

FINAL

**Total Maximum Daily Loads of Fecal Bacteria  
for the Cherry Creek Sub-basin in the Youghiogeny River Basin  
in Garrett County, Maryland**

**FINAL**



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**List of Abbreviations**

ARCC	Average rates of correct classification
ARA	Antibiotic Resistance Analysis
BMP	Best Management Practice
BST	Bacteria Source Tracking
cfs	Cubic Feet per Second
CFR	Code of Federal Regulations
CFU	Colony Forming Units
COMAR	Code of Maryland Regulations
CWA	Clean Water Act
DNR	Maryland Department of Natural Resources
EPA	United States Environmental Protection Agency
GIS	Geographic Information System
LA	Load Allocation
MACS	Maryland Agricultural Cost Share Program
MDE	Maryland Department of the Environment
MDP	Maryland Department of Planning
MGD	Millions of Gallons per Day
ml	Milliliter(s)
MOS	Margin of Safety
MPN	Most Probable Number
MPR	Maximum Practicable Reduction
MS4	Municipal Separate Storm Sewer System
MST	Microbial Source Tracking
NPDES	National Pollutant Discharge Elimination System
RCC	Rates of Correct Classification
TMDL	Total Maximum Daily Load
USGS	United States Geological Survey
WQIA	Water Quality Improvement Act
WLA	Wasteload Allocation
WQLS	Water Quality Limited Segment
WWTP	Wastewater Treatment Plant

## EXECUTIVE SUMMARY

This document, upon approval by the U.S. Environmental Protection Agency (EPA), establishes a Total Maximum Daily Load (TMDL) for fecal bacteria in the Cherry Creek watershed (basin number 05-02-02-01-00-02). Section 303(d)(1)(C) of the federal Clean Water Act (CWA) and EPA's implementing regulations direct each state to identify and list waters, known as water quality limited segments (WQLSs), in which current required controls of a specified substance are inadequate to achieve water quality standards. For each WQLS, states are required to either establish a Total Maximum Daily Load (TMDL) of the specified substance that the waterbody can receive without violating water quality standards or demonstrate that water quality standards are being met.

The Maryland Department of the Environment (MDE) has identified the Cherry Creek watershed and its tributaries on the State of Maryland's 303(d) List as impaired by fecal bacteria (listed in 2008) and impacts to biological communities (listed in 2002). Cherry Creek has been designated as Use III-P (Nontidal Cold Water and Public Water Supply). All other stream segments of the watershed have been designated as Use I (Water Contact Recreation, and Protection of Nontidal Warmwater Aquatic Life). See Code of Maryland Regulations (COMAR) 26.08.02.08S(4) and 26.08.02.07F(5). This document proposes to establish a TMDL for fecal bacteria in the Cherry Creek watershed that will allow for attainment of the beneficial use designation of water contact recreation. The listing for impacts to biological communities will be addressed in a separate document. MDE monitored the Cherry Creek watershed from 2003-2004 for fecal bacteria. A data solicitation for fecal bacteria was conducted by MDE in 2003, and all readily available data from the past five years were considered.

For this TMDL analysis, the pollutant loads set forth in this document are for the Cherry Creek watershed. To establish baseline and allowable pollutant loads for this TMDL, a flow duration curve approach was employed, using bacteria data from MDE and flow strata estimated from United States Geological Survey (USGS) daily flow monitoring. The sources of fecal bacteria are estimated at one representative station in the Cherry Creek watershed where samples were collected for one year. Multiple antibiotic resistance analysis (ARA) source tracking was used to determine the relative proportion of domestic (pets and human associated animals), human (human waste), livestock (agriculture-related animals), and wildlife (mammals and waterfowl) source categories.

The baseline load is estimated from current monitoring data using a long-term geometric mean and weighting factors from the flow duration curve. The TMDL for fecal bacteria is established after considering two different hydrological conditions: an average annual condition and an average seasonal dry weather condition (the period between May 1<sup>st</sup> and September 30<sup>th</sup> when water contact recreation is more prevalent). This allowable load is reported in units of Most Probable Number (MPN)/year and represents a long-term load estimated over a variety of hydrological conditions.

Two scenarios were developed, with the first assessing if attainment of current water quality standards could be achieved by applying maximum practicable reductions (MPRs), and the

second applying higher reductions than MPRs. Scenario solutions were based on an optimization method where the objective was to minimize the overall risk to human health, assuming that the risk varies over the four bacteria source categories. In this watershed, it was estimated that water quality standards could not be attained with MPRs, thus higher maximum reductions were applied.

The baseline loads are summarized in the following table:

<b>MD 12-Digit Cherry Creek Fecal Bacteria Baseline Loads (Billion MPN <i>E. coli</i>/year)</b>						
<b>Total Baseline Load</b>	=	<b>Nonpoint Source BL</b>	+	<b>NPDES Stormwater BL</b>	+	<b>WWTP BL</b>
541,106	=	541,106	+	0	+	0

The Cherry Creek watershed TMDL Contribution is distributed between a load allocation (LA) for nonpoint sources and waste load allocations (WLA) for point sources. Point sources include any National Pollutant Discharge Elimination System (NPDES) wastewater treatment plants (WWTPs) and NPDES regulated stormwater (SW) discharges, including county and municipal separate storm sewer systems (MS4s). The margin of safety (MOS) has been incorporated using a conservative assumption by estimating the loading capacity of the stream based on a water quality endpoint concentration more stringent than the applicable MD water quality standard criterion. The *E. coli* water quality criterion concentration was reduced by 5%, from 126 MPN/100ml to 119.7 MPN/100ml.

The MD 12-digit Cherry Creek TMDL of fecal bacteria is presented in the following table:

<b>MD 12-Digit Cherry Creek Fecal Bacteria TMDL (Billion MPN <i>E. coli</i>/year)</b>								
<b>TMDL</b>	=	<b>LA</b>	+	<b>WLA</b>			+	<b>MOS</b>
				<b>SW WLA</b>	+	<b>WWTP WLA</b>		
51,125	=	51,125	+	0	+	0	+	Incorporated

The long-term annual average TMDL (51,125 billion MPN *E. coli*/year) represents a reduction of approximately 90.6% from the baseline load of 541,106 billion MPN *E. coli*/year.

Pursuant to recent EPA guidance (US EPA 2006a), maximum daily load (MDL) expressions of the long-term annual average TMDLs are also provided, as shown in the following table:



<b>MD 12-Digit Cherry Creek Fecal Bacteria MDL Summary (Billion MPN <i>E. coli</i>/day)</b>								
<b>MDL</b>	<b>=</b>	<b>LA</b>	<b>+</b>	<b>WLA</b>			<b>+</b>	<b>MOS</b>
				<b>SW WLA</b>	<b>+</b>	<b>WWTP WLA</b>		
695	=	695	+	0	+	0	+	Incorporated

Once EPA has approved a TMDL, MDE intends for the required reductions to be implemented in an iterative process that first addresses those sources with the largest impacts to water quality and creating the greatest risks to human health, with consideration given to ease and cost of implementation. In addition, follow-up monitoring plans will be established to track progress and to assess the implementation efforts. As previously stated, water quality standards cannot be attained in the Cherry Creek watershed using the MPR scenario. MPRs may not be sufficient in watersheds where wildlife is a significant component or where very high reductions of fecal bacteria loads are required to meet water quality standards. In these cases, it is expected that the MPR scenario will be the first stage of TMDL implementation. Progress will be made through the iterative implementation process described above, and the situation will be reevaluated in the future.

## 1.0 INTRODUCTION

This document, upon approval by the U.S. Environmental Protection Agency (EPA), establishes a Total Maximum Daily Load (TMDL) for fecal bacteria in the Cherry Creek watershed (basin number 05-02-02-01-00-02). Section 303(d)(1)(C) of the federal Clean Water Act (CWA) and EPA's implementing regulations direct each state to develop a TMDL for each impaired water quality limited segment (WQLS) on the Section 303(d) List, taking into account seasonal variations and a protective margin of safety (MOS) to account for uncertainty. A TMDL reflects the total pollutant loading of the impairing substance a waterbody can receive and still meet water quality standards.

TMDLs are established to achieve and maintain water quality standards. A water quality standard is the combination of a designated use for a particular body of water and the water quality criteria designed to protect that use. Designated uses include activities such as swimming, drinking water supply, and shellfish propagation and harvest. Water quality criteria consist of narrative statements and numeric values designed to protect the designated uses. Criteria may differ among waters with different designated uses.

The Maryland Department of the Environment (MDE) has identified the Cherry Creek watershed and its tributaries on the State of Maryland's 303(d) List as impaired by fecal bacteria (listed in 2008) and impacts to biological communities (listed in 2002). Cherry Creek has been designated as Use III-P (Nontidal Cold Water and Public Water Supply). All other stream segments of the watershed have been designated as Use I (Water Contact Recreation, and Protection of Nontidal Warmwater Aquatic Life). See Code of Maryland Regulations (COMAR) 26.08.02.08S(4) and 26.08.02.07F(5). This document proposes to establish a TMDL for fecal bacteria in the Cherry Creek watershed that will allow for attainment of the beneficial use designation of water contact recreation. The listing for impacts to biological communities will be addressed in a separate document. MDE monitored the Cherry Creek watershed from 2003-2004 for fecal bacteria. A data solicitation for fecal bacteria was conducted by MDE in 2003, and all readily available data from the past five years were considered.

Fecal bacteria are microscopic single-celled organisms (primarily fecal coliform and fecal streptococci) found in the wastes of warm-blooded animals. Their presence in water is used to assess the sanitary quality of water for body-contact recreation, for consumption of molluscan bivalves (shellfish), and for drinking water. Excessive amounts of fecal bacteria in surface water used for recreation are known to indicate an increased risk of pathogen-induced illness to humans. Infections due to pathogen-contaminated recreation waters include gastrointestinal, respiratory, eye, ear, nose, throat, and skin diseases (US EPA 1986).

In 1986, EPA published "Ambient Water Quality Criteria for Bacteria," in which three indicator organisms were assessed to determine their correlation with swimming-associated illnesses. Fecal coliform, *E. coli* and enterococci were the indicators used in the analysis. Fecal coliform bacteria are a subgroup of total coliform bacteria and *E. coli* bacteria are a subgroup of fecal coliform bacteria. Most *E. coli* are harmless and are found in great quantities in the intestines of people and warm-blooded animals. However, certain pathogenic strains may cause illness.

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Enterococci are a subgroup of bacteria in the fecal streptococcus group. Fecal coliform, *E. coli* and enterococci can all be classified as fecal bacteria. The results of the EPA study demonstrated that fecal coliform showed less correlation to swimming-associated gastroenteritis than did either *E. coli* or enterococci.

Based on EPA's guidance (US EPA 1986), adopted by Maryland in 2004, the State has revised the bacteria water quality criteria and it is now based on water column limits for either *E. coli* or enterococci. Because multiple monitoring datasets are available within this watershed for various pathogen indicators, the general term "fecal bacteria" will be used to refer to the impairing substance throughout this document. The TMDL will be based on the pathogen indicator organisms specified in Maryland's current bacteria water quality criteria, either *E. coli* or enterococci. The indicator organism used in the Cherry Creek TMDL analysis was *E. coli*.

## 2.0 SETTING AND WATER QUALITY DESCRIPTION

### 2.1 General Setting

#### Location

The MD 12-digit Cherry Creek watershed is located in the Youghiogheny River region of Maryland and is part of the MD 8-digit Youghiogheny River watershed (See Figure 2.1.1). The watershed encompasses 10,685 acres (16.7 square miles) in Garrett County. The headwaters of Cherry Creek, which is one of the major tributaries of the Youghiogheny River, begin in southern Garrett County, flowing north into the Youghiogheny River downstream of Underwood Road. The watershed includes the town of Gortner. Tributaries to Cherry Creek include Fox Run, Clark Run, Douglass Run, Ambrose Run, and Frozen Camp Run.

#### Land Use

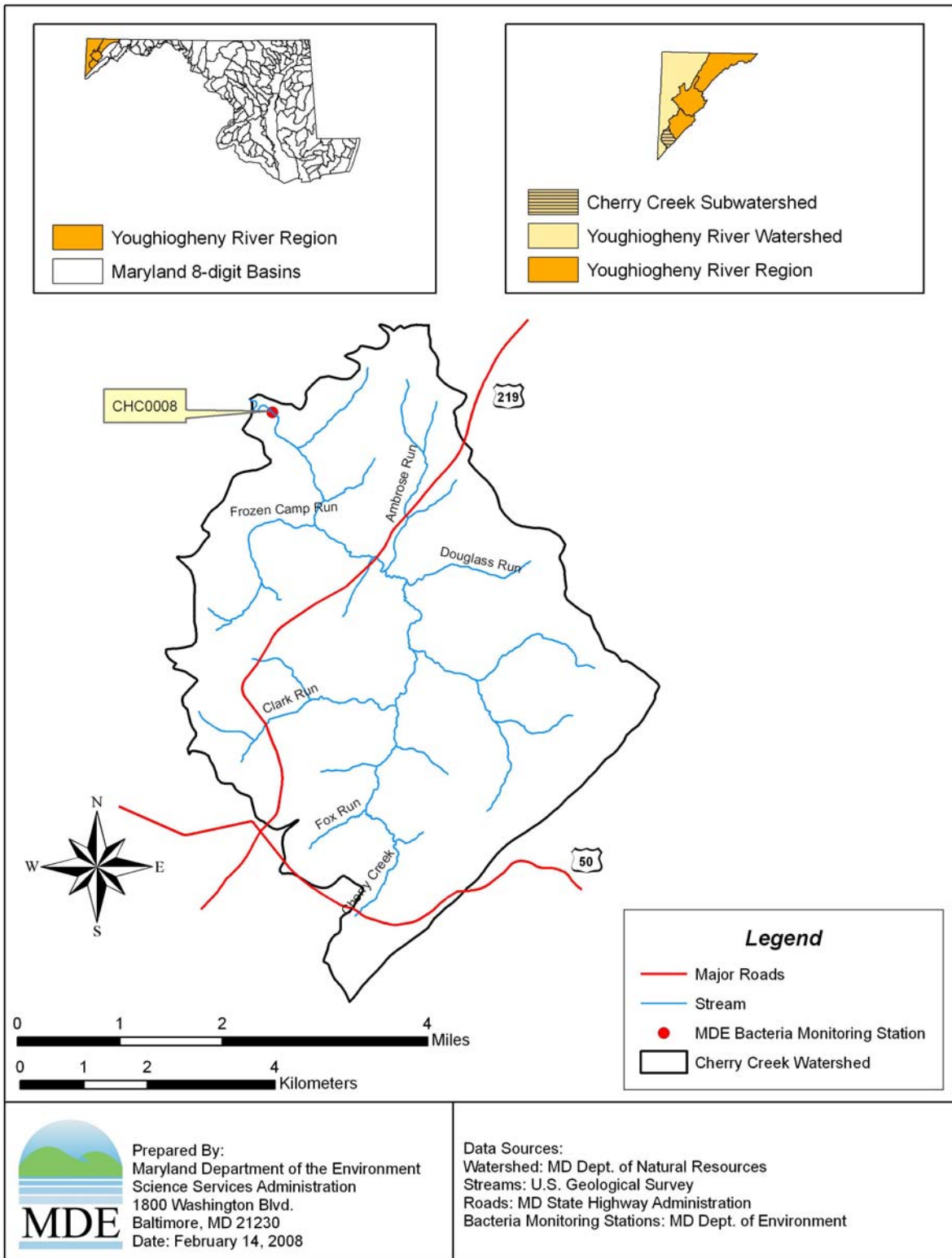
Based on the 2002 Maryland Department of Planning (MDP) land use/land cover data the Cherry Creek watershed is predominantly agricultural and forest. There is minimal residential land. The land use acreage and percentage distribution is shown in Table 2.1.1, and spatial distributions for each land use are shown in Figure 2.1.2.

**Table 2.1.1: Land Use Distribution for the Cherry Creek Watershed**

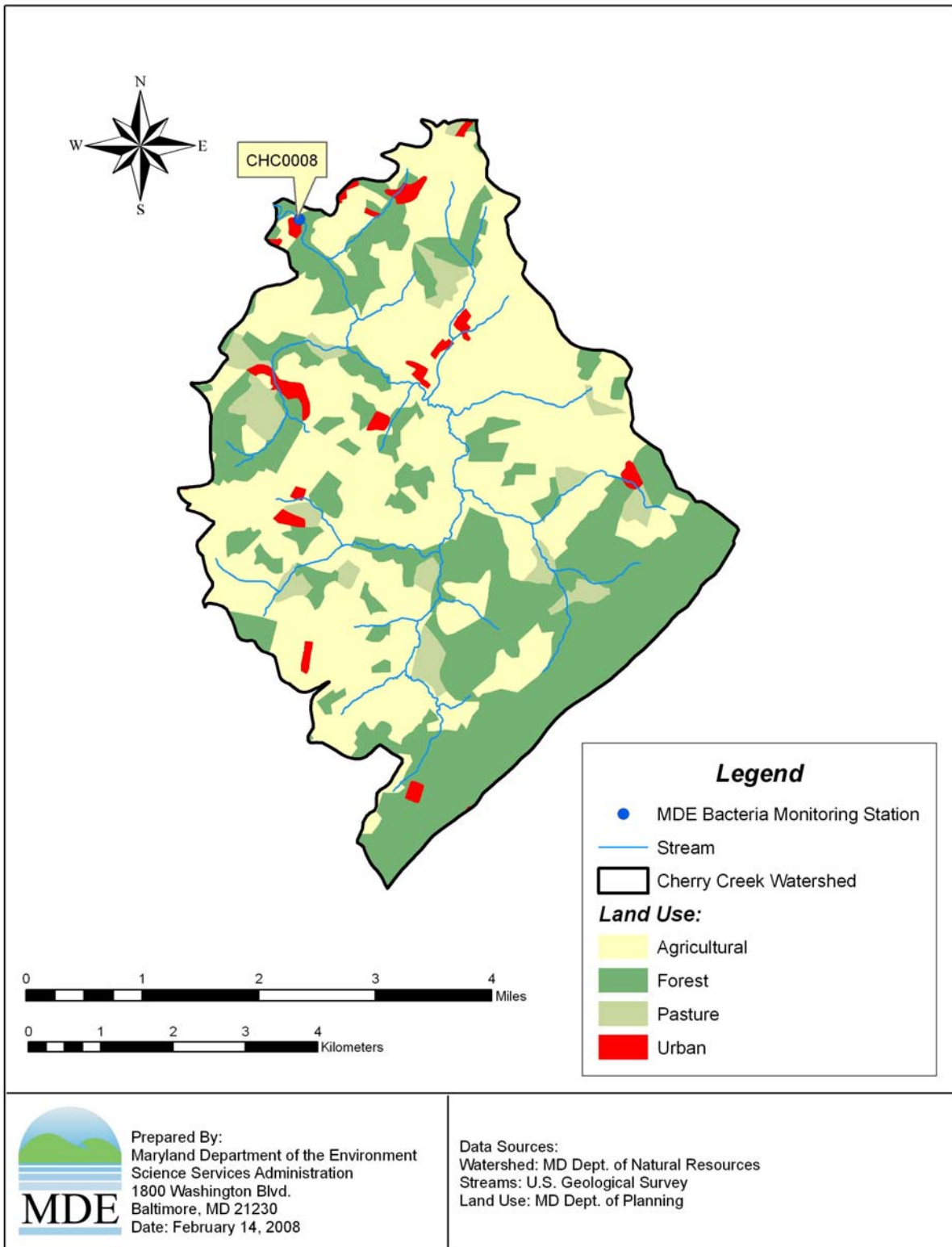
<b>Land Type</b>	<b>Acres</b>	<b>%</b>
Agricultural	5,870	54.9
Forest	4,002	37.5
Pasture	585	5.5
Urban	228	2.1
<b><i>Total</i></b>	<b>10,685</b>	<b>100</b>

