

Bacteria Total Maximum Daily Load
Task Force Report
June 4, 2007

Prepared for:

Texas Commission on Environmental Quality

and

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Table of Contents

Executive Summary	1
Introduction	4
Bacteria Fate and Transport Models	6
Bacteria Source Tracking	23
Recommended Approach for Bacteria TMDL and Implementation Plan Development	36
Research and Development Needs	51
References	64
Appendix 1: Bacteria TMDL Task Force Members and Expert Advisors	70
Appendix 2: Models Used in Bacteria Projects as Described in EPA Publications	72
Appendix 3: EPA Bacteria TMDL Guidelines	77
Appendix 4: State Approaches to Bacteria TMDL Development	87
Appendix 5: Comments from Expert Advisory Group	100

Executive Summary

In September 2006, the Texas Commission on Environmental Quality (TCEQ) and Texas State Soil and Water Conservation Board (TSSWCB) charged a seven-person Bacteria Total Maximum Daily Load (TMDL) Task Force with:

- examining approaches that other states use to develop and implement bacteria TMDLs,
- recommending cost-effective and time-efficient methods for developing TMDLs,
- recommending effective approaches for developing TMDL Implementation Plans (I-Plans),
- evaluating a variety of models and bacteria source tracking (BST) methods available for developing TMDLs and I-Plans, and recommending under what conditions certain methods are more appropriate, and
- developing a roadmap for further scientific research needed to reduce uncertainty about how bacteria behave under different water conditions in Texas.

The Task Force, assisted by an Expert Advisory Group of approximately 50 stakeholders and agency staff, held two two-hour meetings/teleconferences and developed two drafts of the report. These drafts were shared by e-mail and on a Web site and feedback received from the Expert Advisory Group was also made available on the Web site.

The Task Force report describes the characteristics, as well as some of the strengths and weaknesses of several models that have been used and/or are under development to assist bacteria TMDL and I-Plan analysis. These include:

- load duration curves (LDC),
- spatially explicit statistical models, including Arc Hydro, SPARROW and SELECT,
- the mass balance models BLEST and BIT, and
- the mechanistic hydrologic/water quality models HSPF, SWAT, SWMM and WASP.

The Task Force report also describes and makes recommendations for effective use of BST methods that have been used in Texas and elsewhere for TMDL development. These include ERIC-PCR, Ribotyping, PFGE, KB-ARA, CSU and *Bacteroidales* PCR. Based on recent experience in Texas and elsewhere, the Task Force recommends using library-independent methods like *Bacteroidales* PCR for preliminary qualitative analyses and more expensive and time-consuming library-dependent methods if more quantitative data are required for TMDL or I-Plan development.

Based on the discussions of bacteria models and source tracking, as well as extensive input from the Expert Advisory Group, the Task Force recommends a three-tier approach to implementing bacteria TMDLs and I-Plans.

Tier 1 is a one-year process that includes the formation of a representative stakeholder group, development of a comprehensive geographic information system (GIS) of the watershed, a survey of potential bacterial sources, calculation of load duration curves from existing monitoring data and analysis by agency personnel and stakeholders of data collected for Tier 1. After reviewing information from Tier 1, the group may choose to complete and submit a draft TMDL for agency approval, request an evaluation of the designated use of the water body (an use attainability analysis) or proceed to Tier 2.

Tier 2 is a one-to-two-year effort designed to collect targeted monitoring data to fill gaps in previously collected data, conduct qualitative library-independent BST data to determine whether humans and/or a few major classes of animals are sources and develop simple spatially explicit or mass balance models of bacteria in the watershed. After analysis of Tier 1 and Tier 2 data, the group may chose to complete and submit the draft TMDL (or I-Plan if a TMDL was developed after Tier 1), request an evaluation of the designated use (an use attainability analysis), or initiate a “phased TMDL” and proceed with Tier 3 analysis.

Tier 3 is a two-to-three-year process designed to continue strong stakeholder involvement, implement more extensive targeted monitoring, conduct quantitative

library-dependent BST analysis and develop a detailed hydrologic/water quality model for the watershed. Tier 3 should be implemented only when this level of detailed analysis is needed for I-Plan development or for TMDL development for particularly complex watersheds for which consensus cannot be reached after Tier 2.

The Task Force emphasizes that the agencies and stakeholders may choose to deviate from these recommendations if they reach consensus that a more time- and cost-effective approach is feasible.

The Task Force concludes its report by summarizing a number of research activities needed to strengthen the scientific tools available for TMDL and I-Plan development. The needed research falls into the following categories: characterization of sources, characterization of kinetic rates and transport mechanisms, enhancements to bacteria fate and transport models and bacteria source tracking, determination of effectiveness of control mechanisms and quantification of uncertainty and risk.

