their relative locations within the watersheds, and the fate of these contaminants. These sampling studies combined with monitoring of stream and groundwater levels also assisted in gaining a better understanding of how perchlorate moves through the Bosque and Leon River Watersheds overall. Study findings for streams and lakes are discussed further below.

8.1.1 Streams

8.1.1.1 Stream Surface Water

Conclusions pertaining to occurrence, fate, and transport of perchlorate in stream water are listed below. These conclusions are based on information contained in Section 5.1.1 Perchlorate Occurrence in Streams, Section 5.1.3 Groundwater/Surface Water Interactions, and Chapter 6 Updated Hydrologic Conceptual Site Model. Locations of monitoring stations discussed in this section are shown on **Plate 1**. The method detection limit in all surface water samples tested for perchlorate was 1 µg/L.

- Perchlorate does emanate from NWIRP and is regularly detected in Tributary M, Station Creek downstream of Tributary M, the South Bosque River, and Harris Creek. Perchlorate concentrations were found to decrease with distance downstream from NWIRP.
- No perchlorate was detected in the 262 samples collected from the Cowhouse Creek (CHC1) sampling station, which receives surface runoff from Fort

Hood. Based on this limited sampling, surface runoff from Fort Hood is not thought to contribute perchlorate to Lake Belton.

- Perchlorate concentrations detected at sampling locations in small tributaries and creeks were variable with time, except for sampling location Station Creek 1 (SC1). No perchlorate was detected in any of the samples collected at station SC1. Also, with the exception of one perchlorate detection in a duplicate sample, no perchlorate was detected in any of the samples collected at the Onion Creek 1 (OC1) monitoring station.
- Perchlorate concentrations in both watersheds are diluted to below detectable levels by the time surface runoff reaches the monitoring stations located on major streams including the Middle Bosque (MBR1) and Leon Rivers (LR1). All samples collected at the stations along these rivers during this study (218 samples on the Middle Bosque River and 230 samples on the Leon River) were below the method detection limit for perchlorate (< 1 µg/L). All detected perchlorate concentrations were well below estimated levels necessary to cause detectable concentrations in the lakes.
- No sampling station had detectable levels of perchlorate 100% of the time.
- Perchlorate travels further from NWIRP along the South Bosque River and along Harris Creek than along Station Creek. However, perchlorate concentrations in surface water are significantly lower in the Bosque River watershed compared to the Leon River Watershed.
- Lower perchlorate concentrations were observed during storm events, likely due to an increase in water volume and flow which contributed to further dilution of perchlorate.
- A first flushing effect, where perchlorate concentrations increase immediately or shortly after a rainfall event, was observed at monitoring stations SBR1 and SC3 during the first storm event, evidenced by a small spike in perchlorate concentrations following the storm. However, perchlorate concentrations were diluted rapidly due to increased flow. No other monitoring stations showed a first flush effect, but some uncertainty on possible first-flush effects remains. The two storm events sampled during the study were not the largest storms that occurred during the study period.
- Dynamic interaction between surface water and groundwater was observed at almost all locations along streams where monitoring stations and wells were installed. This interaction provides a pathway for contamination in groundwater to move to surface water and vice versa.
- A groundwater connection is evident between monitoring stations TRM1 and OC1. These two monitoring stations are on separate streams and located approximately two miles apart. Monitoring station SC3 also appears to be a key groundwater/surface water interaction area, as fluctuations in stream levels at station TRM1 are sometimes evident in groundwater at station SC3, approximately one mile downstream.

- Station Creek, draining to the Leon River, is seasonal and goes dry during certain times of the year, particularly in the summer months. Conversely, the streams draining to the Middle Bosque River do not typically go dry, even during the summer months or periods of low precipitation.

8.1.1.2 Stream Sediment Pore Water

Conclusions pertaining to occurrence and fate of perchlorate in stream sediment pore water are listed below. These conclusions are based on information contained in Section 5.2 Sediment. The perchlorate detection limit in all stream sediment pore water samples was 4 µg/L.