#### Population

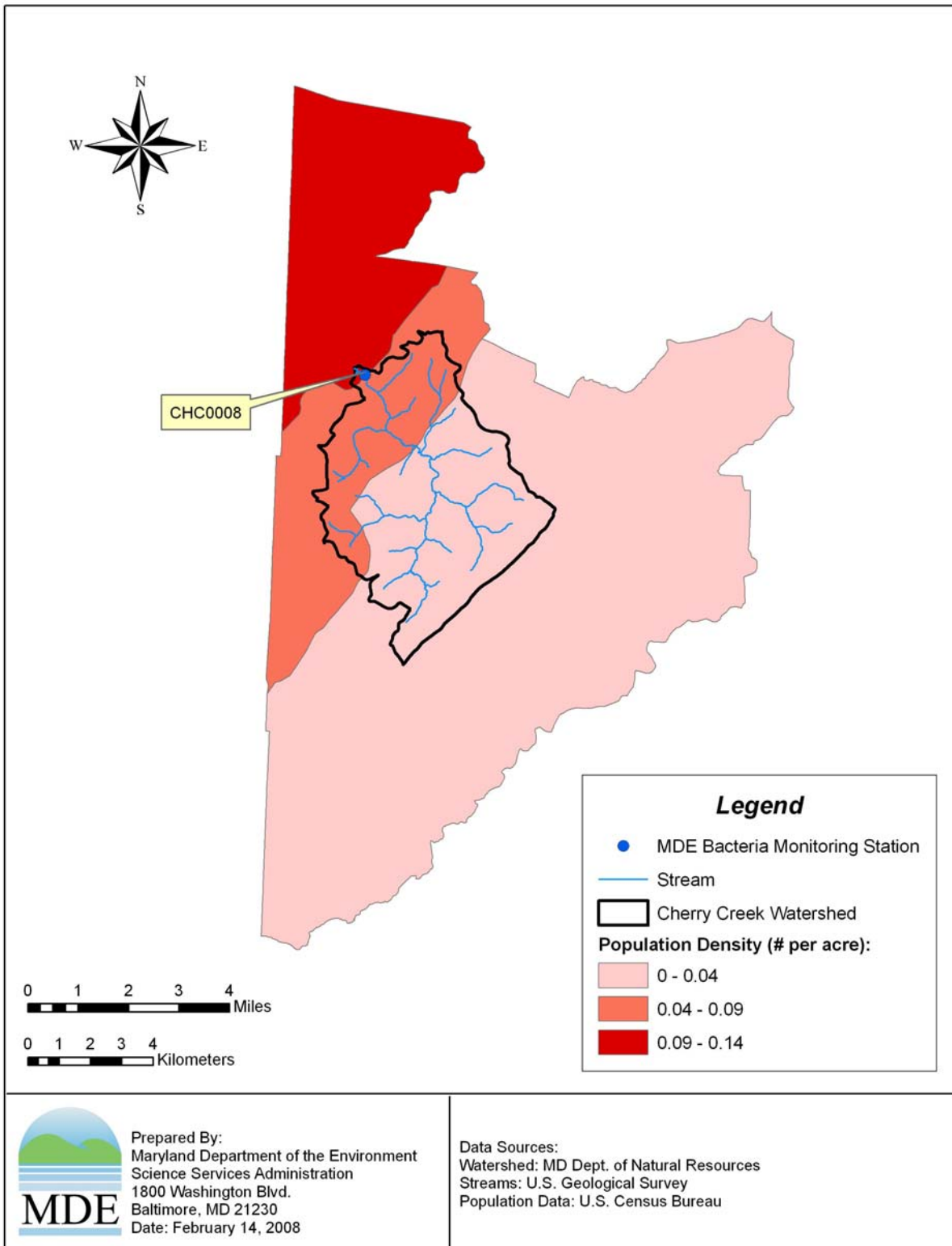
The total population in the Cherry Creek watershed is estimated to be 613 people. Figure 2.1.3 depicts the population density in the area. The population in the Maryland portion of the watershed was estimated based on a weighted average from the Census block groups and the 2007 MDP Property View, a geographic information system (GIS) dataset that provides a holistic assessment of all the taxable properties within the State.



**Figure 2.1.1: Location Map of the Cherry Creek Watershed**



**Figure 2.1.2: Land Use of the Cherry Creek Watershed**



**Figure 2.1.3: Population Density in the Cherry Creek Watershed**

## 2.2 Water Quality Characterization

EPA's guidance document, "Ambient Water Quality Criteria for Bacteria" (1986), recommended that states use *E. coli* (for fresh water) or enterococci (for fresh or salt water) as pathogen indicators. Fecal bacteria, *E. coli*, and enterococci were assessed as indicator organisms for predicting human health impacts. A statistical analysis found that the highest correlation to gastrointestinal illness was linked to elevated levels of *E. coli* and enterococci in fresh water (enterococci in salt water).

As per EPA's guidance, Maryland has adopted the new indicator organisms, *E. coli* and enterococci, for the protection of public health in Use I, II, III and IV waters. These bacteria listings were originally assessed using fecal coliform bacteria. The analysis was based on a geometric mean of the monitoring data, where the result had to be less than or equal to 200 MPN/100ml. From EPA's analysis (US EPA 1986), this fecal coliform geometric mean target equates to an approximate risk of 8 illnesses per 1,000 swimmers at fresh water beaches and 19 illnesses per 1,000 swimmers at marine beaches (enterococci only), which is consistent with MDE's revised Use I bacteria criteria. Therefore, the original 303(d) List fecal coliform listings can be addressed using the refined bacteria indicator organisms to ensure that risk levels are acceptable.

### **Bacteria Monitoring**

Table 2.2.1 lists the historical monitoring data available for the Cherry Creek watershed. MDE conducted bacteria monitoring at one station in the Cherry Creek watershed from November 2003 through October 2004. One USGS gage station, located near the Cherry Creek watershed, was used in deriving the surface water flow. The locations of these stations are shown in Tables 2.2.2 and 2.2.3 and in Figure 2.2.1. Observations recorded from the MDE monitoring station are provided in Appendix A.

Bacteria counts are highly variable, which is typical due to the nature of bacteria and their relationship to flow. The *E. coli* counts for the MDE station ranged between 109 and 6,867 MPN/100 ml.



**Table 2.2.1: Historical Monitoring Data in the Cherry Creek Watershed**

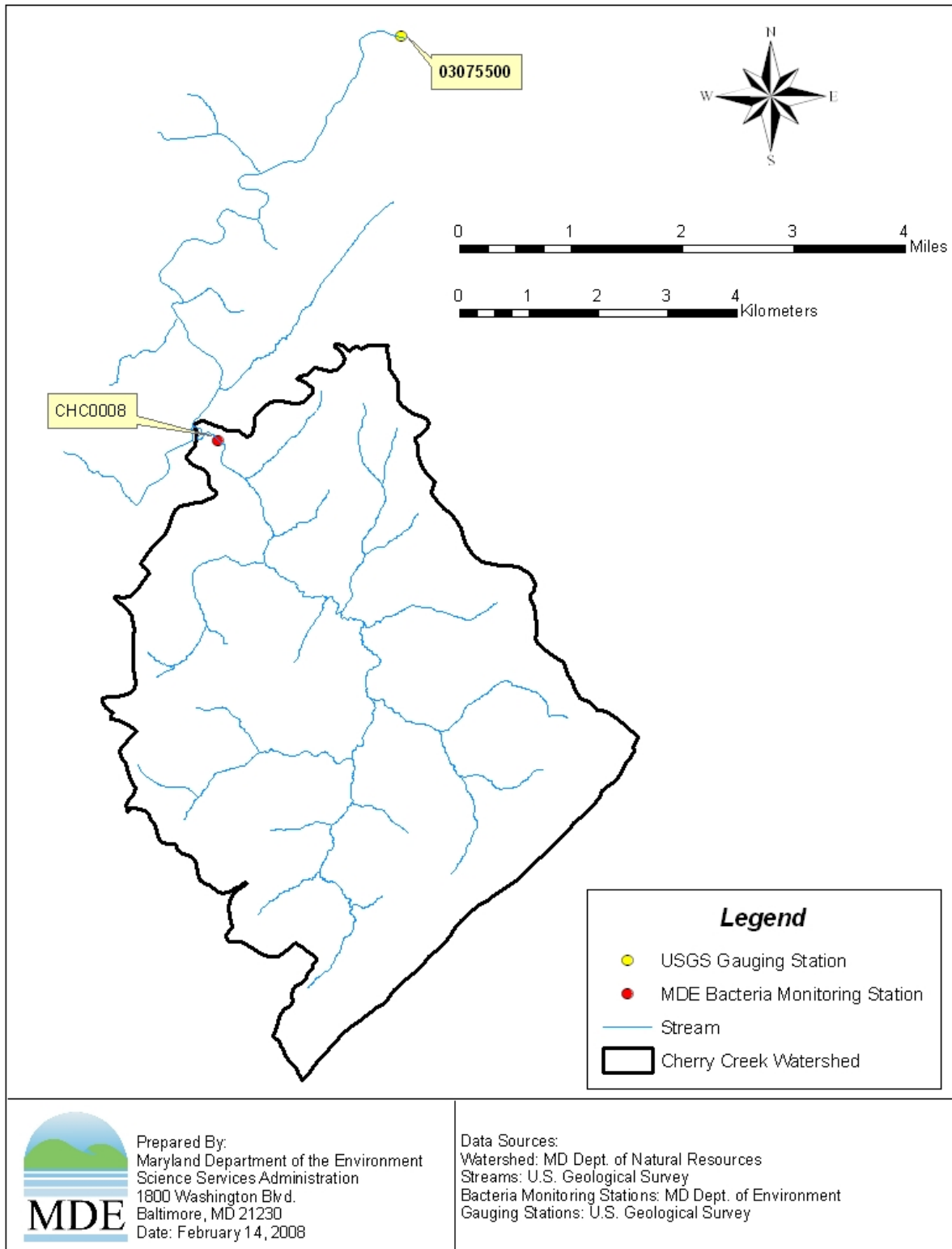
<b>Organization</b>	<b>Date</b>	<b>Parameter</b>	<b>Summary</b>
MDE	11/2003 through 10/2004	<i>E. coli</i>	1 station 2 samples per month
MDE	11/2003 through 10/2004	BST ( <i>Enterococcus</i> )	1 station 1 sample per month

**Table 2.2.2: Location of MDE Monitoring Station in the Cherry Creek Watershed**

<b>Tributary</b>	<b>Station</b>	<b>Observation Period</b>	<b>Total Observations</b>	<b>Latitude (Decimal Degrees)</b>	<b>Longitude (Decimal Degrees)</b>
Cherry Creek	CHC0008	2003-2004	24	39.368	-79.454

**Table 2.2.3: Location of USGS Gauging Station nearest the Cherry Creek Watershed**

<b>Site Number</b>	<b>Observation Period Used</b>	<b>Total Observations</b>	<b>Latitude (Decimal Degrees)</b>	<b>Longitude (Decimal Degrees)</b>
03075500	1982 - 2007	9,131	39.422	-79.424



**Figure 2.2.1: Monitoring Stations for the Cherry Creek Watershed**

## 2.3 Water Quality Impairment

### Designated Uses and Water Quality Standard

The Maryland Surface Water Use Designation in the Code of Maryland Regulations (COMAR) for the waters of the 12-digit Cherry Creek is Use III-P (Nontidal Cold Water and Public Water Supply). All other stream segments of the watershed have been designated as Use I (Water Contact Recreation, and Protection of Nontidal Warmwater Aquatic Life). See Code of Maryland Regulations (COMAR) 26.08.02.08S(4) and 26.08.02.07F(5). The MD 12-digit Cherry Creek watershed was listed on Maryland's 303(d) List [Category 5 if the Integrated Report of Surface Water Quality in Maryland (Integrated Report)] as impaired by fecal bacteria in 2008.

### *Water Quality Criteria*

The State water quality standard for bacteria applicable to freshwater and used in this study is as follows:

**Table 2.3.1: Bacteria Criteria Values**

(Source: COMAR 26.08.02.03-3 Water Quality Criteria Specific to Designated Uses; Table 1)

Indicator	Steady-State Geometric Mean Indicator Density
<i>Freshwater</i>	
<b>E. coli</b>	126 MPN/100ml

### Water Quality Assessment

#### *Interpretation of Bacteria Data for General Recreational Use*

Pursuant to the 2008 Integrated Report, the requirements to confirm a Category 5 listing for fecal bacteria impairment in all Use Waters (Water Contact Recreation and Protection of Aquatic Life) are as follows:

A steady-state geometric mean will be calculated with available data from the previous year two (2) to five (5) years. The data shall be from samples collected during steady-state, dry weather conditions and during the beach season (Memorial Day through Labor Day), to be representative of the critical condition (highest use). If the resulting steady-state geometric mean is greater than 35 cfu/100 ml enterococci in marine/estuarine waters, 33 cfu/100 ml enterococci in freshwater, or 126 cfu/100 ml *E. coli* in freshwater, the waterbody is confirmed as impaired and a TMDL should be established.

Bacteria water quality impairment in the MD 12-digit Cherry Creek watershed was assessed as explained above, by comparing the dry weather steady-state geometric means of *E. coli* concentrations for the Cherry Creek watershed with the water quality criterion. The 1986 EPA criteria guidance document assumed steady-state conditions in determining the risk at various bacterial concentrations, and therefore the chosen criterion value of 126 MPN/100 ml *E. coli* also reflects steady-state conditions (EPA 1986).

The dry weather steady-state geometric mean are calculated using samples taken during non-rainy days and from May 1<sup>st</sup> to September 30<sup>th</sup>, capturing the beach season. Results of these calculations are presented in Table 2.3.2. As shown in the table below, Cherry Creek had a steady-state geometric mean concentration of *E. coli* above the water quality criterion, supporting the 2008 listing for fecal bacteria, and it is therefore concluded that a TMDL is required.

**Table 2.3.2: Cherry Creek Watershed Dry Weather Steady-State Geometric Mean**

<b>Station / Tributary</b>	<b>Number of Samples</b>	<b>Dry Weather Steady State Geometric Mean (MPN/100ml)</b>	<b>Water Quality Criterion (MPN/100ml)</b>
<b>CHC0008</b> Cherry Creek	8	1,170	126

## 2.4 Source Assessment

### Nonpoint Source Assessment

Nonpoint sources of fecal bacteria do not have one discharge point but occur over the entire length of a stream or waterbody. During rain events, surface runoff transports water and fecal bacteria over the land surface and discharges to the stream system. This transport is dictated by rainfall, soil type, land use, and topography of the watershed. Many types of nonpoint sources introduce fecal bacteria to the land surface, including the manure spreading process, direct deposition from livestock during the grazing season, and excretions from pets and wildlife. The deposition of non-human fecal bacteria directly to the stream occurs when livestock or wildlife have direct access to the waterbody. Nonpoint source contributions from human sources generally arise from failing septic systems and their associated drain fields or from leaking infrastructure (i.e., sewer systems).

#### *Sewer Systems*

There is no sewage collection system in the Cherry Creek watershed.

### *Septic Systems*

All households in the Cherry Creek watershed rely on on-site disposal (septic) systems. There are 230 septic systems in the watershed. Figure 2.4.1 displays their location.

### **Point Source Assessment**

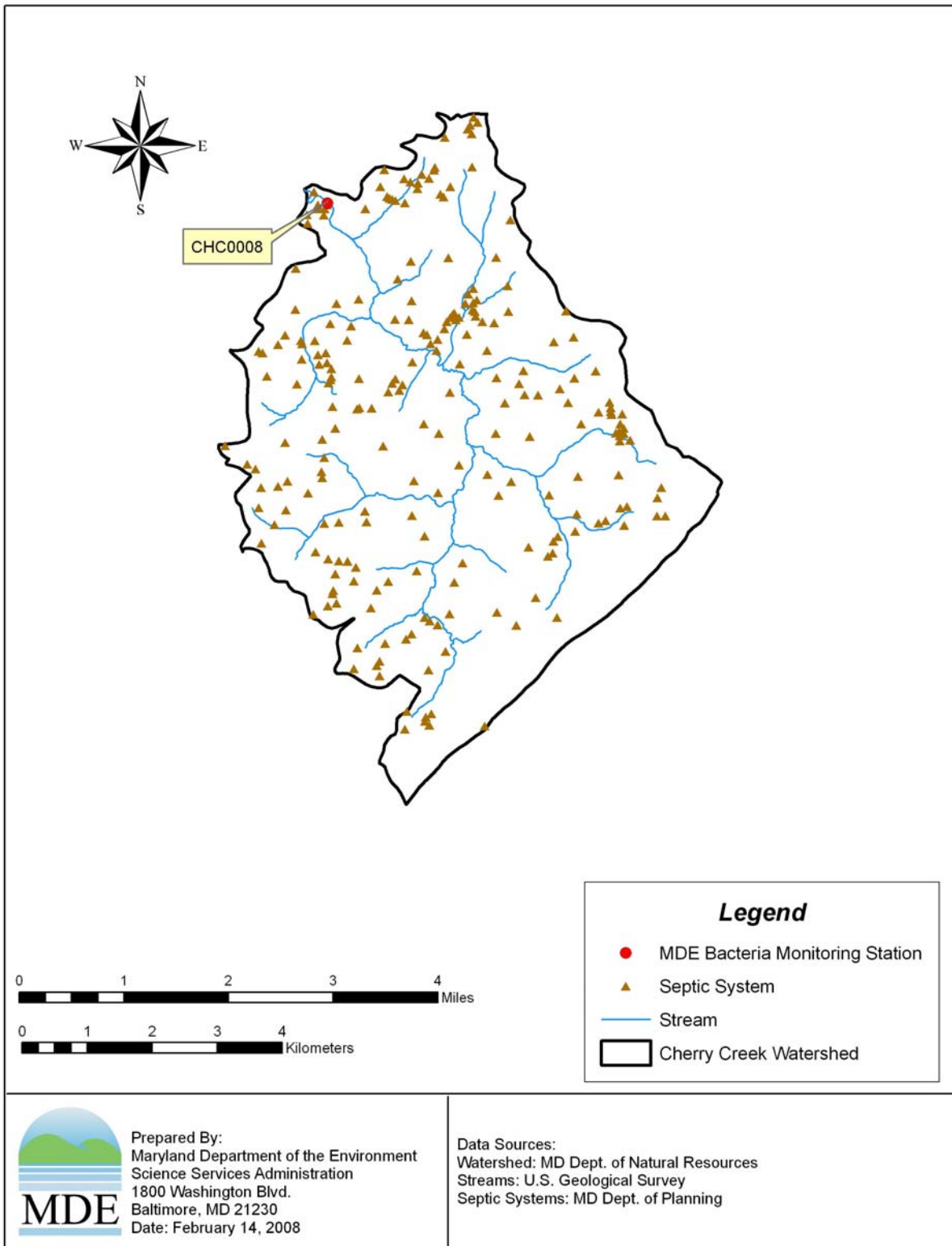
There are two broad types of National Pollutant Discharge Elimination System (NPDES) permits considered in the analysis, individual and general. Both types of permits include industrial and municipal categories. Individual permits are issued for industrial and municipal WWTPs and Phase I municipal separate storm sewer systems (MS4s). MDE general permits have been established for surface water discharges from: Phase II and other MS4 entities, surface coal mines, mineral mines, quarries, borrow pits, ready-mix concrete, asphalt plants, seafood processors, hydrostatic testing of tanks and pipelines, marinas, concentrated animal feeding operations, and stormwater associated with industrial activities.