Finally, the report includes a number of references to relevant scientific literature and studies, as well as five appendices:

Appendix 1	Bacteria TMDL Task Force Members and Expert Advisors
Appendix 2	Models Used in Bacteria TMDLs as Described in EPA Publications
Appendix 3	EPA Bacteria TMDL Guidelines
Appendix 4	State Approaches to Bacteria TMDLs
Appendix 5	Comments from the Expert Advisory Group

Introduction

As of January 2006, 197 water bodies in Texas were impaired because they did not meet bacteria criteria established by the state to protect contact recreation use (freshwater and saltwater) and/or oyster water use. The freshwater contact recreation use criterion used to determine impairment includes both a geometric mean for *Escherichia coli* (*E. coli*) of 126 colonies per 100 ml and a single sample maximum of 394 colonies per 100 ml. The saltwater contact recreation use criterion includes both a geometric mean for *Enterococci* of 35 colonies per 100 ml and a single sample maximum of 89 colonies per 100 ml. Finally, the oyster water use criterion includes a median fecal coliform concentration of 14 colonies per 100 ml and no more than 10% of samples may exceed 43 colonies per 100 ml. TCEQ is currently considering a variety of potential changes to Texas water quality standards. According to the TCEQ Web site, these “revisions to the standards and Standards Implementation Procedures are needed to:

- incorporate recently developed site-specific standards for individual water bodies,
- incorporate new research on the toxicity of specific chemicals,
- improve the way that standards are used and applied.”

As required by Section 303(d) of the Clean Water Act, Texas has committed to complete TMDLs for these bacteria-impaired water bodies within 13 years of the listing date (i.e. 2017 for new bacteria impairments listed on the 2004 list). In order to identify the best and most cost- and time-effective methods to develop bacteria TMDLs and TMDL Implementation Plans (I-Plans), TCEQ and TSSWCB established a joint technical Task Force on Bacteria TMDLs on September 27, 2006. The Task Force was charged with:

- examining approaches that other states use to develop and implement bacteria TMDLs,
- recommending cost-effective and time-efficient methods for developing TMDLs,
- recommending effective approaches for developing I-Plans,

- evaluating the variety of models and bacteria source tracking (BST) methods available for developing TMDLs and I-Plans, and recommending under what conditions certain methods are more appropriate, and
- developing a roadmap for further scientific research needed to reduce uncertainty about how bacteria behave under different water conditions in Texas.

Task Force members are Drs. Allan Jones, Texas Water Resources Institute; George Di Giovanni, Texas Agricultural Experiment Station–El Paso; Larry Hauck, Texas Institute for Applied Environmental Research; Joanna Mott, Texas A&M University–Corpus Christi; Hanadi Rifai, University of Houston; Raghavan Srinivasan, Texas A&M University; and George Ward, The University of Texas at Austin. Dr. Allan Jones was named Task Force Chair by TCEQ and TSSWCB.

Approximately 50 Expert Advisors (Appendix 1) with expertise in bacteria-related issues have also provided significant input to the Task Force during the process. Included in this group are university scientists, environmental consultants and representatives of local, state and federal agencies with jurisdictions impacting bacteria and water quality.

Recommendations from the Task Force are intended to be used by the State of Texas, specifically TSSWCB and TCEQ, to keep Texas as a national leader in water quality protection and restoration.

Bacteria Fate and Transport Models

This section, coordinated by Drs. Hanadi Rifai and Raghavan Srinivasan, describes the strengths and weaknesses of several bacteria fate and transport models that have been used for TMDL and I-Plan development. The discussion is not intended to be a comprehensive review of the numerous models that have been or could be used for bacteria TMDL and I-Plan development. Instead, it briefly characterizes a few of the models that have been developed and/or used in Texas to assist in bacteria TMDL and I-Plan assessments.

Bacterial pollution in surface water bodies is difficult to model because bacterial sources and their fate and transport are complex. In addition, several fate and transport processes control their growth, decay and movement on the land and in streams. Indicators such as *E. coli*, *Enterococcus spp.*, and fecal coliform bacteria, although typically nonpathogenic, are used to identify the potential for the presence of other disease-causing organisms. These bacteria typically originate from mammalian and avian sources and are released into water bodies via point sources (such as wastewater treatment plant [WWTP] effluent and runoff from stormwater drainage networks) as well as dispersed (or nonpoint) sources (such as direct runoff from residential yards and streets, on-site sewage disposal, deposition from non-domestic animals and livestock, and re-suspension of bacteria from stream sediment). Bacteria are present in both water and sediment and experience survival, growth and decay within a water body. Furthermore, bacteria concentrations in streams vary spatially and temporally because of flow variability within the stream network and loads entering the streams from various sources at different times. Because of this complexity, most states use mathematical models to understand bacteria dynamics when developing bacteria TMDLs and I-Plans. Since numerous water quality models are available, selecting an appropriate model or combination of models for bacteria TMDLs is a challenge. Both the characteristics of each watercourse and the nature of its pathogen loads should be considered. Selection of a model or models is an important and critical step that should be undertaken early in the TMDL and I-Plan development processes in consultation with stakeholders and modeling experts.

Since bacteria TMDLs must estimate the maximum bacteria load that a water body can receive and still meet water quality standards, TMDL development involves estimating both existing and allowable loads, the instream water quality effects due to these loads, as well as the reductions that would be required to meet standards. I-plan development, however, involves designing realistic bacteria reduction strategies for different bacteria sources and examining the effects of these strategies on water quality. The different goals of TMDL development and I-Plan development may require the use of different bacteria models with different levels of complexity.