- Perchlorate was detected in stream sediment pore water along impacted streams.
- Perchlorate concentrations vary less in stream sediment pore water than in stream water, indicating that perchlorate is persistent in sediments of streams within the study area.
- A large capacity for natural attenuation of perchlorate exists in sediments of streams within the study area. The presence of nitrate controls the magnitude of perchlorate attenuation in sediments. Microorganisms in sediment prefer nitrate over perchlorate.
- Perchlorate was not detected below 30 cm of depth in the sediment.
- Biodegradation typically occurs in the top 10-20 cm of stream sediment and varies seasonally.
- Perchlorate degradation in sediments decreases during cooler seasons primarily due to less biological activity at cooler temperatures.
- Higher organic content in sediments (which is seasonal) may create anaerobic conditions, which are favorable to perchlorate degradation. There also may be seasonal variations in micro-organism populations which affect perchlorate degradation in sediments.

8.1.2 Lakes

Conclusions pertaining to occurrence, fate and transport of perchlorate in lake water and lake sediments are listed below. These conclusions are based on information contained in Section 5.1.2 Perchlorate Occurrence in Lakes, Section 5.1.4 Flow, and Section 5.2.2 Anoxic Study. The perchlorate method detection limit in all lake water, intake water, and lake sediment pore water samples was 1 µg/L.

- All lake samples collected by the study team during grab sampling and delta area sampling (22 samples) in Lake Waco were below detectable levels for perchlorate. All grab samples, delta area samples and ADCP transect samples (127 samples) collected from Lake Belton were below the method detection limit (1 µg/L) for perchlorate.
- No consistent preferential flow patterns were identified along the old river channel at the bottom of Lake Belton. Most preferential flows identified at the

surveyed transects were generally characterized as inconsistent and disonnected flows. The flows across most transects exhibited very low velocity and uniform flow characteristics.

- No preferential flows towards intakes were identified based on the four seasonal ADCP studies.
- Evidence of indigenous perchlorate-reducing micro-organisms was found in sediment samples collected throughout Lake Belton and in the water column during the anoxic study performed by the project team. Due to the anoxic conditions encountered below the thermocline, and due to the presence of sufficient electron donors, this lake likely has an ability to naturally reduce perchlorate. Lower temperatures decrease the rate of reduction, but reduction was proven to occur based on lab testing of sediment and water column samples.
- Since perchlorate was not detected in either lake during any of the sediment pore water or surface water sampling performed during this study, it appears that perchlorate accumulation is not occurring and perchlorate concentrations are not likely to increase in these lakes.

8.2 EXPOSURE ASSESSMENT RESULTS

Exposure assessment is an analysis of the potential exposure pathways between the source of a chemical or physical contaminant and human or ecological receptors. Using the information provided in the previous sections of this report and the conclusions regarding occurrence, fate and transport previously discussed above, the potential for perchlorate releases from the NWIRP site and associated impacts to various environmental media (soil, sediment, surface water, groundwater and biota) have been assessed. Conclusions regarding these exposures are provided in the following sections.

8.2.1 Human Exposure Findings

The human exposure analysis considered current and future land uses, human activities and receptors consistent with these land uses, and exposure pathways between human receptors and contaminated media. This analysis was intended only to assess the potential for human exposure to perchlorate; it was not intended to determine an appropriate exposure level, set a health level, or evaluate resulting health effects. Based on the study results, the vast majority of potential human receptors (i.e., the public water supply users) are at no risk of exposure to perchlorate. However, NWIRP vicinity residents and recreational users of the area have the potential to be exposed to perchlorate through consumption of plants, terrestrial animals, aquatic wildlife and/or ingestion of perchlorate-contaminated water from streams. More detailed findings related to human exposure to perchlorate are presented below. As discussed in Chapter 7, exposure to perchlorate through dermal contact or inhalation is highly unlikely. Therefore, this section only discusses exposure through ingestion of water and food.