### ***NPDES Regulated Stormwater***

Bacteria sources associated with MS4s and other NPDES regulated stormwater discharges are considered point sources. Stormwater runoff is an important source of water pollution, including bacterial pollution. An MS4 is a conveyance or system of conveyances (roads with drainage systems, municipal streets, catch basins, curbs, gutters, ditches, man-made channels, storm drains) designed or used for collecting or conveying stormwater and delivering it to a waterbody. MS4 programs are designed to reduce the amount of pollution that enters a waterbody from storm sewer systems to the maximum extent practicable. There are no Phase I or Phase II MS4 or other NPDES-regulated stormwater permit holders located in the Cherry Creek watershed.

### ***Municipal and Industrial Wastewater Treatment Plants (WWTPs)***

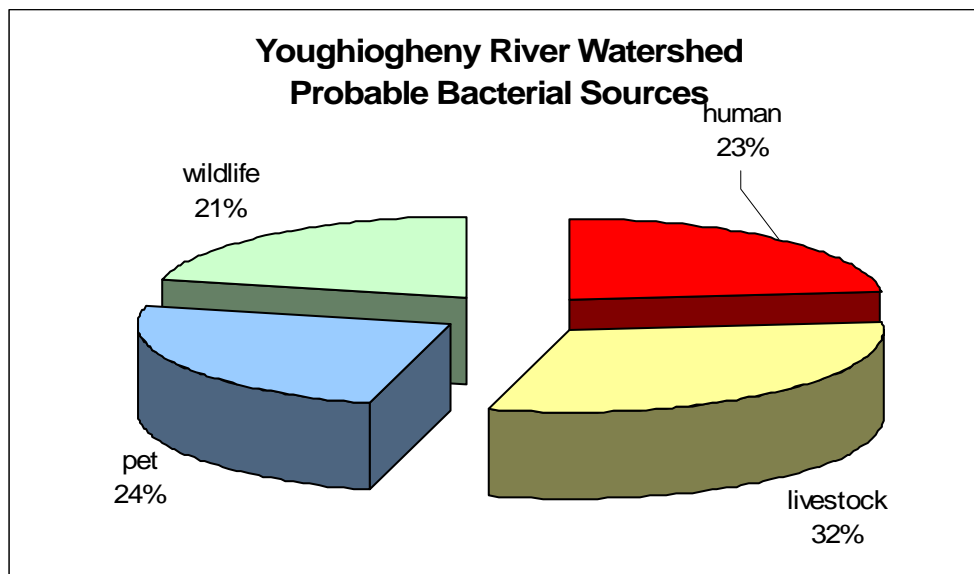
Wastewater treatment plants are designed to treat wastewater before it is discharged to a stream or river. The goals of wastewater treatment are to protect the public health, protect aquatic life, and to prevent harmful substances from entering the environment. There are no WWTPs discharging in the Cherry Creek watershed.



**Figure 2.4.1: Septic Systems in the Cherry Creek Watershed**

### **Bacteria Source Tracking**

Bacteria source tracking (BST) was used to identify the relative contributions of different sources of bacteria to in-stream water samples. As part of the Youghiogheny River bacteria source tracking study, BST monitoring was conducted at one station in the Cherry Creek watershed, where samples were collected once per month for a one-year duration. Sources are defined as domestic (pets and human associated animals), human (human waste), livestock (agricultural animals), and wildlife (mammals and waterfowl). Samples are collected within the watershed from known fecal sources, and a BST technique known as antibiotic resistance analysis (ARA) was used to identify the patterns of antibiotic resistance of these known sources. To identify probable sources, these antibiotic resistance patterns are then compared to isolates of unknown bacteria from ambient water samples. Figure 2.4.2 presents the relative contributions by probable sources of bacteria for the entire Youghiogheny River watershed. Details of the BST methodology and data for the Youghiogheny River and Cherry Creek can be found in Appendix C.



**Figure 2.4.2: Youghiogheny River Watershed Relative Contributions by Probable Sources of Fecal Bacteria Contamination**

### **3.0 TARGETED WATER QUALITY GOAL**

The overall objective of the fecal bacteria TMDL set forth in this document is to establish the loading caps (i.e., the maximum allowable fecal bacteria loads) needed to ensure attainment of water quality standards in the MD 12-digit Cherry Creek watershed. These standards are described fully in Section 2.3, “Water Quality Impairment.”

### **4.0 TOTAL MAXIMUM DAILY LOADS AND SOURCE ALLOCATION**

#### **4.1 Overview**

This section provides an overview of the non-tidal fecal bacteria TMDL development, with a discussion of the many complexities involved in estimating bacteria concentrations, loads and sources. The second section presents the analysis framework and how the hydrological, water quality and BST data are linked together in the TMDL process. The third section describes the analysis for estimating a representative geometric mean fecal bacteria concentration and baseline loads. This analysis methodology is based on available monitoring data and is specific to a free-flowing stream system. The fourth section addresses the critical condition and seasonality. The fifth section presents the margin of safety. The sixth section discusses annual average TMDL loading caps and how maximum daily loads are estimated. The seventh section presents TMDL scenario descriptions. The eighth section presents the load allocations. Finally, in section nine, the TMDL equation is summarized.

To be most effective, the TMDL provides a basis for allocating loads among the known pollutant sources in the watershed so that appropriate control measures can be implemented and water quality standards achieved. By definition, the TMDL is the sum of the individual waste load allocations (WLAs) for point sources and load allocations (LAs) for non point sources and natural background sources. A margin of safety (MOS) is also included and accounts for the uncertainty in the analytical procedures used for water quality modeling, and the limits in scientific and technical understanding of water quality in natural systems. Although this formulation suggests that the TMDL be expressed as a load, the Code of Federal Regulations (40 CFR 130.2(i)) states that the TMDL can be expressed in terms of “mass per time, toxicity or other appropriate measure.”

For many reasons, bacteria are difficult to simulate in water quality models. They reproduce and die off in a non-linear fashion as a function of many environmental factors, including temperature, pH, turbidity (UV light penetration) and settling. They occur in concentrations that vary widely (i.e., over orders of magnitude) and an accurate estimation of source inputs is difficult to develop. Finally, limited data are available to characterize the effectiveness of any program or practice at reducing bacteria loads (Schueler 1999).



Bacteria concentrations, determined through laboratory analysis of in-stream water samples for bacteria indicators (e.g., enterococci), are expressed in either colony forming units (CFU) or most probable number (MPN) of colonies. The first method (Method 1600) is a direct estimate of the bacteria colonies (US EPA 1985). The second method is a statistical estimate of the number of colonies (ONPG MUG Standard Method 9223B, AOAC 991.15). Sample results indicate the extreme variability in the total bacteria counts (see Appendix A). The distribution of the sample results tends to be lognormal, with a strong positive skew of the data. Estimating loads of constituents that vary by orders of magnitude can introduce much uncertainty and result in large confidence intervals around the final results.

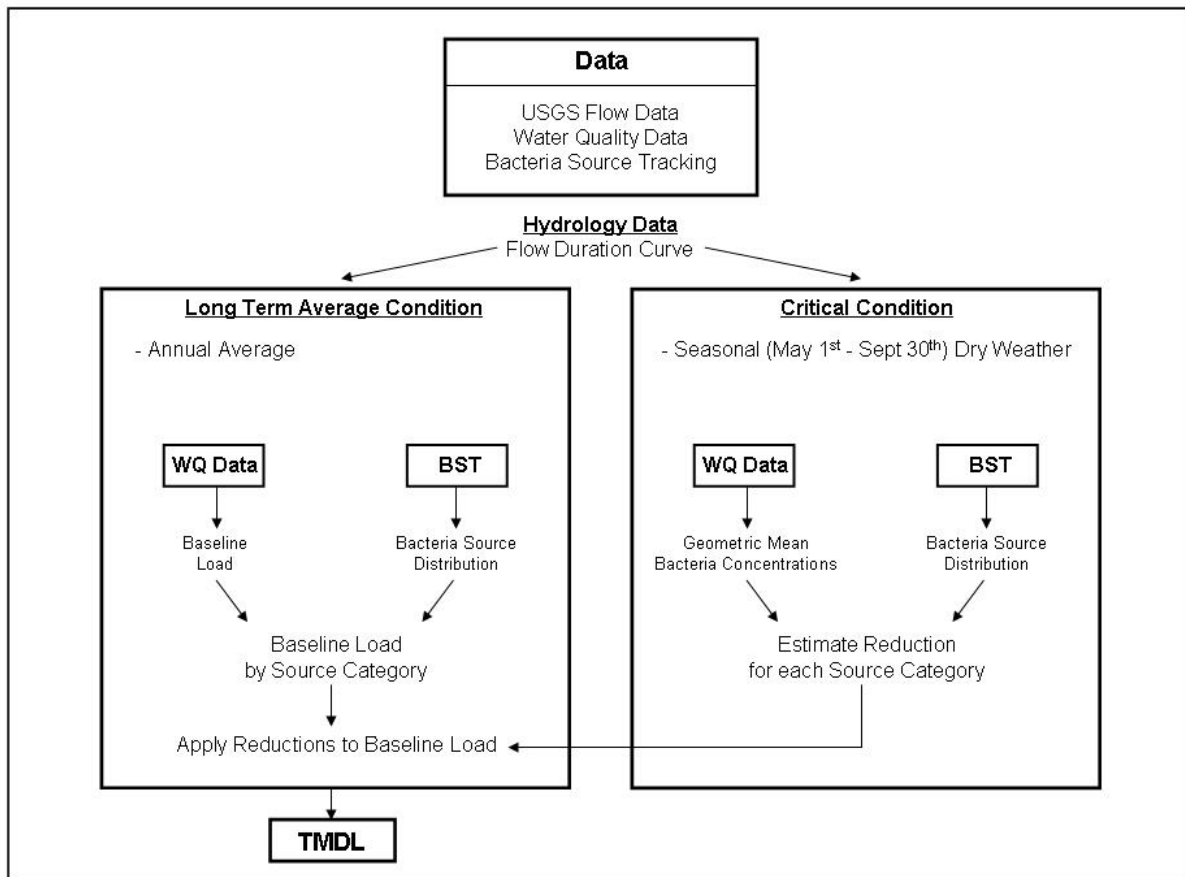
Estimating bacteria sources can also be problematic due to the many assumptions required and the limited data available. Lack of specific numeric and spatial location data for several source categories, from failing septic systems to domestic animals, livestock, and wildlife populations, can create many potential uncertainties in traditional water quality modeling. For this reason, MDE applies an analytical method combined with the bacteria source tracking described above for the calculation of this TMDL.

## **4.2 Analytical Framework**

This TMDL analysis uses flow duration curves to identify flow intervals that are used as indicators of hydrological conditions (i.e., annual average and critical conditions). This analytical method, combined with water quality monitoring data and BST, provides reasonable results (Cleland 2003), a better description of water quality than traditional water quality modeling, and also meets TMDL requirements.

In brief, baseline loads are estimated first for each subwatershed by using bacteria monitoring data and long-term flow data. These baseline loads are divided into four bacteria source categories using the results of the BST analysis. Next, percent reductions required to meet the water quality criterion in each subwatershed are estimated after accounting for critical condition and seasonality. Critical condition and seasonality are determined by assessing annual and dry weather seasonal hydrological conditions. Finally, TMDLs for each subwatershed are estimated by applying these percent reductions to the baseline loads.

Figure 4.2.1 illustrates how the hydrological (flow duration curve), water quality and BST data are linked together for the TMDL development.



**Figure 4.2.1: Diagram of the Non-Tidal Bacteria TMDL Analysis Framework**

### 4.3 Estimating Baseline Loads

The baseline load estimated in this TMDL analysis is reported as a long-term average annual load. This load is estimated using the geometric mean concentration and bias correction factor (calculated from bacteria monitoring data) and daily average flow (estimated from long-term flow data).

#### Estimating Weighted Annual Average Geometric Mean Concentrations

The weighted annual average geometric mean used in the calculation of the baseline load can be estimated either by monitoring design or by statistical analysis as follows:

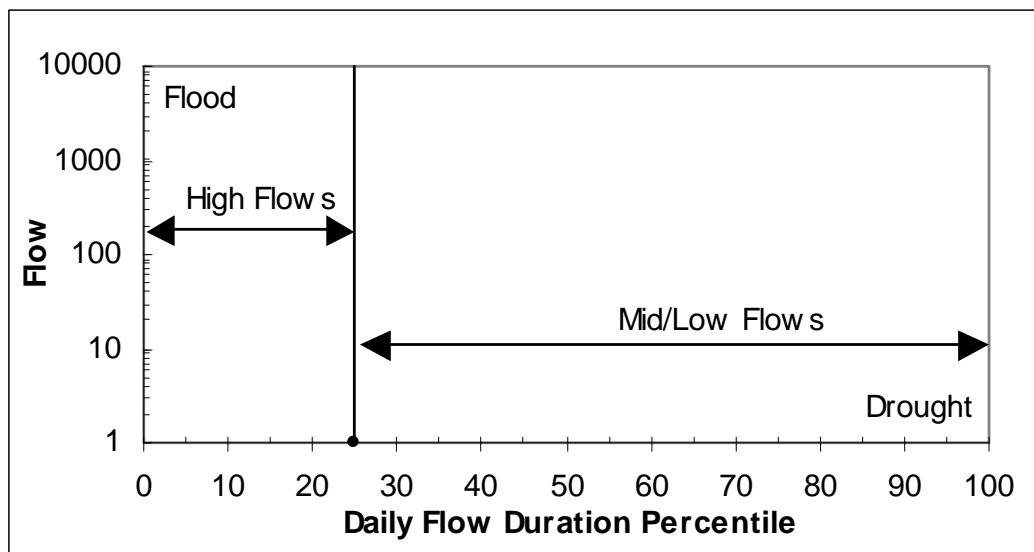
1. A stratified monitoring design is used where the number of samples collected is proportional to the duration of high flows, mid flows, and low flows within the watershed. This sample design allows a geometric mean to be calculated directly from the monitoring data without bias.
2. Routine monitoring typically results in samples from varying hydrologic conditions (i.e., high flows, mid flows, and low flows) where the numbers of samples are not proportional to the

duration of those conditions. Averaging data without consideration of the sampling conditions results in a biased estimate of geometric means. The potential bias of these geometric means can be reduced by weighting the sampling results collected during high flow, mid flow, and low flow regimes by the proportion of time each flow regime is expected to occur. This ensures that the high flow and low flow conditions are proportionally balanced.

3. If (1) the monitoring design was not stratified based on flow regime or (2) flow information is not available to weight the samples accordingly, then a geometric mean of sequential monitoring data can be used as an estimate of the geometric mean for the specified period.

A routine monitoring design was used to collect bacteria data in the Cherry Creek watershed. To estimate the weighted geometric mean, the monitoring data were first reviewed by plotting the sample results versus their corresponding daily flow duration percentile.

To calculate the weighted geometric mean with routine monitoring data, a conceptual model was developed by dividing the daily flow frequency for the stream segment into strata that are representative of hydrologic conditions. A conceptual continuum of flows is illustrated in Figure 4.3.1.



**Figure 4.3.1: Conceptual Diagram of Flow Duration Zones**

During high flows, a significant portion of the total stream flow is from surface flow contributions. Low flow conditions represent periods with minimal rainfall and surface runoff. There is typically a transitional mid flow period between the high and low flow durations, representative of varying contributions of surface flow inputs that result from differing rainfall volumes and antecedent soil moisture conditions. Because the bacteria samples were taken during a routine monitoring design and not a stratified monitoring design, the division of the entire flow regime into strata enables the estimation of a less flow-biased geometric mean. Based on flow data of USGS gage 03075500 it was determined that the long-term average daily flow corresponds to a daily flow duration of 30.7%. Hence for this analysis it is defined that

flows greater than the 30.7 percentile flow represent high flows, and flows less than the 30.7 percentile flow represent mid/low flows. A detailed method of how the flow strata were defined is presented in Appendix B.

Factors for estimating a weighted geometric mean are based on the frequency of each flow stratum. The weighting factor accounts for the proportion of time that each flow stratum represents. The weighting factors for an average hydrological year used in the Cherry Creek TMDL analysis are presented in Table 4.3.1.

**Table 4.3.1: Weighting Factors for Average Hydrology Year Used for Estimation of Geometric Means in the Patapsco River Lower North Branch Watershed**

Flow Duration Zone	Duration Interval	Weighting Factor
High Flows	0 – 30.7%	0.307
Mid/Low Flows	30.7 – 100%	0.693

Bacteria enumeration results for samples within a specified stratum will receive their corresponding weighting factor. The weighted geometric mean is calculated as follows:

$$M = \sum_{i=1}^2 M_i * W_i \quad (1)$$

where,

$$M_i = \frac{\sum_{j=1}^{n_i} \log_{10}(C_{i,j})}{n_i} \quad (2)$$

- M = log weighted mean
- M<sub>i</sub> = log mean concentration for stratum i
- W<sub>i</sub> = proportion of stratum i
- C<sub>i,j</sub> = concentration for sample j in stratum i
- n<sub>i</sub> = number of samples in stratum

Finally, the weighted geometric mean concentration is estimated using the following equation:

$$C_{gm} = 10^M \quad (3)$$

where,

C<sub>gm</sub> = Weighted geometric mean concentration

For the seasonal analysis only, the overall geometric mean for the period was applied due to an insufficient number of samples during low flow conditions. Table 4.3.2 presents the annual maximum and minimum concentrations, the annual average geometric means by stratum, and the annual average weighted geometric mean for the Cherry Creek watershed. Table 4.3.3 presents the seasonal dry weather steady-state maximum and minimum concentrations and the geometric mean concentration for the watershed. Graphs illustrating these results can be found in Appendix B.