Efforts to formulate useful TMDLs have led to the development of many predictive tools for the estimation of necessary reductions to meet water quality goals. States required to develop TMDLs as part of consent decrees have been under a great deal of pressure to produce TMDLs quickly and cost-effectively to comply with federal law. States faced with budgetary constraints need an economical means to develop TMDLs to restore impaired waters. As a result, relatively simple and cost-effective approaches to identify sources of pollution and allocate loads are needed to identify bacteria load reductions to meet water quality standards.

These approaches include load duration curves, spatially explicit statistical models and mass balance models that rely on available flow and water quality data. In other cases states have chosen to use more complex mechanistic (process or physically based) hydrologic/water quality models combined with landscape-loading models. Some of the most common models used for bacteria TMDLs and I-Plans are described below. Other modeling tools described in EPA publications are summarized in Appendix 2.

Load Duration Curve (LDC) Models

Load Duration Curve (LDC) methodologies are acknowledged as a cost-effective, useful tool for addressing bacteria impairments because they are easy to understand, produce reasonable results and have minimal data requirements (Cleland 2002 and 2003, Bonta 2002, Stiles 2001).

LDCs graphically represent streamflow data in terms of pollutant loadings. The analysis begins with a flow duration curve where the x-axis is based on the frequency of exceedance of specific flows (y-axis) during the entire period of record represented in the data (Figure 1). The resulting graph depicts the range of flows (expressed as an exceedance frequency) experienced at a single monitoring over time.

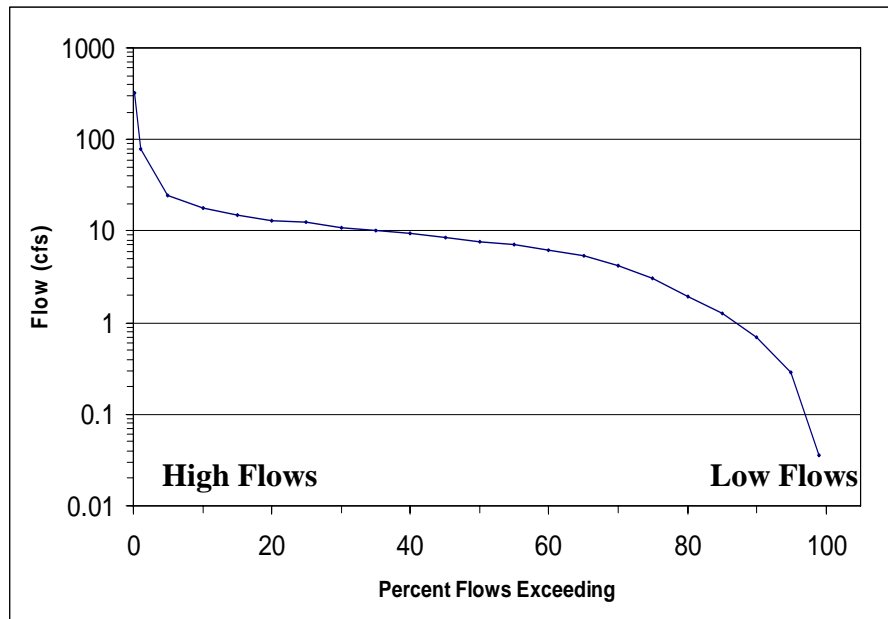


Figure 1: Example flow duration curve

In order to make this approach useful for TMDL purposes, it is necessary to convert the flow duration curve to an LDC. This conversion is accomplished by multiplying the flow (at each frequency interval) by the water quality criterion (Figure 2), in this case the single sample criterion. The resulting plot represents the maximum pollutant load for every flow experienced in the specific stream, in essence the total maximum daily load. The plotted line in Figure 2 is equal to the water quality criterion (in this case, for a single sample) times the flow.

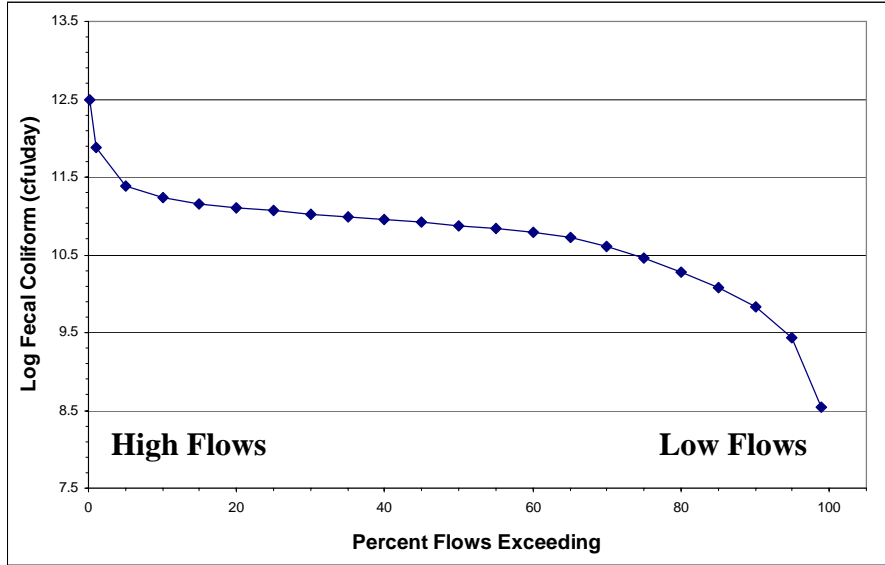


Figure 2: Example load duration curve using the single sample criterion

Monitoring data can be added to the graph to identify those flow conditions where pollutant levels may be above allowable loadings. This step is done by multiplying the water quality sample bacteria concentrations by the daily average flow that occurred when the sample was taken. The data points can then be plotted at the appropriate flow frequency and compared directly to the relevant water quality criterion obtained from the LDC (Figure 3).