8.2.1.1 Through Water

- Public water supply users are at no risk of exposure to perchlorate from their water source based on information collected during this study. No perchlorate was detected during public water supply intake sampling performed by the project team. A total of 77 samples were collected from water intakes within Lake Belton and Lake Waco and downstream of Lake Belton. All samples were below the method detection limit for perchlorate (1 µg/L).
- Historical data further support that public water supply users are not at risk of exposure to perchlorate from the water supply. The U.S. Navy collected 243 samples from intakes between February 1999 and December 2002. Only three of these samples had detectable perchlorate concentrations, all collected prior to July 2000. No detections were identified at any intakes since July 2000.
- Perchlorate does emanate from NWIRP and is regularly detected in Tributary M, Station Creek downstream of Tributary M, the South Bosque River, and Harris Creek. Therefore, any NWIRP vicinity residents or recreational users who use these streams as a drinking water source or for garden irrigation could be exposed to perchlorate.
- Because detectable concentrations of perchlorate have not been found in either Lake Waco or Lake Belton since August 2000, recreational users of Lake Waco and Lake Belton are not considered to be susceptible to exposure via incidental ingestion while swimming in the lakes. However, exposure via incidental ingestion during swimming or wading could occur elsewhere in the watersheds where perchlorate is present at detectable concentrations.

8.2.1.2 Through Food

<u>Through Plants</u>

- Based on field and lab data, the highest potential exposure to perchlorate from plants is most likely from leafy vegetation of plants growing near perchlorate-impacted streams.
- Based on laboratory and field data, people who irrigate vegetable/edible plant gardens with water containing perchlorate and consume the vegetables/edible plants produced will be exposed to perchlorate. The magnitude of that exposure decreases with distance from the NWIRP boundary.
- People who consume wild grapes, wild salads, etc. from perchlorate-impacted areas may be exposed to perchlorate. This exposure source is limited to plants along streams where perchlorate is detected regularly in surface water. Perchlorate uptake into vegetation decreases with increased distance from the impacted stream.

<u>Through Fish</u>

- Human exposure to perchlorate is possible through the consumption of fish, as perchlorate was detected in fish throughout both watersheds, including within Lake Belton and Lake Waco. However, exposure is unlikely due to the low

frequency of detections even in highly impacted areas. Most of the fish collected from streams in the study area (the most perchlorate-impacted areas) were not of legal size (>8 inches), and the streams identified to have the highest perchlorate concentrations are not large enough to support fish of legal catchable size.

- Lake Waco: There were a total of eight perchlorate detections in both fish fillets and heads. The detection limit varies with species and tissue type, but in general is around 50 ppb for fish tissues. Three of the detections were in fillets, out of 65 total fillets analyzed. The other five detections were in heads, out of 24 heads analyzed. The fish in which perchlorate was detected were large mouth bass, channel catfish, and black crappie. All of these fish were of legal size.
- Lake Belton: There were a total of six perchlorate detections in fish fillets out of 54 fillets analyzed. The detection limit varies with species and tissue type, but in general is around 50 ppb for fish tissues. There were no detections in the 18 fish heads analyzed. The fish in which perchlorate was detected were large mouth bass, channel catfish, spotted gar, and drum. All of these fish were of legal size, although spotted gar and drum are not typically desired species for human consumption.

Through Beef/Cattle/Livestock

- There was no indication of potential human exposure to perchlorate through consumption of beef from NWIRP area cattle. Although perchlorate was detected in the blood plasma of one animal, perchlorate was not detected in edible tissues typically consumed by people (detection limit of 23 ppb). These cattle had constant exposure to perchlorate in water for 14 weeks.
- No commercial dairies were identified within watershed areas where perchlorate impacts were identified. Non-commercial use of milk from local cattle or goats in the study area remains an area of uncertainty.
- Potential exposure through consumption of products from other livestock (chickens, pigs, etc.) remains an area of uncertainty.

8.2.2 Biological Exposure Findings

Based on the study results, plants within the study area have the potential to be exposed to perchlorate through uptake of contaminated water. Likewise, animals present within the study area have the potential to be exposed to perchlorate through consumption of plants, other animals within the food chain, and water from perchlorate-contaminated streams or springs. Findings regarding the plant and animal studies performed by the project team are presented below.