**Table 4.3.2: Cherry Creek Watershed Annual Weighted Geometric Mean**

Station / Tributary	Flow Stratum	Number of Samples	<i>E. coli</i> Minimum Concentration (MPN/100ml)	<i>E. coli</i> Maximum Concentration (MPN/100ml)	Annual Steady State Geometric Mean (MPN/100ml)	Annual Weighted Geometric Mean (MPN/100ml)
CHC0008 Cherry Creek	High	5	109	5,794	491	826
	Low	19	160	6,867	1,040	

\* Used for estimating average annual baseline loads

**Table 4.3.3: Cherry Creek Watershed Seasonal (May 1 - September 30) Dry Weather Period Steady-State Geometric Means**

Station / Tributary	Number of Samples	<i>E. coli</i> Minimum Concentration (MPN/100ml)	<i>E. coli</i> Maximum Concentration (MPN/100ml)	Seasonal Steady State Geometric Mean (MPN/100ml)
CHC0008 Cherry Creek	8	576	4,106	1,170

\* Used for estimating reductions needed to meet water quality standards

The weighted annual average geometric mean concentration is calculated from the log transformation of the raw data. Statistical theory tells us that when back-transformed values are used to calculate average daily loads or total annual loads, the loads will be biased low (Richards 1998). To avoid this bias, a factor should be added to the log-concentration before it is back-transformed. There are several methods of determining this bias correction factor, ranging from parametric estimates resulting from the theory of the log-normal distribution to non-parametric estimates using a bias correction factor [Ferguson 1986; Cohn et al. 1989; Duan 1983]. There is much literature on the applicability and results from these various methods with a summary provided in Richards (1998). Each has advantages and conditions of applicability. A non-parametric estimate of the bias correction factor (Duan 1983) was used in this TMDL analysis.

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With calculated geometric means and arithmetic means for each flow stratum, the bias correction factors are estimated as follows:

$$F_{1i} = A_i/C_i \quad (4)$$

where,

- $F_{1i}$  = bias correction factor for stratum  $i$
- $A_i$  = long-term annual arithmetic mean for stratum  $i$
- $C_i$  = long-term annual geometric mean for stratum  $i$

Daily average flows are estimated for each flow stratum using the watershed area ratio approach, since nearby long-term monitoring data are available.

The loads for each stratum are estimated as follows:

$$L_i = Q_i * C_i * F_{1i} * F_2 \quad (5)$$

where,

- $L_i$  = daily average load (Billion MPN/day) at monitoring station for stratum  $i$
- $Q_i$  = daily average flow (cfs) for stratum  $i$
- $C_i$  = geometric mean for stratum  $i$
- $F_{1i}$  = bias correction factor for stratum  $i$
- $F_2$  = unit conversion factor (0.0245)

Finally, for each subwatershed, the baseline load is estimated as follows:

$$L = \sum_{i=1}^2 L_i * W_i \quad (6)$$

where,

- $L$  = daily average load at station (MPN/day)
- $W_i$  = proportion of stratum  $i$

In the Cherry Creek watershed, weighting factors of 0.307 for high flow and 0.693 for mid/low flows were used to estimate the annual baseline load expressed as Billion MPN *E. coli*/year. Results of the baseline load calculations are presented in Table 4.3.4 and a summary of the baseline loads is given in Table 4.3.5.

**Table 4.3.4: Baseline Load Calculations**

Station	Area (mi <sup>2</sup> )	High Flow			Low Flow			Baseline <i>E. coli</i> Load (Billion MPN/year)
		Average Flow (cfs)	<i>E. coli</i> Concentration (MPN/100ml)	Bias Correction Factor	Average Flow (cfs)	<i>E. coli</i> Concentration (MPN/100ml)	Bias Correction Factor	
CHC0008	16.7	97.5	491	2.86	15.0	1,040	1.72	541,106

**Table 4.3.5: Baseline Loads Summary**

MD 12-Digit Cherry Creek Fecal Bacteria Baseline Loads (Billion MPN <i>E. coli</i> /year)						
Total Baseline Load	=	Nonpoint Source BL	+	NPDES Stormwater BL	+	WWTP BL
541,106	=	541,106	+	0	+	0

#### 4.4 Bacteria Source Tracking

As explained above in the Source Assessment section, ARA was used to identify probable bacterial sources in the Cherry Creek watershed. An accurate representation of the expected contribution of each source at each station is estimated by using a stratified weighted mean of the identified sample results. The weighting factors are based on the log<sub>10</sub> of the bacteria concentration and the percent of time that represents the high stream flow or low stream flow (see Appendix B). The procedure for calculating the stratified weighted mean of the sources per monitoring station is as follows:

1. Calculate the percentage of isolates per source per each sample date (S).
2. Calculate an initial weighted percentage (MS) of each source per flow strata (high/low). The weighting is based on the log<sub>10</sub> bacteria concentration for the water sample.
3. Adjust the weighted percentage based on the classification of known sources.
4. The final weighted mean source percentage, for each source category, is based on the proportion of time in each flow duration zone.

If a hydrological condition (i. e., dry weather seasonal condition) does not have enough samples in each flow duration zone, then the final weighted mean source percentage is not stratified based on flow duration zones and an overall seasonal source percentage is calculated, weighted only by the concentration of the water sample (See Appendix B).

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The weighted mean for each source category is calculated using the following equations:

$$MS_l = \sum_{i=1}^2 MS_{i,l} * W_i \quad (7)$$

where,

$$MS_{i,l} = \sum_{k=1}^5 \frac{A_{l,k} * IMS_{i,k}}{P_k} \quad (8)$$

where,

$$IMS_{i,k} = \frac{\sum_{j=1}^{n_i} \log_{10}(C_{i,j}) * S_{i,j,k}}{\sum_{j=1}^{n_i} \log_{10}(C_{i,j})} \quad (9)$$

and where,

- $MS_l$  = weighted mean proportion of isolates of source  $l$
- $MS_{i,l}$  = adjusted weighted mean proportion of isolates for source  $l$  in stratum  $i$
- $IMS_{i,k}$  = initial weighted mean proportion of isolates for source  $k$  in stratum  $i$
- $W_i$  = proportion covered by stratum  $i$
- $A_{l,k}$  = number of known source  $l$  isolates initially predicted as source  $k$
- $P_k$  = number of total known isolates initially predicted as source  $k$
- $i$  = stratum
- $j$  = sample
- $k$  = source category (1=human, 2=domestic, 3=livestock, 4=wildlife, 5=unknown)
- $l$  = final source category (1=human, 2=domestic, 3=livestock, 4=wildlife)
- $C_{i,j}$  = concentration for sample  $j$  in stratum  $i$
- $S_{i,j,k}$  = proportion of isolates for sample  $j$ , of source  $k$  in stratum  $i$
- $n_i$  = number of samples in stratum  $i$

The complete distributions of the annual and seasonal period source loads are listed in Tables 4.4.1 and 4.4.2. Details of the BST data and tables with the BST analysis results can be found in Appendix C.

**Table 4.4.1: Distribution of Fecal Bacteria Source Loads in the Cherry Creek Watershed for the Average Annual Period**

Station	Flow Stratum	% Domestic Animals	% Human	% Livestock	% Wildlife
CHC0008	High	21.5	30.8	19.7	28.0
	Low	15.2	29.4	23.1	32.3
	Weighted	17.1	29.8	22.1	31.0



**Table 4.4.2: Distribution of Fecal Bacteria Source Loads in the Cherry Creek Watershed for the Seasonal (May 1<sup>st</sup> – September 30<sup>th</sup>) Dry Weather Period**

Station	% Domestic Animals	% Human	% Livestock	% Wildlife
CHC0008	10.1	21.5	34.1	34.2

#### 4.5 Critical Condition and Seasonality

Federal regulations (40 CFR 130.7(c)(1)) require TMDLs to take into account critical conditions for stream flow, loading, and water quality parameters. The intent of this requirement is to ensure that the water quality of the waterbody is protected during times when it is most vulnerable.

For this TMDL the critical condition is determined by assessing both the annual and dry weather seasonal conditions. Seasonality is assessed as the time period when water contact recreation is expected, specifically dry weather days during May 1<sup>st</sup> through September 30<sup>th</sup>. The critical condition requirement is met by determining the maximum reduction per bacteria source that satisfies both conditions and meets the water quality standard, thereby minimizing the risk to water contact recreation. It is assumed that the reduction applied to a bacteria source category will be constant through both conditions.

The bacteria monitoring data for the station in the Cherry Creek watershed cover a sufficient temporal span (at least one year) to estimate annual conditions. However, sufficient data were not available for the seasonal period to consider high flow and low flow conditions. Since all samples of the seasonal period were taken during low flow, a geometric mean cannot be established for the high flow condition. Therefore an overall average geometric mean and average flow were used for the seasonal analysis.

The reductions of fecal bacteria required to meet water quality standards in the Cherry Creek watershed are shown in Table 4.5.1.

**Table 4.5.1: Required Fecal Bacteria Reductions (by Condition) to Meet Water Quality Standards**

Station	Condition	Domestic Animals %	Human %	Livestock %	Wildlife %
CHC0008	Annual	98.0	98.0	98.0	57.6
	Seasonal	98.0	98.0	98.0	73.9
	<b>Maximum Source Reduction</b>	<b>98.0</b>	<b>98.0</b>	<b>98.0</b>	<b>73.9</b>

#### 4.6 Margin of Safety

A margin of safety (MOS) is required as part of this TMDL in recognition of the many uncertainties in the understanding and simulation of bacteriological water quality in natural systems and in statistical estimates of indicators. As mentioned in Section 4.1, it is difficult to estimate stream loadings for fecal bacteria due to the variation in loadings across sample locations and time. Load estimation methods should be both precise and accurate to obtain the true estimate of the mean load. Refined precision in the load estimation is due to using a stratified approach along the flow duration intervals, thus reducing the variation in the estimates. Moreover, Richards (1998) reports that averaging methods are generally biased, and the bias increases as the size of the averaging window increases. Finally, accuracy in the load estimation is based on minimal bias in the final result when compared to the true value.

Based on EPA guidance, the MOS can be achieved through two approaches (US EPA 1991a). One approach is to reserve a portion of the loading capacity as a separate term in the TMDL (i.e.,  $TMDL = LA + WLA + MOS$ ). The second approach is to incorporate the MOS as conservative assumptions used in the TMDL analysis. The second approach was used for this TMDL by estimating the loading capacity of the stream based on a reduced (more stringent) water quality criterion concentration. The *E. coli* water quality criterion concentration was reduced by 5%, from 126 *E. coli* MPN/100ml to 119.7 *E. coli* MPN/100ml.

#### 4.7 Scenario Descriptions

##### Source Distribution

The final bacteria source distribution and corresponding baseline loads are derived from the source proportions listed in Table 4.4.1. The source distribution and baseline loads used in the TMDL scenarios are presented in Table 4.7.1.

**Table 4.7.1: Bacteria Source Distributions and Corresponding Baseline Loads Used in the Annual Average TMDL Analysis**

Station	Domestic		Human		Livestock		Wildlife		Total Load (Billion <i>E. coli</i> MPN/year)
	%	Load (Billion <i>E. coli</i> MPN/year)	%	Load (Billion <i>E. coli</i> MPN/year)	%	Load (Billion <i>E. coli</i> MPN/year)	%	Load (Billion <i>E. coli</i> MPN/year)	
CHC0008	17.1	92,760	29.8	161,407	22.1	119,400	31.0	167,539	541,106

### **First Scenario: Fecal Bacteria Practicable Reduction Targets**

The maximum practicable reduction (MPR) for each of the four source categories is listed in Table 4.7.2. These values are based on review of the available literature and best professional judgment. It is assumed that human sources would potentially have the highest risk of causing gastrointestinal illness and therefore should have the highest reduction. If a domestic WWTP is located in the upstream watershed, this is considered in the MPR so as to not violate the permitted loads. The domestic animal category includes sources from pets (e.g., dogs) and the MPR is based on an estimated success of education and outreach programs.

**Table 4.7.2: Maximum Practicable Reduction Targets**

<b>Max Practicable Reduction per Source</b>	<b>Human</b>	<b>Domestic</b>	<b>Livestock</b>	<b>Wildlife</b>
	95%	75%	75%	0%
<b>Rationale</b>	(a) Direct source inputs. (b) Human pathogens more prevalent in humans than animals. (c) Enteric viral diseases spread from human to human. <sup>1</sup>	Target goal reflects uncertainty in effectiveness of urban BMPs <sup>2</sup> and is also based on best professional judgment	Target goal based on sediment reductions from BMPs <sup>3</sup> and best professional judgment	No programmatic approaches for wildlife reduction to meet water quality standards.  Waters contaminated by wild animal wastes offer a public health risk that is orders of magnitude less than that associated with human waste. <sup>4</sup>

<sup>1</sup>Health Effects Criteria for Fresh Recreational Waters. EPA-600/1-84-004. U.S. Environmental Protection Agency, Washington, DC. EPA. 1984.

<sup>2</sup>Preliminary Data Summary of Urban Storm Water Best Management Practices. EPA-821-R-99-012. U.S. Environmental Protection Agency, Washington, DC. EPA. 1999.

<sup>3</sup>Agricultural BMP Descriptions as Defined for The Chesapeake Bay Program Watershed Model. Nutrient Subcommittee Agricultural Nutrient Reduction Workshop. EPA. 2004.

<sup>4</sup>Environmental Indicators and Shellfish Safety. 1994. Edited by Cameron, R., Mackeney and Merle D. Pierson, Chapman & Hall.

As previously stated, these maximum practicable reduction targets are based on the available literature and best professional judgment. There is much uncertainty with estimated reductions from best management practices (BMP). The BMP efficiency for bacteria reduction ranged from -6% to +99% based on a total of 10 observations (US EPA 1999). The MPR to agricultural lands was based on sediment reductions identified by EPA (US EPA 2004).

The practicable reduction scenario was developed based on an optimization analysis whereby a subjective estimate of risk was minimized and constraints were set on maximum reduction and allowable background conditions. Risk was defined on a scale of one to five, where it was assumed that human sources had the highest risk (5), domestic animals and livestock next (3), and wildlife the lowest (1) (See Table 4.6.2). The model was defined as follows:

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$$\text{Risk Score} = \text{Min} \sum_{i=1}^4 P_j * W_j \quad (10)$$

where,

$$P_j = \frac{(1 - R_i) * P_{b_j}}{1 - TR} \quad (11)$$

and,

$$TR = \frac{C - C_{cr}}{C} \quad (12)$$

Therefore the risk score can be represented as:

$$\text{Risk Score} = \text{Min} \sum_{i=1}^4 \left[ \frac{(1 - R_j) * P_{b_j}}{\left(1 - \frac{C - C_{cr}}{C}\right)} * W_j \right] \quad (13)$$

where,

- $i$  = hydrological condition
- $j$  = bacteria source category = human, domestic animal, livestock and wildlife
- $P_j$  = % of each source category (human, domestic animals, livestock and wildlife) in final allocation
- $W_j$  = weight of risk per source category = 5, 3 or 1
- $R_j$  = percent reduction applied by source category (human, domestic animals, livestock and wildlife) for the specified hydrological condition (variable)
- $P_{b_j}$  = original (baseline) percent distribution by source category (variable)
- $TR$  = total reduction (constant within each hydrological condition) = Target reduction
- $C$  = in-stream concentration
- $C_{cr}$  = water quality criterion

The model is subject to the following constraints:

$$\begin{aligned} C &= C_{cr} \\ 0 \leq R_{\text{human}} &\leq 95\% \\ 0 \leq R_{\text{pets}} &\leq 75\% \\ 0 \leq R_{\text{livestock}} &\leq 75\% \\ R_{\text{wildlife}} &= 0 \\ P_j &\geq 1\% \end{aligned}$$

For the Cherry Creek watershed the constraints of this scenario could not be satisfied, indicating there was not a practicable solution. A summary of the first scenario analysis results is presented in Table 4.7.3.

**Table 4.7.3: Practicable Reduction Scenario Results**

Station	Applied Reductions				Total Reduction %	Target Reduction %
	Domestic %	Human %	Livestock %	Wildlife %		
CHC0008	75.0	95.0	75.0	0.0	57.7	90.6

**Second Scenario: Fecal Bacteria Reductions Higher Than MPRs**

The TMDL must specify load allocations that will meet the water quality standards. In the practicable reduction targets scenario, it is possible for the watershed to meet water quality standards based on MPRs.

To further develop the TMDL, a second scenario was analyzed in which the constraints of the MPRs were relaxed. The maximum allowable reduction was increased to 98% for all sources, including wildlife. A similar optimization procedure as before was used to minimize risk. Again, the objective is to minimize the sum of the risk for all conditions while meeting the scenario reduction constraints. The model was defined in the same manner as considered in the practicable reduction scenario but subject to the following constraints:

$$\begin{aligned}
 C &= C_{cr} \\
 0 \leq R_{human} &\leq 98\% \\
 0 \leq R_{pets} &\leq 98\% \\
 0 \leq R_{livestock} &\leq 98\% \\
 0 \leq R_{wildlife} &\leq 98\% \\
 P_j &\geq 1\%
 \end{aligned}$$

A summary of the results from this second scenario analysis is presented in Table 4.7.4.

**Table 4.7.4: Reduction Results Based on Optimization Model Allowing up to 98% Reduction**

Station	Applied Reductions				Total Reduction %	Target Reduction %
	Domestic %	Human %	Livestock %	Wildlife %		
CHC0008	98.0	98.0	98.0	73.9	90.6	90.6

#### 4.8 TMDL Loading Caps

The TMDL loading cap is an estimate of the assimilative capacity of the monitored watershed. Estimation of the TMDL requires knowledge of how bacteria concentrations vary with flow rate or the flow duration interval. This relationship between concentration and flow is established using the strata defined by the flow duration curve. The TMDL loading caps are provided in billion MPN *E. coli*/year.

##### Annual Average TMDL Loading Caps

As explained in the sections above, the annual average TMDL loading caps are estimated by first determining the baseline or current condition loads with the associated geometric mean from the available monitoring data. This annual average baseline load is estimated using the geometric mean concentration and the long-term annual average daily flow for each flow stratum. The loads from these two strata are then weighted to represent average conditions (see Table 4.3.1), based on the proportion of each stratum, to estimate the total long-term loading rate.

Next, the percent reduction required to meet the water quality criterion is estimated from the observed bacteria concentrations accounting for the critical conditions (See Section 4.5). A reduction in concentration is proportional to a reduction in load; thus the TMDL is equal to the current baseline load multiplied by one minus the required reduction. This reduction, estimated as explained in Section 4.5, represents the maximum reduction per source that satisfies the two hydrological conditions, and that is required to meet water quality standards.

$$\text{TMDL Loading Cap} = L_b * (1 - R) \quad (14)$$

where,

- $L_b$  = current or baseline load estimated from monitoring data
- $R$  = reduction required from baseline to meet water quality criterion.

The annual average bacteria TMDL loading cap is shown in Tables 4.8.1 and 4.8.2.