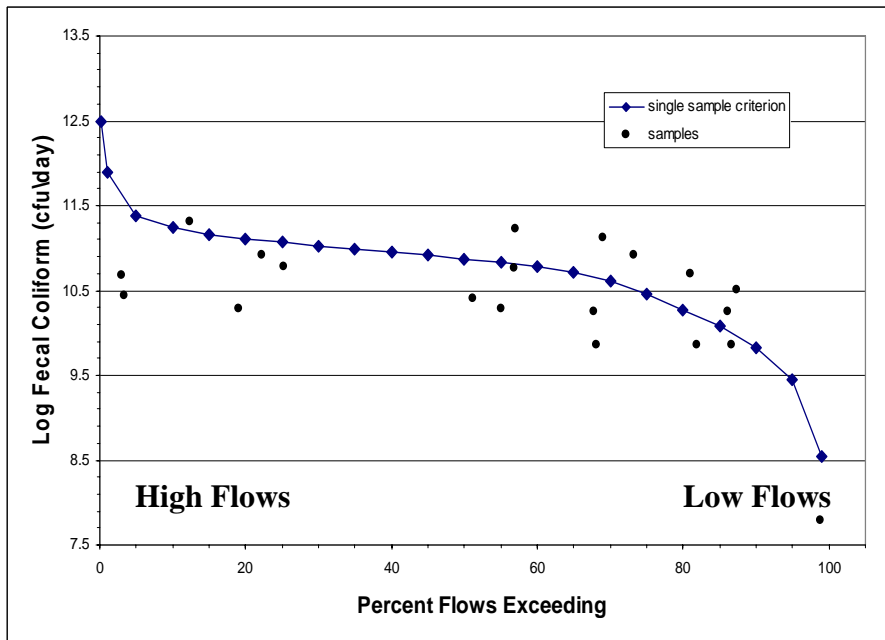


Figure 3: Data assessment using load duration curve

LDCs provide several types of useful information for the TMDL development process. An LDC can differentiate between point and nonpoint sources of bacteria related to different flow conditions (Figure 4). Generally speaking, monitored loads that exceed the allowable load at low flows (i.e., high frequency of exceedance) are likely to be the result of point source discharges, such as a WWTP discharging bacteria into a low flow stream. Resident populations of waterfowl could also contribute to chronic exceedances during low flows. In contrast, monitored loads exceeding the criteria at the mid-range and high flows are typically caused by nonpoint source inputs or point sources (such as stormwater systems) that channel rainfall runoff into water bodies. An example might be surface runoff carrying bacteria from livestock or non-domestic animal sources into a high flow stream. Finally, resuspension of indicator bacteria surviving in streambed sediments could produce exceedances under both high and low flow conditions.

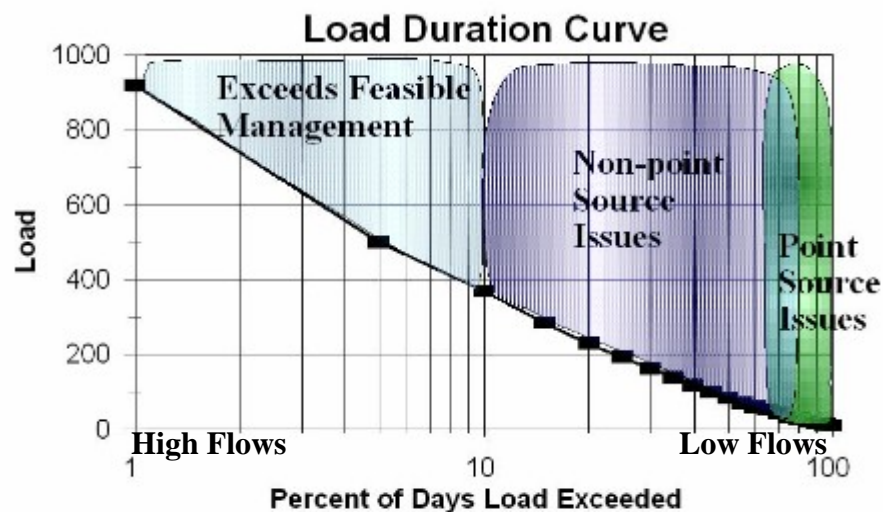


Figure 4: Source identification using load duration curve (Cleland 2003)

Developing load reduction scenarios based upon the LDC requires the examination of relationships between the data and the allowable loads expressed in the curve. This usually requires comparisons between statistical representations of the data (means,

regression lines, confidence intervals) and the LDC, with the difference between the two values representing the required reduction. Statistical estimates of the data may include all of the loads or just those that exceed the TMDL line.