8.2.2.1 Plants

Perchlorate uptake was found to occur in algae, aquatic plants, and terrestrial plants in areas near NWIRP where surface water tested positive for perchlorate. However, no data

collected during this study suggested that perchlorate is toxic to plants. Findings relating to the nature of perchlorate uptake in plants are listed below. These conclusions are based on information contained in Section 5.3 Plants and Section 7.3 Ecological Exposure Analysis.

- Perchlorate concentrations in riparian and aquatic plants are less variable than concentrations in flowing water. Vegetative uptake of perchlorate is a function of both length of exposure and perchlorate concentration in the source water. Perchlorate uptake varies by species in both aquatic and terrestrial plants.
- Limited data indicate a bioconcentration factor (BCF) for algae from 1 to 15 (lower than terrestrial plant BCFs), but potentially as high as 300-400.
- Perchlorate accumulates in leafy, above-ground or above-water vegetation. Based on limited field data, perchlorate does not appear to concentrate as much in nuts and fruits as in leaves.
- In terrestrial plants, the perchlorate concentration increases throughout the growing season. In contrast, perchlorate concentrations appear to equilibrate in aquatic plants and do not change with season.
- Perchlorate concentrations found in tree leaves decreased with distance from contaminated surface water.
- Nitrate and perchlorate compete for uptake by plants; nitrate is preferred. In areas with high background concentrations of nitrate (such as the study area), excess nitrate will reduce perchlorate uptake by plants.
- Lab data support the field observations such as accumulation in above ground vegetation, BCFs, variation by species, and variation with length of exposure and concentration.
- Modeling data are consistent with field data, and predict perchlorate accumulation in leaves and roots. However, the actual relative concentrations between fruits and leaves appear to be reversed from those predicted by the model.
- To characterize potential exposure to higher organisms, plants may be a better indicator than water because of lower variability in perchlorate concentrations.
- Perchlorate is released from tree leaves after they fall.

8.2.2.2 Aquatic Animals

8.2.2.2.1 Fish

Perchlorate uptake was found to occur in fish in areas throughout the watersheds, even where surface water did not test positive for perchlorate. However, detections were sporadic, occurring only in some species and in a few fish at each location. Findings relating to the nature of perchlorate uptake in fish are listed below. These conclusions are based on information contained in Section 5.4.1 Fish and in Section 7.3 Ecological Exposure Analysis.

- Laboratory studies indicate that perchlorate is taken up by fish faster than it is excreted. Perchlorate is first detected in fish within hours or exposure, but levels in fish reach steady-state in days.
- In fish, perchlorate was found more in small insectivorous species.
- Algae are a good food source for aquatic organisms. Because of the algal BCFs, consumption of algae may be a significant exposure pathway of perchlorate to fish.
- When found in fish, perchlorate concentrations were almost always higher than that of the water from which they were collected; however, no bioconcentration of perchlorate was observed in laboratory studies at extreme perchlorate concentrations. These observations support food as another source of perchlorate exposure for fish.
- Fish thyroid histopathology indicates impact from perchlorate, increasing with higher observed perchlorate concentrations in water.
- Laboratory data show significant individual variation in perchlorate elimination rates for fish. This finding helps explain some of the field data variation in fish tissue residues.
- Laboratory tissue distribution studies indicate that uptake of perchlorate into the fillet is the slowest. Elimination of perchlorate from the fillet is also the slowest.
- Modeling and laboratory data are consistent on tissue distribution of perchlorate in fish. Modeled perchlorate uptake phase is five days, but laboratory data indicate that perchlorate uptake in fish occurs much faster than predicted by the model.
- Predicted thyroid impacts from the model are consistent with field data. Modeling data predict a decrease in thyroid hormones at the "worst-case" perchlorate exposure scenario (S Creek). This result could explain observed alterations of fish thyroid histology.
- Because population level and community level endpoints were not investigated in this study, the relationship between changes in thyroid histopathology and ecological impacts is unknown.

8.2.2.2.2 Frogs

Laboratory studies indicate that frogs can take up perchlorate and be affected by it, but the greatest potential exposure levels near NWIRP do not appear to be large enough to affect frog metamorphosis. Findings related to the nature of perchlorate impacts in frogs are listed below. These conclusions are based on information contained in Section 5.4.2 Frogs and in Section 7.3 Ecological Exposure Analysis.