**Table 4.8.1: Annual Average TMDL Loading Caps**

Station	<i>E. coli</i> Baseline Load (Billion MPN/year)	Long-Term Average <i>E. coli</i> TMDL Load (Billion MPN/year)	% Target Reduction
CHC0008	541,106	51,125	90.6

**Table 4.8.2: Annual Average TMDL Loading Caps by Source Category**

Station	Domestic		Human		Livestock		Wildlife		Total Load (Billion <i>E. coli</i> MPN/year)
	%	Load (Billion <i>E. coli</i> MPN/year)	%	Load (Billion <i>E. coli</i> MPN/year)	%	Load (Billion <i>E. coli</i> MPN/year)	%	Load (Billion <i>E. coli</i> MPN/year)	
CHC0008	3.6	1,855	6.3	3,228	4.7	2,388	85.4	43,654	51,125

### **Maximum Daily Loads**

Recent EPA guidance (US EPA 2006a) recommends that maximum daily load (MDL) expressions of long-term annual average TMDLs should also be provided as part of the TMDL analysis and report. Selection of an appropriate method for translating a TMDL based on a longer time period into one using a daily time period requires decisions regarding 1) the level of resolution, and 2) the level of protection. The level of resolution pertains to the amount of detail used in specifying the maximum daily load. The level of protection represents how often the maximum daily load (MDL) is expected to be exceeded. Draft EPA/TetraTech guidance on daily loads (Limno-Tech 2007) provides three categories of options for both level of resolution and level of protection, and discusses these categories in detail.

For the Cherry Creek watershed MDLs, a “representative daily load” option was selected as the level of resolution, and a value “that will be exceeded with a pre-defined probability” was selected as the level of protection. In these options, the MDLs have an upper bound percentile that accounts for the variability of daily loads. The upper bound percentile and the MDLs were estimated following EPA’s “*Technical Support Document for Water Quality-Based Toxics Control*” (1991 TSD) (US EPA 1991); and “*Approaches For Developing a Daily Load Expression for TMDLs Computed for Longer Term Averages*” (US EPA 2006).

There are three steps to the overall process of estimating these MDLs. First, all the data available from each monitoring station are examined together by stratum and the percentile rank of the highest observed concentration (for each stratum at each station) is computed. The highest computed percentile rank is the upper bound percentile to be used in estimating the MDLs.

Secondly, the long-term annual average TMDL (see Table 4.7.1) concentrations are estimated for both high-flow and low-flow strata. This is conducted for each station using a statistical methodology (the “Statistical Theory of Rollback,” or “STR,” described more fully in Appendix D).

Third, based on the estimated long-term average (LTA) TMDL concentrations, the MDL for each flow stratum at each station is estimated using the upper boundary percentile computed in the first step above. Finally, MDLs are computed from these MDL concentrations and their corresponding flows.

Results of the fecal bacteria MDL analysis for the Cherry Creek watershed are shown in Table 4.8.3.

**Table 4.8.3: Cherry Creek Watershed Maximum Daily Loads Summary**

Station	Flow Stratum	Maximum Daily Load (Billion <i>E. coli</i> MPN/day)	
		by Stratum	Weighted by Stratum
CHC0008	High	1,727	695
	Low	237	

See Appendix D for a more detailed explanation of the procedure for obtaining these daily loads.

#### 4.9 TMDL Allocation

The Cherry Creek watershed fecal bacteria TMDL has the following components:

$$\text{TMDL} = \text{LA} + \text{WLA} + \text{MOS} \quad (15)$$

where,

LA = Load Allocation  
WLA = Waste Load Allocation  
MOS = Margin of Safety

The TMDL allocation includes load allocations (LA) for nonpoint sources and waste load allocations (WLA) for point sources including WWTPs and NPDES-regulated stormwater



discharges. The Stormwater (SW) WLA includes any nonpoint source loads deemed to be transported and discharged by regulated stormwater systems. An explanation of the distribution of nonpoint source loads and point source loads to the LA and to the SW-WLA and WWTP-WLA is provided in the subsections that follow.

The margin of safety (MOS) is explicit and is incorporated in the analysis using a conservative assumption; it is not specified as a separate term. The assumption is that a 5% reduction of the criterion concentration established by MD to meet the applicable water quality standard will result in more conservative allowable loads of fecal bacteria, and thus provide the MOS. The final loads are based on average hydrological conditions, with reductions estimated based on critical hydrological conditions. The load reduction scenario results in load allocations that will achieve water quality standards. The State of Maryland reserves the right to revise these allocations provided such revisions are consistent with the achievement of water quality standards.

**Bacteria Source Categories and Allocation Distributions**

The bacteria sources are grouped into four categories that are also consistent with divisions for various management strategies. The categories are human, domestic animal, livestock and wildlife. TMDL allocation rules are presented in Table 4.9.1. This table identifies how the TMDL will be allocated among the LA (those nonpoint sources or portions thereof not transported and discharged by stormwater systems) and the WLA (point sources including WWTPs and NPDES regulated stormwater discharges). Only the final LA or WLA is reported in this TMDL.

**Table 4.9.1: Potential Source Contributions for TMDL Allocation Categories**

Source Category	TMDL Allocation Categories		
	LA	WLA	
		WWTP	Stormwater
Human	X		
Domestic	X		
Livestock	X		
Wildlife	X		

**LA**

All four bacteria source categories could potentially contribute to nonpoint source loads. For human sources, if the watershed has no Phase I or Phase II MS4 or other NPDES-regulated stormwater discharges, the nonpoint source contribution is estimated by subtracting any WWTP

and/or CSO loads from the TMDL human load, and is then assigned to the LA. However, in watersheds covered by NPDES-regulated stormwater permits, any such nonpoint sources of human bacteria (i.e., beyond the reach of the sanitary sewer systems) are distributed amongst the SW-WLA and LA.

Livestock loads are all assigned to the LA. Since there are no MS4 or other NPDES-regulated stormwater systems, bacteria loads from domestic animal, human and wildlife sources are entirely assigned to the LA.

### WLA

#### *NPDES Regulated Stormwater*

EPA's guidance document, *Establishing Total Maximum Daily Load (TMDL) Wasteload Allocations (WLAs) for Storm Water Sources and NPDES Permit Requirements Based on Those WLAs* (November 2002), advises that all individual and general NPDES Phase I and Phase II stormwater permits are point sources subject to WLA assignment in the TMDL. The document acknowledges that quantification of rainfall-driven nonpoint source loads is uncertain, stating that available data and information usually are not detailed enough to determine WLAs for NPDES-regulated stormwater discharges on an outfall-specific basis; therefore, the EPA guidance allows the stormwater WLA to be expressed as an aggregate allotment.

There are no Phase I or Phase II MS4 permits or other NPDES-regulated stormwater entities in the Cherry Creek watershed.

#### *Municipal and Industrial WWTPs*

There are no municipal or industrial NPDES permitted point source facilities with permits regulating the discharge of fecal bacteria in the Cherry Creek watershed.

## 4.10 Summary

The long-term annual average TMDL and TMDL allocations are presented in Table 4.10.1. Table 4.9.2 presents the maximum daily loads for the watershed.

**Table 4.10.1: Cherry Creek Watershed Annual Average TMDL**

Station	Total Allocation	LA	SW-WLA	WWTP-WLA
		(Billion MPN <i>E. coli</i> /year)		
CHC0008	51,125	51,125	N/A	N/A
<b>Total</b>	<b>51,125</b>	<b>51,125</b>	<b>N/A</b>	<b>N/A</b>

**Table 4.10.2: Cherry Creek Watershed Maximum Daily Loads**

Station	Total Allocation	LA	SW-WLA	WWTP-WLA
		(Billion MPN <i>E. coli</i> /day)		
CHC0008	695	695	N/A	N/A
<b>Total</b>	<b>695</b>	<b>695</b>	<b>N/A</b>	<b>N/A</b>

The long-term annual average fecal bacteria TMDL summary for the Cherry Creek watershed is presented in Table 4.10.3.

**Table 4.10.3: MD 12-Digit Cherry Creek Watershed Annual Average TMDL Summary**

(Billion MPN <i>E. coli</i> /year)								
TMDL	=	LA	+	WLA			+	MOS
				SW WLA	+	WWTP WLA		
51,125	=	51,125	+	0	+	0	+	Incorporated

The maximum daily loads of fecal bacteria for the Cherry Creek watershed are summarized in Table 4.10.4.

**Table 4.10.4: MD 12-Digit Cherry Creek Watershed MDL Summary**

(Billion MPN <i>E. coli</i> /day)								
MDL	=	LA	+	WLA			+	MOS
				SW WLA	+	WWTP WLA		
695	=	695	+	0	+	0	+	Incorporated

In certain watersheds, the goal of meeting water quality standards may require very high reductions that are not achievable with current technologies and management practices. In this situation, where there is no feasible TMDL scenario, MPRs are increased to provide estimates of the reductions required to meet water quality standards. In the Cherry Creek watershed, water quality standards cannot be achieved with the maximum practicable reduction rates specified in Table 4.7.3. The TMDLs shown in Tables 4.10.1 and 4.10.2 represent reductions from current bacteria loadings that are beyond practical reductions. In cases where such high reductions are required to meet standards, it is expected that the first stage of implementation will be to carry out the MPR scenario.

## 5.0 ASSURANCE OF IMPLEMENTATION

Section 303(d) of the Clean Water Act and current EPA regulations require reasonable assurance that the TMDL load and waste load allocations can and will be implemented. In the Cherry Creek watershed, the TMDL analysis indicates that the reduction of fecal bacteria loads are beyond the MPR targets. These MPR targets were defined based on a literature review of BMPs effectiveness and assuming a zero reduction for wildlife sources. Cherry Creek and its tributaries may not be able to attain water quality standards. The fecal bacteria load reductions required to meet water quality criteria in the Cherry Creek watershed are not feasible by implementing effluent limitations and cost-effective, reasonable BMPs to nonpoint sources. Therefore, MDE proposes a staged approach to implementation beginning with the MPR scenario, with regularly scheduled follow-up monitoring to assess the effectiveness of the implementation plan.

Additional reductions will be achieved through the implementation of BMPs; however, the literature reports considerable uncertainty concerning the effectiveness of BMPs in treating bacteria. As an example, pet waste education programs have varying results based on stakeholder involvement. Additionally, the extent of wildlife reduction associated with various BMPs methods (e.g., structural, non-structural, etc.) is uncertain. Therefore, MDE intends for the required reductions to be implemented in an iterative process that first addresses those sources with the largest impact on water quality and human health risk, with consideration given to ease of implementation and cost. The iterative implementation of BMPs in the watershed has several benefits: tracking of water quality improvements following BMP implementation through follow-up stream monitoring; providing a mechanism for developing public support through periodic updates on BMP implementation; and helping to ensure that the most cost-effective practices are implemented first.

Potential funding sources for implementation include the Maryland's Agricultural Cost Share Program (MACS), which provides grants to farmers to help protect natural resources, and the Environmental Quality and Incentives Program, which focuses on implementing conservation practices and BMPs on land involved with livestock and production. Though not directly linked, it is assumed that the nutrient management plans from the Water Quality Improvement Act of 1998 (WQIA) will have some reduction of bacteria from manure application practices.

### **Implementation and Wildlife Sources**

It is expected that, in some waters for which TMDLs will be developed, the bacteria source analysis indicates that after controls are in place for all anthropogenic sources, the waterbody will not meet water quality standards. Managing the overpopulation of wildlife remains an option for state and local stakeholders.

After developing and implementing, to the maximum extent possible, a reduction goal based on the anthropogenic sources identified in the TMDL, Maryland anticipates that implementation to reduce the controllable nonpoint sources may also reduce some wildlife inputs to the waters.

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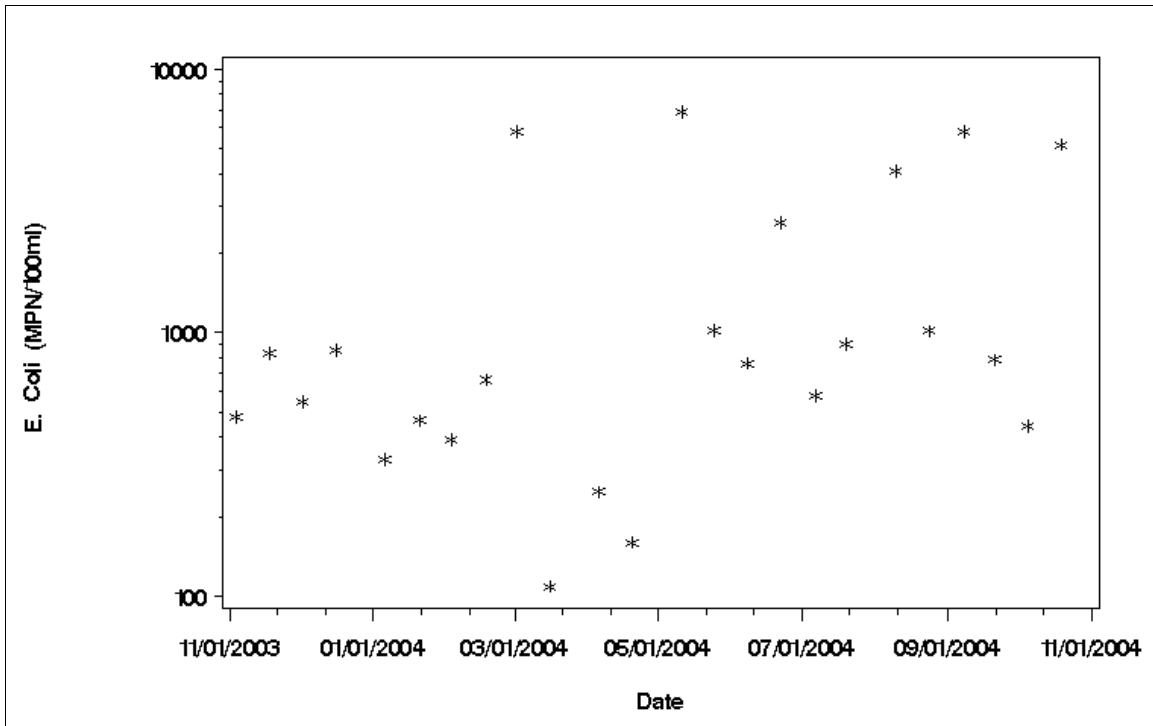
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## Appendix A – Bacteria Data

Table A-1: Measured Bacteria Concentration and Daily Flow Frequency

Station	Date	Daily flow frequency	<i>E. coli</i> Concentration (MPN/100ml)
CHC0008	11/04/2003	50.5475	480
	11/18/2003	31.4608	833
	12/02/2003	30.1029	547
	12/16/2003	32.6544	855
	01/06/2004	3.0771	331
	01/21/2004	60.1621	464
	02/03/2004	70.5869	394
	02/18/2004	51.6426	663
	03/02/2004	3.0771	5794
	03/16/2004	23.1932	109
	04/06/2004	11.1586	249
	04/20/2004	35.5344	160
	05/11/2004	68.1121	6867
	05/25/2004	38.7538	1019
	06/08/2004	60.4358	766
	06/22/2004	40.7140	2613
	07/07/2004	81.6579	576
	07/20/2004	75.9527	907
	08/10/2004	88.0311	4106
	08/24/2004	62.7135	1014
09/08/2004	33.3990	5794	
09/21/2004	53.9203	789	
10/05/2004	78.8108	441	
10/19/2004	64.8270	5172	



**Figure A-1: *E. coli* Concentration vs. Time for MDE Monitoring Station CHC0008**



## Appendix B – Flow Duration Curve Analysis to Define Strata

The Cherry Creek watershed was assessed to determine hydrologically significant strata. The purpose of these strata is to apply weights to monitoring data and thus reduce bias associated with the monitoring design. The strata group hydrologically similar water quality samples and provide a better estimate of the mean concentration at the monitoring station.

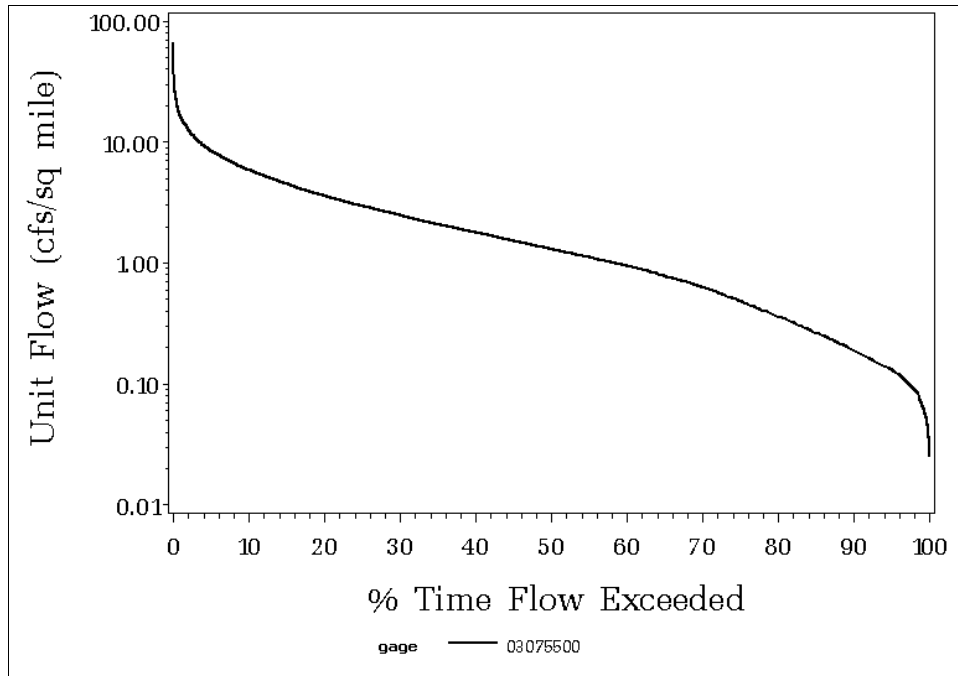
The flow duration curve for a watershed is a plot of all possible daily flows, ranked from highest to lowest, versus their probability of exceedance. In general, the higher flows will tend to be dominated by excess runoff from rain events and the lower flows will result from drought type conditions. The mid-range flows are a combination of high base flow with limited runoff and lower base flow with excess runoff. The range of these mid-level flows will vary with antecedent soil moisture conditions. The purpose of the following analysis is to identify hydrologically significant groups, based on the previously described flow regimes, within the flow duration curve.

### *Flow Analysis*

A USGS gage station is not present within the Cherry Creek watershed thus a nearby gage station was used for the analysis. The site is listed in Table B-1 and the flow duration curve for the site is presented in Figure B-1.

**Table B-1: USGS Sites Near the Cherry Creek Watershed**

USGS Site #	Dates Used	Location
03075500	10/01/1982 – 9/30/2007	Youghiogheny River near Oakland, MD



**Figure B-1: Flow Duration Curve for USGS Gage 03075500**

Based on the flow data from the Youghiogheny River gage station the long-term average daily unit flow is 2.41 cfs/sq. mile, which corresponds to a flow frequency of 30.7%. Using the definition of a high flow condition as occurring when flows are higher than the long-term average flow and a low flow condition as occurring when flows are lower than the long-term average flow, the 30.7 percentile threshold was selected to define the limits between high flows and low flows in this watershed. Therefore, a high flow condition will be defined as occurring when the daily flow duration percentile is less than 30.7% and a low flow condition will be defined as occurring when the daily flow duration percentile is greater than 30.7%. Definitions of high and low range flows are presented in Table B-2.