The segmentation of the LDC allows for the development of appropriate implementation strategies that target specific flow conditions (Cleland 2002). Exceedances occurring at the low flows may require regulatory actions to control point sources. At the mid range and high flows, management measures directed towards nonpoint sources could be developed. At some point in the flow frequency, control of pollutant sources becomes unfeasible. Pollutant loadings at these high flow events typically exceed design specifications for control actions. For this reason, it may be reasonable to exclude data and loadings that occur at flooding conditions.

Several states have developed bacteria TMDLs using LDCs and have produced reasonable scenarios to address impairments. Oklahoma developed TMDLs for bacteria in the Upper Canadian River in several nonpoint source-dominated watersheds. In Maryland, a TMDL for bacteria was developed for Cabin John Creek. Maryland also used BST to further refine source loadings. Kansas used the load duration methodology to develop a bacteria TMDL for the Kansas River. Texas is currently developing several TMDLs using LDCs to address bacteria impairments (Upper Oyster Creek, Gilleland Creek, Guadalupe River above Canyon Lake, among others).

Recognizing the importance and practical use of LDCs, the U.S. Environmental Protection Agency's Watershed Branch in the Office of Wetlands, Oceans and Watersheds is producing a detailed technical document on how to use LDCs for establishing TMDLs. The draft of "An Approach for Using Load Duration Curves in Developing TMDLs" is available on the Task Force's Web site at: <http://twri.tamu.edu/bacteriatmdl/>.

As with all predictive tools used in TMDL development, the LDC approach has both strengths and weaknesses. The primary strengths of this approach are the minimal data

requirements (although large datasets are preferable in all cases), its simplicity and its usefulness as an illustrative model. The model's simple nature can also be considered a weakness in that very few inputs are considered for estimating resulting bacteria loads, potentially increasing uncertainty, though this could also be considered a strength in that other uncertainties are reduced by using fewer assumptions. This method also does not work very well in tidally influenced areas, and intermittent streams tend to produce truncated curves. Though the load duration approach can help differentiate between point and nonpoint sources, more mechanistic models provide more detailed assessments of specific sources of bacteria loads. On the other hand, specific source identification may not be necessary since a TMDL, in its most basic form, only requires differentiation between point (Waste Load Allocation [WLA]) and nonpoint (Load Allocation [LA]) sources.

Further refinements may be desirable for developing measures to control sources in the I-Plan following the TMDL. Additional tools (targeted monitoring, bacteria source surveys, BST and more complex models) used in conjunction with load duration methods have the potential to significantly refine source identification and increase the power of this analytical tool.

Spatially Explicit Statistical Models

The three models described below estimate the spatial distributions of potential pollutant sources throughout a watershed, estimate loadings from each source into water bodies and calculate the change in pollutant concentration within the water body. Key parameters are calibrated to mimic pollutant concentrations measured in the watershed.

Arc Hydro Model (Maidment)

The Arc Hydro Model, developed by Dr. David Maidment of the Center for Research in Water Resources at The University of Texas at Austin, can be defined as a geographic database containing a geographic information system (GIS) representation of a hydrological information system. Arc Hydro uses a case-specific database design that is

extensible, flexible and adaptable to user requirements. It takes advantage of the next generation of spatial data in relational database management systems (an RDBMS-based GIS system), the geodatabase model. Conceptually, it is a combination of GIS objects enhanced with the capabilities of a relational database to allow for relationships, topologies and geometric networks.

This model has been used to develop bacteria TMDLs for Galveston and Copano Bays (Gibson, C.J. et al., 2006) The Copano Bay TMDL model input was developed within a GIS framework. Point sources were located in the GIS and accounted for in the model. Nonpoint source loadings were estimated from livestock, non-domestic animals and domestic animal populations per county. These populations were assumed to be equally distributed within their appropriate land use categories within each county. Loadings were then calculated by combining population, geographic and precipitation data. This information was used to estimate bacterial loadings for each watershed.

Septic systems were accounted for in a similar manner. It was determined, however, that systems located immediately adjacent to the bay were of particular interest. Increased efforts were made, therefore, to accurately account for systems in these areas.

A first order decay rate was assumed for bacterial degradation as pollutants moved through the watershed, into streams/channels and eventually into the bay. Once in the bay, degradation was modeled as four independent continuous flow, stirred tank reactors.

Once the loading to the bay was obtained, a Monte Carlo simulation was used to determine the necessary load reductions. Monte Carlo simulations use random number probability distributions to simulate random behavior, providing estimates of uncertainty in a given outcome. Accurate estimates of measured bacteria concentrations only required calibration of stream residence times and a multiplication factor.

SPAtially Referenced Regressions On Watershed attributes (SPARROW)

SPARROW is maintained by the U.S. Geological Survey. It spatially references various watershed components, such as stream monitoring data, pollutant sources, etc., to surface

water flow paths that are defined by a digital drainage network. It then imposes mass balance constraints to empirically estimate terrestrial and aquatic rates of pollutant flux. Applications of SPARROW include estimation of the spatial distributions of pollutant yields, pollutant sources and the potential for delivery of those yields to receiving waters. This information can be used to:

- predict ranges in pollutant levels in surface waters,
- identify the environmental variables that are significantly correlated to the pollutant levels in streams,
- evaluate monitoring efforts for better determination of pollutant loads, and
- evaluate various management options for reducing pollutant loads to achieve water-quality goals.