- Native frog thyroid histology shows possible positive correlation in impact with perchlorate concentration. However, since the impact observed was on follical height alone, not on colloid depletion, and because only about 40% of

the impact could be explained by the presence of perchlorate, other factors may be involved.

- There is no evidence to suggest that perchlorate concentrations in the study area are high enough to affect frog metamorphosis.
- There is no evidence of alteration of sex ratios (ratio of males to females) in native frogs or lab frogs at the perchlorate concentrations detected within the study area.

8.2.2.3 Terrestrial Animals/Birds

8.2.2.3.1 Small Mammals and Birds

This study demonstrated that animals and birds in affected areas can be exposed to perchlorate either directly from drinking contaminated stream water or indirectly by consuming plants that have been exposed to perchlorate. Laboratory studies (Thuett et al., 2002) indicate the highest potential exposure levels near NWIRP may be sufficient to cause thyroid histopathological impacts in small mammals. These conclusions are based on information contained in Section 5.5.1 Small Mammals and Birds and in Section 7.3 Ecological Exposure Analysis.

- Residue data indicate perchlorate exposure to birds and small mammals.
- Bird exposure to perchlorate was limited to an area adjacent to the unnamed tributary near the wastewater treatment plant. Lack of perchlorate exposure to birds may be due to limited bird occurrence in other areas. Similar bird habitat existed near other contaminated streams, but bird trapping in these areas had limited success.
- Two areas were characterized as high exposure risk to terrestrial wildlife: the unnamed tributary near the wastewater treatment plant and the spring on Oglesby Road.
- An apparent exposure gradient exists for small mammals and birds, with exposure decreasing with distance from affected streams.
- Small mammal exposures to perchlorate determined in the field were large enough to cause thyroid histopathological impacts based on laboratory dosing studies with similar species.
- Consumption of vegetation appears to be the primary pathway of perchlorate exposure for small mammals.
- Modeling data for a “worst-case” perchlorate exposure (S Creek) predict perchlorate increases in small mammal tissue but not enough to suppress thyroid hormone production. No small mammals were captured at this location to support or refute the modeling data.
- Model results predict that with observed exposure levels, perchlorate would also be detected in bird eggs. However, monitoring data from sites with similar perchlorate contamination levels indicate little exposure to bird eggs.

8.2.2.3.2 Medium and Large Mammals

These conclusions are based on information included in Section 5.5.2 Medium Mammals.

- Perchlorate was not detected in medium mammals. Raccoons and opossum captured near the most contaminated stream in the study area (S Creek) did not contain perchlorate in blood (detection limit approximately 14 ppb).
- Raccoons and opossum captured near contaminated streams in the study area showed no signs of thyroid histology abnormalities.
- Thyroid hormone levels in medium-sized mammals captured near contaminated streams in the study area appeared normal, consistent with residue and thyroid histology data.
- Perchlorate exposure (approximately 25 ppb) to large mammals (cattle) in the study area did not adversely affect thyroid hormones.

8.3 SUMMARY

Based on all of the investigations completed during this study, as well as evidence that the current and historical remediation activities conducted by the U.S. Navy are having a significant and positive effect, the project team concluded that the approximately 500,000 public water supply users in the communities surrounding Lake Belton and Lake Waco are at no risk of exposure to perchlorate from this source. However, NWIRP vicinity residents/visitors could potentially be exposed to perchlorate if they consume produce from gardens irrigated with impacted stream or spring water, gather and consume wild edible vegetation near impacted streams, or drink water from impacted wells or streams. Similarly, people who consume fish caught in these watersheds could also potentially be exposed to perchlorate in fish fillets, although the risk of this exposure is thought to be low. This study also demonstrated that animals in affected areas can be exposed to perchlorate either directly from drinking contaminated stream water or indirectly by consuming plants or animals that have been exposed to perchlorate. An overview map of perchlorate detections in the study area is included on **Plate 6**.

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