**Table B-2: Definition of Flow Regimes**

<b>High Flow</b>	Represents conditions where stream flow tends to be dominated by surface runoff.
<b>Low Flow</b>	Represents conditions where stream flow tends to be more dominated by groundwater flow.

The final analysis to define the daily flow duration intervals (flow regions, strata) includes the bacteria monitoring data. Bacteria (*E. coli*) monitoring data are “placed” within the regions (strata) based on the daily flow duration percentile of the date of sampling. Figures B-2 and B-3

show the Cherry Creek watershed *E. coli* monitoring data with corresponding flow frequency for the average annual and the dry weather seasonal conditions.

Maryland's water quality standards for bacteria state that, when available, the geometric mean indicator should be based on at least five. Therefore, in situations in which fewer than five samples "fall" within a particular flow regime interval, the interval and the adjacent interval will be joined. In the Cherry Creek watershed, for the annual average flow condition, there are sufficient samples in both the high flow and low flow strata to estimate the geometric means. However, in the dry weather seasonal (May 1<sup>st</sup> – September 30<sup>th</sup>) flow condition, there are no samples within the high flow strata; therefore, for this condition an overall geometric mean will be calculated.

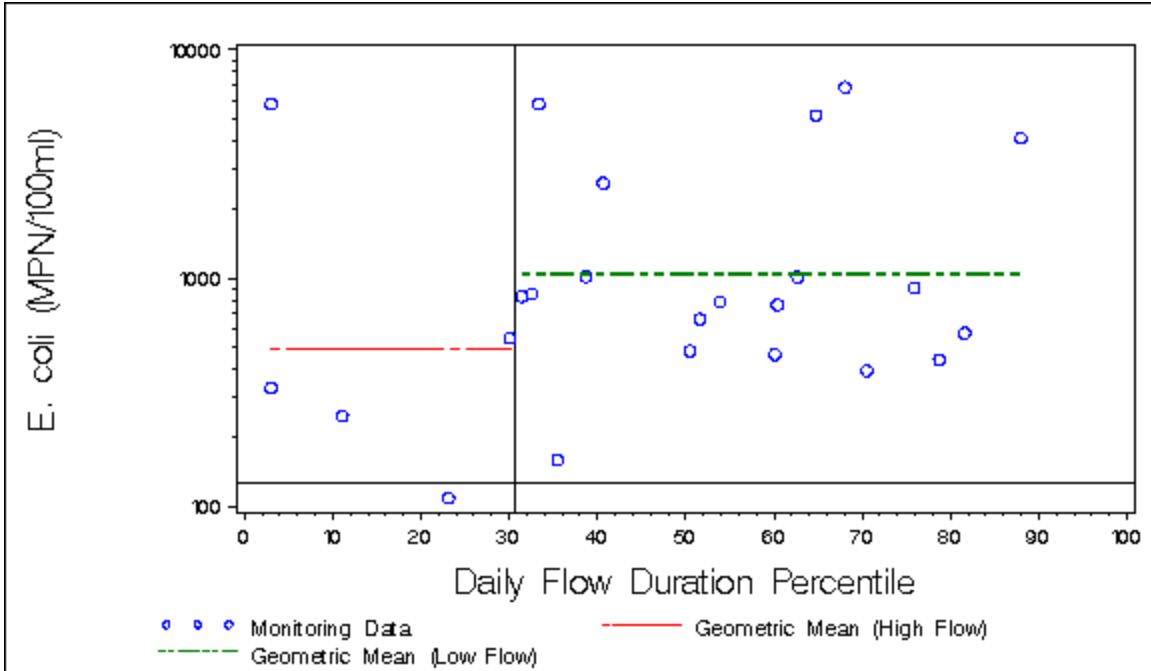
Weighting factors for estimating a weighted geometric mean are based on the frequency of each flow stratum during the averaging period. The weighting factors for the averaging periods and hydrological conditions are presented in Table B-3. Averaging periods are defined in this report as:

- (1) Average Annual Hydrological Condition
- (2) Dry Weather Seasonal (May 1st – September 30th) Condition

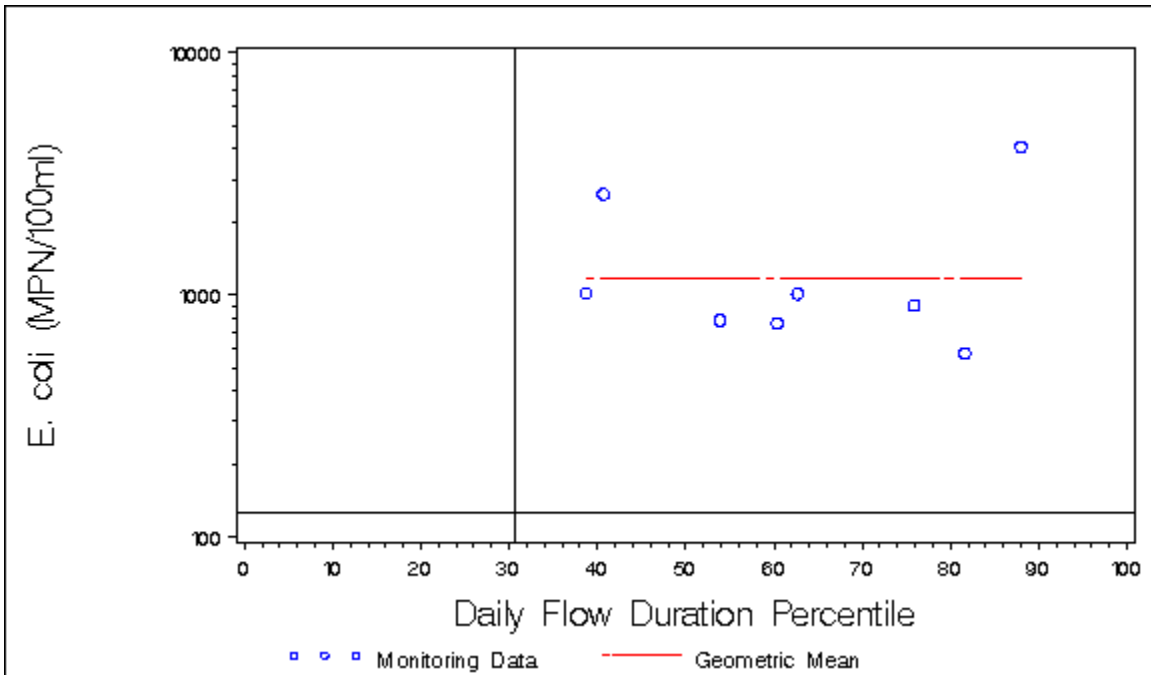
Weighted geometric means for the average annual and the overall seasonal conditions geometric means are plotted with the monitoring data on Figures B-2 and B-3.

**Table B-3: Weighting Factors for Estimation of Geometric Mean**

Condition	Averaging Period	Water Quality Data Used	Fraction High Flow	Fraction Low Flow
Annual Average	365 days	All	0.307	0.693
Dry Weather Seasonal	May 1 <sup>st</sup> – Sept. 30 <sup>th</sup>	Dry Weather Samples During May 1 <sup>st</sup> – Sept. 30 <sup>th</sup>	1.000	



**Figure B-2: *E. coli* Concentration vs. Flow Duration for Monitoring Station CHC0008 (Annual Condition)**



**Figure B-3: *E. coli* Concentration vs. Flow Duration for Monitoring Station CHC0008 (Seasonal Condition)**

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**Appendix C - BST Report**

**Maryland Department of the Environment**

**Identifying Sources of Fecal Pollution in  
Shellfish and Nontidal Waters in  
Maryland Watersheds**

**November 2005 – June 2007**

**Mark F. Frana, Ph.D. and Elichia A. Venso, Ph.D.  
Department of Biological Sciences and Environmental Health Science  
Salisbury University, Salisbury, MD**

**Final Report  
June 30, 2007**

*Cherry Creek (Non-tidal) TMDL Fecal Bacteria  
Document version: July 2, 2009*

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## INTRODUCTION

**Microbial Source Tracking.** Microbial Source Tracking (MST) is a relatively recent scientific and technological innovation designed to distinguish the origins of enteric microorganisms found in environmental waters. Several different methods and a variety of different indicator organisms (both bacteria and viruses) have successfully been used for MST, as described in recent reviews (Scott *et al.*, 2002; Simpson *et al.*, 2002). When the indicator organism is bacteria, the term Bacterial Source Tracking (BST) is often used. Some common bacterial indicators for BST analysis include: *E. coli*, *Enterococcus* spp., *Bacteroides-Prevotella*, and *Bifidobacterium* spp.

Techniques for MST can be grouped into one of the following three categories: molecular (genotypic) methods, biochemical (phenotypic) methods, or chemical methods. Ribotyping, Pulsed-Field Gel Electrophoresis (PFGE), and Randomly-Amplified Polymorphic DNA (RAPD) are examples of molecular techniques. Biochemical methods include Antibiotic Resistance Analysis (ARA), F-specific coliphage typing, and Carbon Source Utilization (CSU) analysis. Chemical techniques detect chemical compounds associated with human activities, but do not provide any information regarding nonhuman sources. Examples of this type of technology include detection of optical brighteners from laundry detergents or caffeine (Simpson *et al.*, 2002).

Many of the molecular and biochemical methods of MST are “library-based,” requiring the collection of a database of fingerprints or patterns obtained from indicator organisms isolated from known sources. Statistical analysis determines fingerprints/patterns of known sources species or categories of species (*i.e.*, human, livestock, pets, wildlife). Indicator isolates collected from water samples are analyzed using the same MST method to obtain their fingerprints or patterns, which are then statistically compared to those in the library. Based upon this comparison, the final results are expressed in terms of the “statistical probability” that the water isolates came from a given source (Simpson *et al.* 2002).

In this BST project, we studied the following Maryland nontidal watersheds: Deep Creek, Dividing Creek, Little Youghiogheny River, Patapsco River, Pretty Boy Reservoir, and the Youghiogheny River. Also included in the study were the following tidal shellfish harvesting areas: the Chester River, Corsica River, Herring and Turnville Creeks, Laws and Upper Thorofare, Manokin River, and the Pocomoke River watersheds. The methodology used was the ARA with *Enterococcus* spp. as the indicator organism. Previous BST publications have demonstrated the predictive value of using this particular technique and indicator organism (Hagedorn, 1999; Wiggins, 1999). A pilot study using PFGE, a genotypic BST method, was used on a subset of known-source isolates collected from the Pocomoke River Watershed.

**Antibiotic Resistance Analysis.** A variety of different host species can potentially contribute to the fecal contamination found in natural waters. Many years ago, scientists speculated on the possibility of using resistance to antibiotics as a way of determining the sources of this fecal contamination (Bell *et al.*, 1983; Krumperman, 1983). In ARA, the premise is that bacteria

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isolated from different hosts can be discriminated based upon differences in the selective pressure of microbial populations found in the gastrointestinal tract of those hosts (humans, livestock, pets, wildlife) (Wiggins, 1996). Microorganisms isolated from the fecal material of wildlife would be expected to have a much lower level of resistance to antibiotics than isolates collected from the fecal material of humans, livestock and pets. In addition, depending upon the specific antibiotics used in the analysis, isolates from humans, livestock and pets could be differentiated from each other.

In ARA, isolates from known sources are tested for resistance or sensitivity against a panel of antibiotics and antibiotic concentrations. This information is then used to construct a library of antibiotic resistance patterns from known-source bacterial isolates. Microbial isolates collected from water samples are then tested and their resistance results are recorded. Based upon a comparison of resistance patterns of water and library isolates, a statistical analysis can predict the likely host source of the water isolates. (Hagedorn 1999; Wiggins 1999).

## LABORATORY METHODS

**Isolation of *Enterococcus* from Known-Source Samples.** Fecal samples, identified to source, were delivered to the Salisbury University (SU) BST lab by Maryland Department of the Environment (MDE) personnel. Fecal material suspended in phosphate buffered saline was plated onto selective m-Enterococcus agar. After incubation at 37° C, up to eight (8) *Enterococcus* isolates were randomly selected from each fecal sample for ARA testing.

**Isolation of *Enterococcus* from Water Samples.** Water samples were collected by MDE staff and shipped overnight to MapTech Inc, Blacksburg, Va. Bacterial isolates were collected by membrane filtration. Up to 24 randomly selected *Enterococcus* isolates were collected from each water sample and all isolates were then shipped to the SU BST lab.

**Antibiotic Resistance Analysis.** Each bacterial isolate from both water and scat were grown in Enterococcosel<sup>®</sup> broth (Becton Dickinson, Sparks, MD) prior to ARA testing. *Enterococci* are capable of hydrolyzing esculin, turning this broth black. Only esculin-positive isolates were tested for antibiotic resistance.

Bacterial isolates were plated onto tryptic soy agar plates, each containing a different concentration of a given antibiotic. Plates were incubated overnight at 37° C and isolates then scored for growth (resistance) or no growth (sensitivity). Data consisting of a “1” for resistance or “0” for sensitivity for each isolate at each concentration of each antibiotic was then entered into a spread-sheet for statistical analysis.

The following table includes the antibiotics and concentrations used for isolates in analyses for all the study watersheds.



**Table C-1. Antibiotics and concentrations used for ARA.**

<u>Antibiotic</u>	<u>Concentration (µg/ml)</u>
Amoxicillin	0.625
Cephalothin	10, 15, 30, 50
Chloramphenicol	10
Chlortetracycline	60, 80, 100
Erythromycin	10
Gentamycin	5, 10, 15
Neomycin	40, 60, 80
Oxytetracycline	20, 40, 60, 80, 100
Salinomycin	10
Streptomycin	40, 60, 80, 100
Tetracycline	10, 30, 50, 100
Vancomycin	2.5

### KNOWN-SOURCE LIBRARY

**Construction and Use.** Fecal samples (scat) from known sources in each watershed were collected during the study period by MDE personnel and delivered to the BST Laboratory at SU. *Enterococcus* isolates were obtained from known sources (e.g., human, cow, goat, horse, dog, bear, beaver, deer, duck, fox, goose, heron, opossum, rabbit, raccoon, and squirrel). For each watershed, a library of patterns of *Enterococcus* isolate responses to the panel of antibiotics was analyzed using the statistical software CART<sup>®</sup> (Salford Systems, San Diego, CA).

*Enterococcus* isolate response patterns were also obtained from bacteria in water samples collected at the monitoring stations in each basin. Using statistical techniques, these patterns were then compared to those in the appropriate library to identify the probable source of each water isolate. A combined library of known sources was used for the nontidal Little Youghioghenny River and the Youghioghenny River Watersheds using patterns from scat obtained from both watersheds, and the water isolate patterns of each were compared to the combined library. A combined known-source library was also used for the nontidal Patapsco River and Pretty Boy Reservoir Watersheds, with water isolate patterns of each compared to this combined library. For the tidal watersheds, no combined known-source libraries were used for any shellfish harvesting area; a known-source isolate library collected from each area was used for the particular watershed.

## STATISTICAL ANALYSIS

We applied a tree classification method,<sup>1</sup> CART<sup>®</sup>, to build a model that classifies isolates into source categories based on ARA data. CART<sup>®</sup> builds a classification tree by recursively splitting the library of isolates into two nodes. Each split is determined by the antibiotic variables (antibiotic resistance measured for a collection of antibiotics at varying concentrations). The first step in the tree-building process splits the library into two nodes by considering every binary split associated with every variable. The split is chosen that maximizes a specified index of homogeneity for isolate sources within each of the nodes. In subsequent steps, the same process is applied to each resulting node until a *stopping* criterion is satisfied. Nodes where an additional split would lead to only an insignificant increase in the *homogeneity index* relative to the *stopping* criterion are referred to as *terminal* nodes.<sup>2</sup> The collection of *terminal* nodes defines the classification model. Each *terminal* node is associated with one source, the source isolate with an unknown source), based that is most populous among the library isolates in the node. Each water sample isolate (*i.e.*, an on its antibiotic resistance pattern, is identified with one specific *terminal* node and is assigned the source of the majority of library isolates in that *terminal* node.<sup>3</sup>

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<sup>1</sup> The Elements of Statistical Learning: Data Mining, Inference, and Prediction. Hastie T, Tibshirani R, and Friedman J. Springer 2001.

<sup>2</sup> An ideal split, *i.e.*, a split that achieves the theoretical maximum for homogeneity, would produce two nodes each containing library isolates from only one source.

<sup>3</sup> The CART<sup>®</sup> tree-classification method we employed includes various features to ensure the development of an optimal classification model. For brevity in exposition, we have chosen not to present details of those features, but suggest the following sources: Breiman L, et al. *Classification and Regression Trees*. Pacific Grove: Wadsworth, 1984; and Steinberg D and Colla P. *CART—Classification and Regression Trees*. San Diego, CA: Salford Systems, 1997.

## Youghiogheny River Watershed ARA Results

**Known-Source Library.** A 525 known-source isolate library was constructed from sources in the Youghiogheny River Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the combined library were grouped into four categories: human, livestock (cow, horse), pet (dog), and wildlife (deer, duck, goose) (Table C-2). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns. Average rates of correct classification (ARCC) for the library were found by repeating this analysis using several probability cutoff points, as described above. The number-not-classified for each probability was determined. From these results, the percent unknown and percent correct classification (RCCs) was calculated (Table C-3).