SPARROW has been used previously to estimate the quantities of nutrients delivered to streams and watershed outlets from point and nonpoint sources over a range of watershed sizes (Smith et al., 1997; Alexander et al., 2000, 2001; Preston and Brakebill, 1999). This approach can be used for bacteria TMDLs because it not only uses process-based models to simulate transport of pollutants, but it also uses the actual historical monitoring data and known predictor variables to predict the various model input parameters. In this manner, a more realistic model can be developed that closely describes the conditions of the particular watershed (Schwarz, et al., 2006).

Spatially Explicit Load Enrichment Calculation Tool (SELECT)

SELECT (Teague 2007a, 2007b) spatially references the sources of bacteria contamination and is being developed under an ArcGIS 9 environment. SELECT will calculate and allocate bacteria loading to a stream from various sources in a watershed. All loads will be spatially referenced. In order to allocate the bacteria load throughout the watershed, estimations of the source contributions will be made. This, in turn, allows the sources and locations to be ranked according to their potential contribution. The populations of livestock, non-domestic animals and domestic pets will be calculated and distributed throughout the watershed according to appropriate land use. Furthermore, point sources will be identified and their contribution quantified based on flow and

outfall concentration. Septic system contribution will also be estimated based on criteria including distance to a stream, soil type, failure rate and age of system. Once the watershed profile is developed for each potential source, the information can be aggregated to the sub-watershed level to identify the top contributing areas.

Mass Balance (MB) Methods

These methods, as the name implies, calculate a mass balance between bacteria loads entering the water body and bacteria loads within the stream. Sources are typically inventoried, quantified and compared to existing and allowable in-stream loads at specified points within the stream (typically, where the TMDL is sought) for different flow conditions. MB methods require more data than the LDC method, but are more amenable for use in TMDL implementation. These methods have typically been developed using spreadsheets. The main advantages of the MB methods are that they can be used for tidal and non-tidal water bodies, including both TMDL and I-Plan development. In addition, they can be used for watersheds where both point and nonpoint sources appear to contribute at both low flow and high flow conditions. The main disadvantage is that MB methods, like the LDC method, are static and do not consider temporal variations in loading. The MB method accounts for spatial variations since it estimates the various sources within the watershed. However, estimates of nonpoint source loadings may be difficult to obtain. The output from these methods can often be used in the development of more complex, mechanistic models discussed in the next section.

Bacteria Load Estimator Spreadsheet Tool (BLEST)

In Texas, one of the more recent MB applications is described in Petersen (2006) in which BLEST was used to calculate bacteria loads from all sources and land uses on a subwatershed basis for Buffalo and White Oak Bayous. The loads were accumulated by segment and calculated for low, median and high flow conditions in a stream. Sources include WWTPs, septic tanks, nonpoint source runoff, sanitary sewer overflows and bypasses, sewer leaks and spills, in-stream sediment and non-domestic animals and

domesticated animals. BLEST was used to calculate existing loads and allowable loads and to estimate the load reductions that would be required to meet the standard. The limitations of this tool include the extensive amount of inventory data required, the use of literature values for some of its variables (for example, the rate of failure for septic systems) and the absence of attenuation or loss mechanisms that might affect loads before they reach the water body. Additionally, and due to its static nature, temporal variations in the source loads are not modeled.

Tools similar to BLEST have been developed elsewhere; for example, the Bacteria Source Load Calculator (BSLC) from the Center for TMDL and Watershed Studies at Virginia Tech (<http://www.tmdl.bse.vt.edu/outreach/C85/>).

Bacteria Indicator Tool (BIT)

Another MB tool is the BIT provided by EPA

(<http://www.epa.gov/waterscience/ftp/basins/system/BASINS3/bit.htm>). The BIT is a spreadsheet that can be used to estimate the monthly accumulation rate of fecal coliform bacteria on four land uses (cropland, forested, built-up and pastureland). The tool also estimates the direct input of fecal coliform bacteria to streams from grazing agricultural animals and failing septic systems. Output from BIT can be used as input to the Hydrological Simulation Program Fortran (HSPF) water quality model in BASINS (Better Assessment Science Integrating Point and Nonpoint Sources). BIT has been used in Texas.

Mechanistic Hydrologic/Water Quality Bacteria Models

A number of simulation models have been used to describe (in mathematical form) the mechanisms of water movement as well as loading and transport of pollutants. Both researchers and managers desire to have a means to create scenarios to simulate environmental outcomes in response to specific management practices. Models are used to predict the water quality in a water body based upon changes in pollutant loading and various allocation strategies. Current models have been adapted for use in bacteria TMDLs from other models originally designed for more conventional pollutants

(suspended sediments, nutrients, dissolved salts). These models can be used for both TMDL and I-Plan development to evaluate spatial and temporal variation of bacteria loading within a watershed. These models, however, have extensive data requirements and their level of sophistication requires a significant investment of resources for parameterization, calibration and training of personnel to use the models effectively.