**Table C-2: Category, total number, and number of unique patterns in the Youghiogheny River known-source library.**

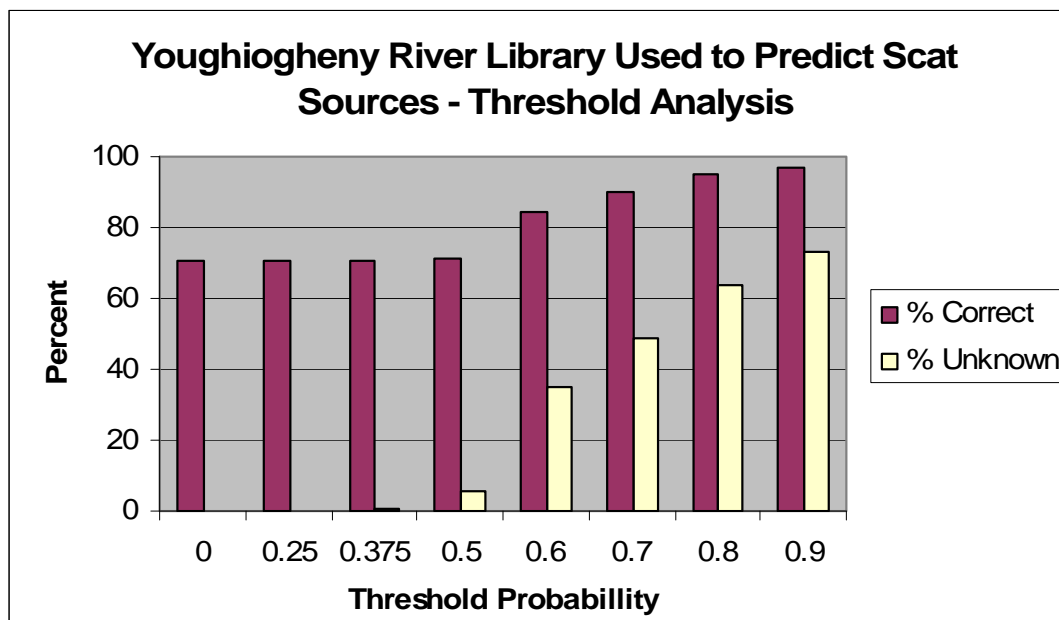
Category	Potential Sources	Total Isolates	Unique Patterns
human	human	158	113
livestock	cow, horse	127	32
pet	dog	73	47
wildlife	deer, duck, goose	167	59
Total		525	251

For Youghiogheny River Watershed, a cutoff probability of 0.60 (60%) was shown to yield an overall rate of correct classification of 79% (Table C-3). The resulting rates of correction classification (RCCs) for the four categories of sources in the Youghiogheny River portion of the library are shown in Table C-4.

**Table C-3: Number of isolates not classified, percent unknown, and percent correct for eight (8) cutoff probabilities for Youghiogheny River known-source isolates using the Youghiogheny River known-source library.**

Threshold	0	0.25	0.375	0.5	<b>0.6</b>	0.7	0.8	0.9
% correct	67.2%	67.2%	68.6%	71.5%	<b>79.3%</b>	83.2%	92.7%	98.4%
% unknown	0.0%	0.0%	3.0%	10.5%	<b>38.5%</b>	51.2%	66.3%	76.4%
# not classified	0	0	16	55	<b>202</b>	269	348	401

**Figure C-1: Youghiogheny River Classification Model: Percent Correct versus Percent Unknown using the Youghiogheny River library.**



**Table C-4: Actual species categories versus predicted categories, at 60% probability cutoff, with rates of correct classification (RCC) for each category.**

Actual	Predicted					Total	RCC*
	Human	Livestock	Pet	Wildlife	Unknown		
human	108	7	8	3	32	158	85.7%
livestock	2	47	2	9	67	127	78.3%
pet	1	2	43	1	26	73	91.5%
wildlife	13	13	6	58	77	167	64.4%
Total	124	69	59	71	202	525	

\*RCC = Actual number of predicted species category / Total number predicted.

Example: 163 pet correctly predicted / 175 total number predicted for pet = 163/175 = 93%.

**Youghiogheny River Water Samples.** Monthly monitoring from nine (9) monitoring stations on Youghiogheny River was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 1,453 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category, shown in Table C-5, indicate that 64% of the water isolates were able to be classified to a probable host source when using a 0.60 (60%) probability threshold.

**Table C-5: Probable host sources of water isolates by species category, number of isolates, and percent isolates classified at a cutoff probability of 60%.**

Source	Count	Percent	Percent Without Unknowns
human	215	14.8%	23.3%
livestock	285	19.6%	30.9%
pet	225	15.5%	24.4%
wildlife	198	13.6%	21.5%
unknown	530	36.5%	
Total	1453	100.0%	100.0%

**% classified** 63.5%

\*Percentages may not add up to 100% due to rounding.

The seasonal distribution of water isolates from samples collected at each sampling station is shown below in Table C-6.

**Table C-6: *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons at the Youghiogeny River's nine (9) monitoring stations.**

Station	Spring	Summer	Fall	Winter	Total
BUF0021	49	37	59	31	176
MXL0010	14	51	52	54	171
BRC0011	37	52	38	48	175
YOU0918	18	28	34	34	114
YOU0979	23	29	22	44	118
MYC0002	15	26	23	32	96
YOU1069	18	25	43	47	133
YOU1159	31	34	68	72	205
CHC0008	67	61	65	72	265
Total	272	343	404	434	1453

## FINAL

Tables C-7 and C-8 on the following pages show the number and percent of the probable sources for each monitoring station by month.

<b>Table C-7: BST Analysis: Number of Isolates per Station per Date.</b>							
Station	Date	Predicted Source					Total
		Human	Livestock	Pet	Wildlife	Unknown	
BUF0021	11/18/03	3	3	2	2	9	19
MXL0010	11/18/03	4	3	3	1	12	23
BRC0011	11/18/03	7	2	0	2	9	20
YOU0918	11/18/03	0	1	0	1	6	8
YOU0979	11/18/03	5	0	2	0	1	8
MYC0002	11/18/03	1	2	1	2	3	9
YOU1069	11/18/03	3	0	2	0	10	15
YOU1159	11/18/03	1	5	2	5	8	21
CHC0008	11/18/03	11	0	7	0	2	20
BUF0021	12/02/03	2	1	8	1	11	23
MXL0010	12/02/03	2	1	1	5	11	20
BRC0011	12/02/03	0	1	1	0	8	10
YOU0918	12/02/03	1	3	0	2	6	12
YOU0979	12/02/03	3	0	0	0	2	5
MYC0002	12/02/03	0	1	0	4	2	7
YOU1069	12/02/03	1	0	6	7	9	23
YOU1159	12/02/03	8	2	1	2	10	23
CHC0008	12/02/03	7	2	5	2	5	21
BUF0021	01/06/04	1	2	10	2	8	23
MXL0010	01/06/04	6	2	1	2	13	24
BRC0011	01/06/04	3	2	12	1	1	19
YOU0918	01/06/04	4	0	8	3	7	22
YOU0979	01/06/04	3	0	6	5	7	21
MYC0002	01/06/04	0	4	0	3	1	8
YOU1069	01/06/04	4	0	5	2	12	23
YOU1159	01/06/04	11	1	2	1	9	24
CHC0008	01/06/04	12	1	9	0	2	24
BUF0021	02/03/04	0	1	2	0	1	4
MXL0010	02/03/04	0	0	5	0	1	6
BRC0011	02/03/04	4	1	0	0	0	5
MYC0002	02/03/04	0	3	0	0	9	12
YOU1159	02/03/04	7	0	4	5	8	24
CHC0008	02/03/04	8	3	5	2	6	24
BUF0021	03/02/04	0	0	1	1	2	4
MXL0010	03/02/04	4	2	7	3	8	24
BRC0011	03/02/04	11	4	4	5	0	24
YOU0918	03/02/04	2	2	2	1	5	12
YOU0979	03/02/04	8	1	3	2	9	23

**Table C-7: BST Analysis: Number of Isolates per Station per Date (continued).**

Station	Date	Predicted Source					Total
		Human	Livestock	Pet	Wildlife	Unknown	
MYC0002	03/02/04	1	1	3	1	6	12
YOU1069	03/02/04	4	1	2	10	7	24
YOU1159	03/02/04	5	3	8	2	6	24
CHC0008	03/02/04	3	4	3	6	8	24
BUF0021	04/06/04	1	22	0	1	0	24
BRC0011	04/06/04	1	1	0	1	2	5
YOU0918	04/06/04	0	1	0	1	2	4
YOU0979	04/06/04	3	1	0	0	0	4
MYC0002	04/06/04	1	1	1	0	1	4
YOU1069	04/06/04	0	2	0	0	5	7
YOU1159	04/06/04	1	0	0	1	3	5
CHC0008	04/06/04	2	5	6	4	2	19
BUF0021	05/11/04	0	0	0	0	1	1
MXL0010	05/11/04	2	2	0	0	1	5
BRC0011	05/11/04	0	10	2	1	1	14
YOU0918	05/11/04	0	1	0	0	4	5
YOU0979	05/11/04	0	3	0	0	1	4
MYC0002	05/11/04	0	1	0	3	3	7
YOU1069	05/11/04	0	0	0	0	1	1
YOU1159	05/11/04	2	1	1	2	2	8
CHC0008	05/11/04	4	0	3	5	12	24
BUF0021	06/08/04	0	10	0	1	13	24
MXL0010	06/08/04	0	1	0	3	5	9
BRC0011	06/08/04	0	12	0	1	5	18
YOU0918	06/08/04	0	6	0	1	2	9
YOU0979	06/08/04	0	4	1	2	8	15
MYC0002	06/08/04	1	0	0	2	1	4
YOU1069	06/08/04	0	1	0	3	6	10
YOU1159	06/08/04	2	3	0	2	11	18
CHC0008	06/08/04	3	7	0	7	7	24
BUF0021	07/07/04	0	4	0	0	1	5
MXL0010	07/07/04	0	3	0	0	2	5
BRC0011	07/07/04	0	6	1	1	7	15
YOU0918	07/07/04	0	1	0	0	0	1
YOU0979	07/07/04	0	0	0	0	2	2
MYC0002	07/07/04	0	1	0	0	1	2
YOU1069	07/07/04	0	0	0	0	1	1
YOU1159	07/07/04	0	0	0	4	0	4
CHC0008	07/07/04	1	4	1	2	6	14
BUF0021	08/10/04	0	4	0	0	4	8
MXL0010	08/10/04	0	12	0	1	9	22
BRC0011	08/10/04	0	9	0	0	4	13

**Table C-7: BST Analysis: Number of Isolates per Station per Date (continued).**

Station	Date	Predicted Source					Total
		Human	Livestock	Pet	Wildlife	Unknown	
YOU0918	08/10/04	0	1	0	0	2	3
YOU0979	08/10/04	0	1	0	0	2	3
YOU1159	08/10/04	0	5	0	0	1	6
CHC0008	08/10/04	4	6	2	1	10	23
BUF0021	09/08/04	0	14	3	2	5	24
MXL0010	09/08/04	1	11	0	7	5	24
BRC0011	09/08/04	0	12	1	4	7	24
YOU0918	09/08/04	0	14	0	6	4	24
YOU0979	09/08/04	3	6	0	5	10	24
MYC0002	09/08/04	1	0	7	0	16	24
YOU1069	09/08/04	0	0	20	1	3	24
YOU1159	09/08/04	6	2	2	4	10	24
CHC0008	09/08/04	0	1	3	6	14	24
BUF0021	10/05/04	0	3	9	2	3	17
MXL0010	10/05/04	0	1	0	1	7	9
BRC0011	10/05/04	1	0	1	0	6	8
YOU0918	10/05/04	0	1	8	2	3	14
YOU0979	10/05/04	0	0	4	1	4	9
MYC0002	10/05/04	0	0	1	0	6	7
YOU1069	10/05/04	1	0	1	0	3	5
YOU1159	10/05/04	1	3	1	10	9	24
CHC0008	10/05/04	13	0	3	4	4	24
Total		215	285	225	198	530	1453

**Table C-8: BST Analysis: Percent of Isolates per Station per Date.**

Station	Date	Predicted Source					Total
		Human	Livestock	Pet	Wildlife	Unknown	
BUF0021	11/18/03	15.8%	15.8%	10.5%	10.5%	47.4%	100.0%
MXL0010	11/18/03	17.4%	13.0%	13.0%	4.3%	52.2%	100.0%
BRC0011	11/18/03	35.0%	10.0%	0.0%	10.0%	45.0%	100.0%
YOU0918	11/18/03	0.0%	12.5%	0.0%	12.5%	75.0%	100.0%
YOU0979	11/18/03	62.5%	0.0%	25.0%	0.0%	12.5%	100.0%
MYC0002	11/18/03	11.1%	22.2%	11.1%	22.2%	33.3%	100.0%
YOU1069	11/18/03	20.0%	0.0%	13.3%	0.0%	66.7%	100.0%
YOU1159	11/18/03	4.8%	23.8%	9.5%	23.8%	38.1%	100.0%
CHC0008	11/18/03	55.0%	0.0%	35.0%	0.0%	10.0%	100.0%
BUF0021	12/02/03	8.7%	4.3%	34.8%	4.3%	47.8%	100.0%
MXL0010	12/02/03	10.0%	5.0%	5.0%	25.0%	55.0%	100.0%
BRC0011	12/02/03	0.0%	10.0%	10.0%	0.0%	80.0%	100.0%
YOU0918	12/02/03	8.3%	25.0%	0.0%	16.7%	50.0%	100.0%



**Table C-8: BST Analysis: Percent of Isolates per Station per Date (continued).**

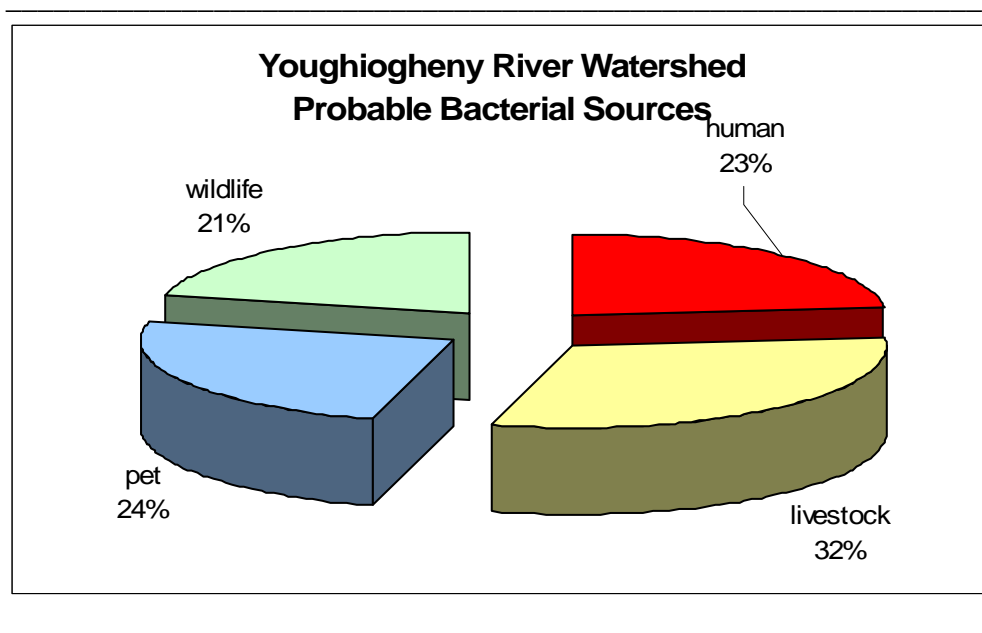
Station	Date	Predicted Source					Total
		Human	Livestock	Pet	Wildlife	Unknown	
YOU0979	12/02/03	60.0%	0.0%	0.0%	0.0%	40.0%	100.0%
MYC0002	12/02/03	0.0%	14.3%	0.0%	57.1%	28.6%	100.0%
YOU1069	12/02/03	4.3%	0.0%	26.1%	30.4%	39.1%	100.0%
YOU1159	12/02/03	34.8%	8.7%	4.3%	8.7%	43.5%	100.0%
CHC0008	12/02/03	33.3%	9.5%	23.8%	9.5%	23.8%	100.0%
BUF0021	01/06/04	4.3%	8.7%	43.5%	8.7%	34.8%	100.0%
MXL0010	01/06/04	25.0%	8.3%	4.2%	8.3%	54.2%	100.0%
BRC0011	01/06/04	15.8%	10.5%	63.2%	5.3%	5.3%	100.0%
YOU0918	01/06/04	18.2%	0.0%	36.4%	13.6%	31.8%	100.0%
YOU0979	01/06/04	14.3%	0.0%	28.6%	23.8%	33.3%	100.0%
MYC0002	01/06/04	0.0%	50.0%	0.0%	37.5%	12.5%	100.0%
YOU1069	01/06/04	17.4%	0.0%	21.7%	8.7%	52.2%	100.0%
YOU1159	01/06/04	45.8%	4.2%	8.3%	4.2%	37.5%	100.0%
CHC0008	01/06/04	50.0%	4.2%	37.5%	0.0%	8.3%	100.0%
BUF0021	02/03/04	0.0%	25.0%	50.0%	0.0%	25.0%	100.0%
MXL0010	02/03/04	0.0%	0.0%	83.3%	0.0%	16.7%	100.0%
BRC0011	02/03/04	80.0%	20.0%	0.0%	0.0%	0.0%	100.0%
MYC0002	02/03/04	0.0%	25.0%	0.0%	0.0%	75.0%	100.0%
YOU1159	02/03/04	29.2%	0.0%	16.7%	20.8%	33.3%	100.0%
CHC0008	02/03/04	33.3%	12.5%	20.8%	8.3%	25.0%	100.0%
BUF0021	03/02/04	0.0%	0.0%	25.0%	25.0%	50.0%	100.0%
MXL0010	03/02/04	16.7%	8.3%	29.2%	12.5%	33.3%	100.0%
BRC0011	03/02/04	45.8%	16.7%	16.7%	20.8%	0.0%	100.0%
YOU0918	03/02/04	16.7%	16.7%	16.7%	8.3%	41.7%	100.0%
YOU0979	03/02/04	34.8%	4.3%	13.0%	8.7%	39.1%	100.0%
MYC0002	03/02/04	8.3%	8.3%	25.0%	8.3%	50.0%	100.0%
YOU1069	03/02/04	16.7%	4.2%	8.3%	41.7%	29.2%	100.0%
YOU1159	03/02/04	20.8%	12.5%	33.3%	8.3%	25.0%	100.0%
CHC0008	03/02/04	12.5%	16.7%	12.5%	25.0%	33.3%	100.0%
BUF0021	04/06/04	4.2%	91.7%	0.0%	4.2%	0.0%	100.0%
BRC0011	04/06/04	20.0%	20.0%	0.0%	20.0%	40.0%	100.0%
YOU0918	04/06/04	0.0%	25.0%	0.0%	25.0%	50.0%	100.0%
YOU0979	04/06/04	75.0%	25.0%	0.0%	0.0%	0.0%	100.0%
MYC0002	04/06/04	25.0%	25.0%	25.0%	0.0%	25.0%	100.0%
YOU1069	04/06/04	0.0%	28.6%	0.0%	0.0%	71.4%	100.0%
YOU1159	04/06/04	20.0%	0.0%	0.0%	20.0%	60.0%	100.0%
CHC0008	04/06/04	10.5%	26.3%	31.6%	21.1%	10.5%	100.0%
BUF0021	05/11/04	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%
MXL0010	05/11/04	40.0%	40.0%	0.0%	0.0%	20.0%	100.0%
BRC0011	05/11/04	0.0%	71.4%	14.3%	7.1%	7.1%	100.0%
YOU0918	05/11/04	0.0%	20.0%	0.0%	0.0%	80.0%	100.0%
YOU0979	05/11/04	0.0%	75.0%	0.0%	0.0%	25.0%	100.0%