In-stream water quality models are either steady-state or transient and are hydrologically driven (via rainfall) or hydrodynamically driven (via velocities in the water body). A steady-state model does not allow for variations over time and averages water quality over time. A dynamic or transient model, on the other hand, allows for changes over time and can be used to estimate bacteria loads and concentrations at different points in time anywhere in the stream.

Ward and Benaman (1999) identified a number of models as being appropriate for use in Texas TMDLs. Their list includes: ANSWERS, CE-QUAL-W2, DYNHYD, EFDC, GLEAMS, HSPF, POM, PRMS, QUALTX, SWAT, SWMM, TxBLEND and WASP. Their assessment categorized these models based on the watercourse type and the scale of resolution for time. For example, HSPF, SWAT, PRSM, SWMM and ANSWERS were characterized as watershed type models that can be used for “slow time variation” and “continuous time variation.”

Of the above list of models identified by Ward and Benaman (1999) for use in Texas TMDLs, the most commonly used for bacteria include HSPF, SWAT, SWMM and WASP. HSPF has been the most commonly used of the four, but EPA specifically requested U.S. Department of Agriculture – Agricultural Research Service (USDA–ARS) scientists in Temple, Texas to develop bacteria fate and transport components for SWAT to facilitate its use in TMDL and I-Plan development. These models all require many input variables, a substantial cost and time investment in set-up, calibration and validation time and have a steep learning curve.

Hydrological Simulation Program – FORTRAN (HSPF)

HSPF has been in extensive use since the 1970s, and is distributed by EPA's Center for Exposure Assessment Modeling. This watershed hydrologic model has been commonly used for TMDL development for a variety of conventional water quality parameters in Texas (for indicator bacteria and for dissolved oxygen) and also in other states. The required data include land use; watershed and subwatershed boundaries; location and data for rainfall gages and surface water quality monitoring stations; detailed descriptions of stream geometry and capacity; detailed information about sources within the watershed; sedimentation and re-suspension characteristics and bacteria die-off rates, to name a few. Development of an HSPF model for a given watershed is both complex and time-consuming and involves substantial calibration and validation. HSPF can be used for most types of watersheds (except possibly tidally influenced streams) regardless of the land use, but it requires extensive input from hydrologic and hydraulic models as well as GIS data layers. HSPF allows for a detailed spatial resolution within the watershed and allows for estimation of bacteria loads from runoff from the land surface as well as re-suspension from the streambed and from direct deposition sources. Disadvantages include the inherent difficulty in its application, its poor documentation and inadequate simulation of bacteria fate and transport processes. For example, transport of bacteria associated with sediment, sedimentation and re-suspension, regrowth and die-off processes are simplified and modeled using variables that difficult to measure in field settings. These variables are commonly treated as calibration variables during model development.

Soil and Water Assessment Tool (SWAT)

The SWAT model is a continuation of nearly 30 years of modeling efforts conducted by the USDA-ARS at Temple, Texas. SWAT has gained international acceptance as a robust interdisciplinary watershed modeling tool as evidenced by regular biennial international SWAT conferences (Gassman 2005a), SWAT-related papers presented at numerous other scientific meetings, and dozens of articles published in peer-reviewed journals. The model, like HSPF, has been adopted as part of the EPA Better Assessment Science Integrating Point and Nonpoint Sources (BASINS) software package and is being used by

many federal and state agencies, including the USDA within the Conservation Effects Assessment Project (CEAP). Reviews of SWAT applications and/or components have been previously reported, sometimes in conjunction with comparisons with other models (i.e., Arnold and Fohrer, 2005; Borah and Bera, 2003; Borah and Bera, 2004; Steinhardt and Volk, 2003; Gassman, et. al 2005b).

SWAT was developed primarily to estimate loads from rural and mainly agricultural watersheds; however, the capability for including impervious cover was accomplished by adding urban buildup/washoff equations from SWMM. At the request of EPA, a microbial sub-model was incorporated to SWAT for use at the watershed or river basin levels. The microbial sub-model simulates (1) functional relationships for both the die-off and regrowth rates and (2) release and transport of pathogenic organisms from various sources that have distinctly different biological and physical characteristics. SWAT has been used in Virginia and North Carolina for bacteria TMDL development.

Storm Water Management Model (SWMM)

SWMM was developed primarily for urban areas. It is most commonly used to simulate real storm events based on meteorological and watershed data, although it can also be used for continuous simulations. While SWMM was developed with urban watersheds in mind, it can be used for rural watersheds. The biggest advantage of SWMM is in its ability to model urban infrastructure including drains, detention basins, sewers and related flow controls. However, SWMM does not simulate the in-stream water quality. Linking it to WASP can circumvent this limitation. Perhaps the best application for SWMM is to characterize the bacterial pollution from the urban drainage infrastructure, but this limits its usefulness within a bacterial TMDL context to implementation rather than development.