**Table C-8: BST Analysis: Percent of Isolates per Station per Date (continued)**

Station	Date	Predicted Source					Total
		Human	Livestock	Pet	Wildlife	Unknown	
MYC0002	05/11/04	0.0%	14.3%	0.0%	42.9%	42.9%	100.0%
YOU1069	05/11/04	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%
YOU1159	05/11/04	25.0%	12.5%	12.5%	25.0%	25.0%	100.0%
CHC0008	05/11/04	16.7%	0.0%	12.5%	20.8%	50.0%	100.0%
BUF0021	06/08/04	0.0%	41.7%	0.0%	4.2%	54.2%	100.0%
MXL0010	06/08/04	0.0%	11.1%	0.0%	33.3%	55.6%	100.0%
BRC0011	06/08/04	0.0%	66.7%	0.0%	5.6%	27.8%	100.0%
YOU0918	06/08/04	0.0%	66.7%	0.0%	11.1%	22.2%	100.0%
YOU0979	06/08/04	0.0%	26.7%	6.7%	13.3%	53.3%	100.0%
MYC0002	06/08/04	25.0%	0.0%	0.0%	50.0%	25.0%	100.0%
YOU1069	06/08/04	0.0%	10.0%	0.0%	30.0%	60.0%	100.0%
YOU1159	06/08/04	11.1%	16.7%	0.0%	11.1%	61.1%	100.0%
CHC0008	06/08/04	12.5%	29.2%	0.0%	29.2%	29.2%	100.0%
BUF0021	07/07/04	0.0%	80.0%	0.0%	0.0%	20.0%	100.0%
MXL0010	07/07/04	0.0%	60.0%	0.0%	0.0%	40.0%	100.0%
BRC0011	07/07/04	0.0%	40.0%	6.7%	6.7%	46.7%	100.0%
YOU0918	07/07/04	0.0%	100.0%	0.0%	0.0%	0.0%	100.0%
YOU0979	07/07/04	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%
MYC0002	07/07/04	0.0%	50.0%	0.0%	0.0%	50.0%	100.0%
YOU1069	07/07/04	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%
YOU1159	07/07/04	0.0%	0.0%	0.0%	100.0%	0.0%	100.0%
CHC0008	07/07/04	7.1%	28.6%	7.1%	14.3%	42.9%	100.0%
BUF0021	08/10/04	0.0%	50.0%	0.0%	0.0%	50.0%	100.0%
MXL0010	08/10/04	0.0%	54.5%	0.0%	4.5%	40.9%	100.0%
BRC0011	08/10/04	0.0%	69.2%	0.0%	0.0%	30.8%	100.0%
YOU0918	08/10/04	0.0%	33.3%	0.0%	0.0%	66.7%	100.0%
YOU0979	08/10/04	0.0%	33.3%	0.0%	0.0%	66.7%	100.0%
YOU1159	08/10/04	0.0%	83.3%	0.0%	0.0%	16.7%	100.0%
CHC0008	08/10/04	17.4%	26.1%	8.7%	4.3%	43.5%	100.0%
BUF0021	09/08/04	0.0%	58.3%	12.5%	8.3%	20.8%	100.0%
MXL0010	09/08/04	4.2%	45.8%	0.0%	29.2%	20.8%	100.0%
BRC0011	09/08/04	0.0%	50.0%	4.2%	16.7%	29.2%	100.0%
YOU0918	09/08/04	0.0%	58.3%	0.0%	25.0%	16.7%	100.0%
YOU0979	09/08/04	12.5%	25.0%	0.0%	20.8%	41.7%	100.0%
MYC0002	09/08/04	4.2%	0.0%	29.2%	0.0%	66.7%	100.0%
YOU1069	09/08/04	0.0%	0.0%	83.3%	4.2%	12.5%	100.0%
YOU1159	09/08/04	25.0%	8.3%	8.3%	16.7%	41.7%	100.0%
CHC0008	09/08/04	0.0%	4.2%	12.5%	25.0%	58.3%	100.0%
BUF0021	10/05/04	0.0%	17.6%	52.9%	11.8%	17.6%	100.0%
MXL0010	10/05/04	0.0%	11.1%	0.0%	11.1%	77.8%	100.0%
BRC0011	10/05/04	12.5%	0.0%	12.5%	0.0%	75.0%	100.0%
YOU0918	10/05/04	0.0%	7.1%	57.1%	14.3%	21.4%	100.0%

<b>Table C-8: BST Analysis: Percent of Isolates per Station per Date (continued)</b>							
Station	Date	Predicted Source					Total
		Human	Livestock	Pet	Wildlife	Unknown	
YOU0979	10/05/04	0.0%	0.0%	44.4%	11.1%	44.4%	100.0%
MYC0002	10/05/04	0.0%	0.0%	14.3%	0.0%	85.7%	100.0%
YOU1069	10/05/04	20.0%	0.0%	20.0%	0.0%	60.0%	100.0%
YOU1159	10/05/04	4.2%	12.5%	4.2%	41.7%	37.5%	100.0%
CHC0008	10/05/04	54.2%	0.0%	12.5%	16.7%	16.7%	100.0%
Total		14.8%	19.6%	15.5%	13.6%	36.5%	100.0%

**Figure C-2: Youghiogheny River Watershed relative contributions by probable sources of *Enterococcus* contamination.**



### Youghiogheny River Summary

The use of ARA was successful for identification of probable bacterial sources in the Youghiogheny River Watershed. When water isolates were compared to the library and potential sources predicted, 64% of the isolates were classified as to category by statistical analysis. The highest RCC for the library was 92% (for pet), followed by 86% for human). The RCCs for livestock and wildlife sources were 78% and 64%, respectively.

The largest category of potential sources in the watershed as a whole was livestock (32% of classified water isolates), followed by pet (24%), human (23%), and wildlife (21%) (Fig. C-2).

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### Adjustment of BST Results

As explained in the BST Summary for the Youghiogheny River watershed, the percent of correct classification (RCC) for bacteria sources, especially for the livestock category can introduce a potential misclassification of the more probable sources in the watershed. This is seen in Table C-4, which shows results of the analysis of samples from known sources. For example, out of 525, 158 isolates were known to be of human source but only 108 were classified by the analysis as being of human source. Of those 158, three isolates were classified as wildlife, 8 as pet, 7 as wildlife, and 32 as unknown. Similarly, of the other three categories, two isolates were known to be livestock, one isolate known to be from pets, and 13 isolates from wildlife were classified as human, resulting in a total of 124 of all 525 isolates classified as human of which only 108 were known to be of human source.

The results provided by the BST methodology can be adjusted based on the known source percent of correct classification results provided in Table C-4.

#### **Example:**

The current BST methodology provides the following source percentages for Cherry Creek station CHC0008 during high flow conditions:

<b>Source Category</b>	<b>Original Percentage</b>
Pets	24.73%
Human	25.36 %
Livestock	14.22 %
Wildlife	14.94 %
Unknown	20.74 %

To get the correct human source percentage in the Cherry Creek Watershed the percentages above are redistributed based on the % of correct classification as follows.

From Table C-4:

<b>Source Category</b>	<b>Isolates known to be from Human Source</b>	<b>Total Isolates Predicted for Each category</b>	<b>Percentage</b>
Pets	8	59	13.6%
Human	108	124	87.1%
Livestock	7	69	10.1%
Wildlife	3	71	4.2%
Unknown	32	202	15.8%
<b>Total</b>	<b>158</b>	<b>525</b>	<b>30.1%</b>

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Applying those percentages to the original estimated source distribution presented above will result in the adjusted percentage for human sources:

$$= (13.6 \times 24.73) + (87.1 \times 25.36) + (10.1 \times 14.22) + (4.2 \times 14.94) + (15.8 \times 14.94) = 30.8 \%$$

Thus the correct human source percentage, the value used in the TMDL analysis, is 30.8% and not 25.36%. Corrected percentages are also calculated as above for domestic animal, livestock and wildlife sources. The classification of unknown is eliminated in the process as all known isolates are of known source. For station CHC0008 during high flow condition the corrected source percentages are as follows:

<b>Source Category</b>	<b>Adjusted Percentage</b>
Pets	21.5 %
Human	30.8 %
Livestock	19.7 %
Wildlife	28.0 %

## Appendix D – Estimating Maximum Daily Loads

This appendix documents the technical approach used to define maximum daily loads of fecal bacteria consistent with the annual average TMDL which, when met, are protective of water quality standards in the Cherry Creek watershed. The approach builds upon the TMDL analysis that was conducted to ensure that compliance with the annual average target will result in compliance with the applicable water quality standards. The annual average loading target was converted into allowable *daily* values by using the loadings developed from the TMDL analysis. The approach is consistent with available EPA guidance on generating daily loads for TMDLs.

The available guidance for developing daily loads does not specify a single allowable approach; it contains a range of options. Selection of a specific method for translating a time-series of allowable loads into expression of a TMDL requires decisions regarding both the level of resolution (e.g., single daily load for all conditions vs. loads that vary with environmental conditions) and level of probability associated with the TMDL.

### Level of Resolution

The level of resolution pertains to the amount of detail used in specifying the maximum daily load. The draft EPA guidance on daily loads provides three categories of options for level of resolution.

1. **Representative daily load:** In this option, a single daily load (or multiple representative daily loads) is specified that covers all time periods and environmental conditions.
2. **Flow-variable daily load:** This option allows the maximum daily load to vary based upon the observed flow condition.
3. **Temporally-variable daily load:** This option allows the maximum daily load to vary based upon seasons or times of varying source or water body behavior.

### Probability Level

Essentially all TMDLs have some probability of being exceeded, with the specific probability being either explicitly specified or implicitly assumed. This level of probability reflects, directly or indirectly, two separate phenomena:

1. Water quality criteria consist of components describing acceptable magnitude, duration, and frequency. The frequency component addresses how often conditions can allowably surpass the combined magnitude and duration components.
2. Pollutant loads, especially from wet weather sources, typically exhibit a large degree of variability over time. It is rarely practical to specify a “never to be exceeded value” for a daily load, as essentially any loading value has some finite probability of being exceeded.

The draft daily load guidance states that the probability component of the maximum daily load should be “based on a representative statistical measure” that is dependent upon the specific TMDL and best professional judgment of the developers. This statistical measure represents how often the maximum daily load is expected/allowed to be exceeded. The primary options for selecting this level of protection would be:

1. **The maximum daily load reflects some central tendency:** In this option, the maximum daily load is based upon the mean or median value of the range of loads expected to occur. The variability in the actual loads is not addressed.
2. **The maximum daily load reflects a level of protection implicitly provided by the selection of some “critical” period:** In this option, the maximum daily load is based upon the allowable load that is predicted to occur during some critical period examined during the analysis. The developer does not explicitly specify the probability of occurrence.
3. **The maximum daily load is a value that will be exceeded with a pre-defined probability:** In this option, a “reasonable” upper bound percentile is selected for the maximum daily load based upon a characterization of the variability of daily loads. For example, selection of the 95<sup>th</sup> percentile value would result in a maximum daily load that would be exceeded 5% of the time.

***Selected Approach for Defining Maximum Daily Loads for Nonpoint Sources and MS4***

To calculate the Cherry Creek watershed MDL for nonpoint sources (there are no MS4s in the watershed), a “representative daily load” option was selected as the level of resolution, and a value “that will be exceeded with a pre-defined probability” was selected as the level of protection. In these options, the maximum daily load is one single daily load that covers the two flow strata, with an upper bound percentile that accounts for the variability of daily loads. The upper bound percentile and the maximum daily loads were estimated following EPA’s “Technical Support Document for Water Quality-Based Toxics Control” (1991 TSD) (EPA 1991); and “Approaches For Developing a Daily Load Expression for TMDLs Computed for Longer Term Averages” (EPA 2006).

The 1991 TSD illustrates a way to identify a target maximum daily concentration from a long-term average concentration (LTA) based on a coefficient of variation (CV) and the assumption of a log-normal distribution of the data. The equations for determining both the upper boundary percentile and corresponding maximum daily load described in the TSD are as follows:

$$MDLC = LTA * e^{[Z\sigma - 0.5\sigma^2]} \tag{D1}$$

and,

$$MDL = MDLC * Q * F \tag{D2}$$

where,

- MDLC = maximum daily load concentration (MPN/100ml)
- LTAC = long-term average TMDL concentration (MPN/100ml)
- MDL = Maximum Daily Load (MPN/day)
- Z = z-score associated with upper bound percentile (unitless)
- $\sigma^2$  =  $\ln(CV^2 + 1)$
- CV = coefficient of variation



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Q = flow (cfs)  
F = conversion factor

The first step is to use the bacteria monitoring data to estimate the upper bound percentile as the percentile of the highest observed bacteria concentration in each monitoring station of the Cherry Creek watershed. Using the maximum value of *E. coli* observed in each monitoring station, and solving for the z-score using the above formula, the value of “z” and its corresponding percentile is found as shown below. The percentile associated with the particular value of z can be found in tables in statistics books or using the function NORMSINV(%) in EXCEL<sup>®</sup>.

$$Z = [\log_{10}(\text{MOC}) - \log(\text{AM}) + 0.5\sigma^2]/\sigma \quad (\text{D3})$$

where,

Z = z-score associated with upper bound percentile  
MOC = maximum observed bacteria concentration (MPN/100ml)  
AM = arithmetic mean observed bacteria concentrations (MPN/100ml)  
 $\sigma^2$  =  $\ln(\text{CV}^2 + 1)$   
CV = coefficient of variation (arithmetic)

Note that these equations use arithmetic parameters, not geometric parameters as used in the calculations of the long-term annual average TMDL. Therefore, bias correction factors are not necessary to estimate the loads as will be explained below.

The highest percentile of all the stations analyzed by stratum will define the upper bound percentile to be used in estimating the maximum daily limits. In the case of the Cherry Creek watershed, a value measured during low-flow conditions at the CHC0008 station resulted in the highest percentile of both strata. This value translates to the 96.7<sup>th</sup> percentile, which is the upper boundary percentile to be used in the computation of the maximum daily limits (MDLs) throughout this analysis. Results of the analysis to estimate the recurrence or upper boundary percentile are shown in Table D-1.

**Table D-1: Percentiles of Maximum Observed Bacteria Concentrations**

Station	Flow Stratum	Maximum Observed <i>E. coli</i> Concentration (MPN/100ml)	Percentile (%)
CHC0008	High	5,794	95.0
	Low	6,867	96.7

The 96.7<sup>th</sup> percentile value results in a maximum daily load that would not be exceeded 96.7% of the time, as, in a similar manner, a TMDL that represents the long-term average condition would be expected to be exceeded half the time even after all required controls were implemented.

The MDLCs are estimated based on a statistical methodology referred to as “Statistical Theory of Rollback (STR)”. This method predicts concentrations of a pollutant after its sources have been controlled (post-control concentrations), in this case after annual average TMDL implementation. Using STR, the daily TMDLs are calculated as presented below.

First, the long-term average TMDL concentrations ( $C_{LTA}$ ) by stratum are estimated by applying the required percent reduction to the baseline (monitoring data) concentrations ( $C_b$ ) by stratum as follows:

From Section 4.3, equations (8) and (9):

$$L_b = L_{b-H} + L_{b-L}$$

$$L_b = Q_H * C_{bH} * F_{IH} * W_H + Q_L * C_{bL} * F_{IL} * W_L$$

And from equation (14):

$$\text{Annual Average TMDL} = L_b * (1 - R)$$

Therefore,

$$L_b * (1 - R) = Q_H * C_H * F_{IH} * W_H * (1 - R) + Q_L * C_L * F_{IL} * W_L * (1 - R) \quad (D4)$$

As explained before, a reduction in concentration is proportional to a reduction in load, thus the bacteria concentrations expected after reductions are applied are equal to the baseline concentrations multiplied by one minus the required reduction:

$$C_{LTA-H} = C_{b-H} * (1 - R_H) \quad (D5)$$

$$C_{LTA-L} = C_{b-L} * (1 - R_L) \quad (D6)$$

The TMDL concentrations estimated as explained above are shown in Table D-2.

**Table D-2: Long-term Annual Average (LTA) TMDL Bacteria Concentrations**

Station	Flow Stratum	LTA Geometric Mean <i>E. coli</i> Concentration (MPN/100ml)	LTA Arithmetic Mean* <i>E. coli</i> Concentration (MPN/100ml)
	CHC0008	High	46
Low		98	167

\*Only arithmetic parameters are used in the daily loads analysis.

The next step is to calculate the 96.7<sup>th</sup> percentile (the MDL concentrations) of these expected concentrations (LTA concentrations) using the coefficient of variation of the baseline concentrations. Based on a general rule for coefficient of variations, the coefficient of variation of the distribution of pollutant concentrations does not change after these concentrations have been reduced or controlled by a fixed proportion (Ott 1995). Therefore, the coefficient of variation estimated using the monitoring data concentrations does not change, and it can be used to estimate the 96.7<sup>th</sup> percentile of the long-term average TMDL concentrations (LTAC) using equation (D1). These values are shown in Table D-3.

**Table D-3: Maximum Daily Load (MDL) Concentrations**

Station	Flow Stratum	Coefficient of Variation	MDL <i>E. coli</i> Concentration (MPN/100ml)
CHC0008	High	2.9	724
	Low	1.4	649

With the 96.7<sup>th</sup> percentiles of LTA TMDL bacteria concentrations estimated for both high flow and low flow strata as explained above, the maximum daily load for nonpoint sources for each subwatershed can be now estimated as:

$$\begin{aligned} \text{Daily TMDL (MPN/day)} = & Q_H * (96.7^{\text{th}} C_{LTA-H}) * F_{IH} * W_H \\ & + Q_L * (96.7^{\text{th}} C_{LTA-L}) * F_{IL} * W_L \end{aligned} \quad (D7)$$

***Selected Approach for Defining Maximum Daily Loads for Other Point Sources***

The TMDL also considers contributions from other point sources (i.e., municipal and industrial WWTP) in watersheds that have NPDES permits with fecal bacteria limits. The TMDL analysis that defined the average annual TMDL holds each of these sources constant at their existing NPDES permit limits (daily or monthly) for the entire year. The approach used to determine maximum daily loads is dependent upon whether a maximum daily load is specified within the permit. If a maximum daily load is specified within the permit, then the maximum design flow is multiplied by the maximum daily limit to obtain a maximum daily load. If a maximum daily limit is not specified in the permit, then the maximum daily loads are calculated from guidance in the TSD for Water Quality-based Toxics Control (EPA 1991).

There are no NPDES-regulated point sources to consider in the Cherry Creek watershed.

The Maximum Daily Loads for the Cherry Creek watershed are presented in Table D-4 below.

**Table D-4: Maximum Daily Loads Summary**

Station	Flow Stratum	Maximum Daily Load (Billion <i>E. coli</i> MPN/day)	
		by Stratum	Weighted
CHC0008	High	1,727	695
	Low	237	

#### ***Maximum Daily Loads Allocations***

Using the MDLs estimated as explained above, loads are allocated following the same methodology as the annual average TMDL (See section 4.8). The maximum daily load allocations for the Cherry Creek watershed are presented in Table D-5.

**Table D-5: Maximum Daily Loads**

Station	Total Allocation	LA	SW-WLA	WWTP-WLA
	(Billion MPN <i>E. coli</i> /day)			
CHC0008	695	695	N/A	N/A
<b><i>Total</i></b>	<b>695</b>	<b>695</b>	<b>N/A</b>	<b>N/A</b>

FINAL

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