Water-quality Analysis Simulation Program (WASP)

This model is also distributed by EPA's Center for Exposure Assessment Modeling and is a well-established water quality model incorporating transport and reaction kinetics. Unlike HSPF, however, WASP is driven by flow velocity rather than rainfall, thus it is

usually coupled with a suitable hydrodynamic model such as DYNHYD, EFDC or SWAT. WASP is typically used for main channels, reservoirs and bays and estuaries and not for modeling watershed-scale processes. Problems studied using WASP include biochemical oxygen demand and sources of bacteria, dissolved oxygen dynamics nutrients and eutrophication, organic chemical and heavy metal contamination.

Important Considerations for Bacteria Modeling

- The expectations from using a model for TMDL development or implementation must be realistic and commensurate with the level of data and information available for the watershed in question. The model used will only be as good as the data used to develop it.
- Models should be used as part of the TMDL framework and not as an only tool for decision-making. Models should continually evolve as the knowledge base used in developing them changes.
- In-stream sediment settling and re-suspension processes are not well represented in most models available to date and their roles in bacterial concentrations in water bodies are poorly understood.
- Bacteria regrowth and decay are also not well represented in presently available models. Bacteria death is typically approximated using first-order expressions, and the first-order decay constant is determined from controlled laboratory and/or field experiments.
- Transient (time-varying) models such as HSPF provide bacterial concentrations on a very detailed time scale (minutes or hours), whereas most bacterial measurements are made much less frequently (once a week or once a month or once a quarter) thus complicating calibration and validation of the model.
- The models that are hydrologically driven such as HSPF are biased toward high flow conditions since rainfall is the main driver for flow in the water body. These models have to be fine-tuned to represent bacterial sources in dry weather conditions (under mostly effluent dominated conditions).

- The main advantage of simple models such as LDC, SELECT, BLEST or BIT is in determining required reductions to meet the standard.
- The main value of detailed models is that they allow for spatial and temporal analysis of different reduction strategies (i.e., BMPs) and their effectiveness in improving in-stream water quality.
- Sensitivity and uncertainty in data, parameters and models should be considered and assessed.
- The results of modeling exercises are heavily dependant on the precision of the model as determined by calibration activities. For this reason, calibration specifications for model application should be explicitly stated and standardized throughout all applications.

Table 1 (below) is a matrix describing the applicability and capability of typically used models.

Table 1 Bacteria Modeling Matrix

Model		LDC	Spatial Explicit Statistical Models			Mass Balance Models			Mechanistic/Hydrologic/WQ			
			ArcHydro	SPARROW	SELECT	BLEST	BSLC	BIT	HSPF	SWAT	SWMM	WASP
Watercourse Type	Watersheds		x	x	x	x	x	x	x	x	x	
	River/Stream	x	x	x	x	x	x	x	x	x		x
	Lake/Reservoir		x	x	x	x	x	x				x
	Fresh/Saltwater Estuarine		x	x	x	x	x	x				x
TMDL Phase	Development	x	x	x	x	x	x	x	x	x		x
	Implementation		x			x			x	x		x
Model Type	Analytical	x	x	x	x	x	x	x				
	Numerical								x	x	x	x
Spatial Dimensions	1-D			x	x				x	x	x	x
	2-D											x
	3-D											x
Time Scale	Steady-state			x						x		x
	Time Varying								x	x	x	x
	Single Storm Event				x				x	x	x	
	Continuous in time			x					x	x	x	x
Watershed Characteristics	Rural	x	x	x	x	x	x	x	x	x		
	Urban	x	x	x	x	x	x	x	x	x	x	
	Sediment transport			x	x				x	x		x
In-Stream Processes	Bacteria Regrowth											
	Bacteria Die-off			x					x	x		
	Settling								x	x		
	Re-suspension					x			x	x		
WLA Sources	WWTF			x	x	x			x	x		x
	Storm Sewers			x	x	x			x	x		x
LA Sources	Septic Tanks			x	x	x	x	x	x	x		
	Direct Deposition					x	x	x	x	x		x
	Bed Sediment					x			x			x
Cost		\$	\$\$			\$\$			\$\$\$			

Notes: 1. Shaded areas: not applicable.

Bacteria Source Tracking (BST)

This section, coordinated by Drs. George Di Giovanni and Joanna Mott, describes the strengths and weaknesses of several BST tools that have been used. The EPA has also issued a microbial source tracking guidance document (USEPA 2005) which provides technical details on many different BST methods, quality control measures, discussion of library size and representativeness, statistical analysis, project design and case studies. No consensus has been reached for most of these issues by the source tracking scientific community, and detailed discussions of these topics are outside the scope of this report. However, where appropriate, brief discussion of these issues have been included in this document and considered in the recommendations presented below.

The premise behind BST is that genetic and phenotypic tests can identify bacterial strains that are host specific so that the original host animal and source of the fecal contamination can be identified. Often *E. coli* or *Enterococcus spp.* are used as the bacteria targets in source tracking, as this provides a direct link with water quality standards which are usually based on one of these two indicators (for example, [Parveen, Portier et al. 1999; Dombek, Johnson et al. 2000; Graves, Hagedorn et al. 2002; Griffith, Weisberg et al. 2003; Hartel, Summer et al. 2003; Kuntz, Hartel et al. 2003; Stoeckel, Mathes et al. 2004; Scott, Jenkins et al. 2005]). While there has been some controversy concerning host specificity and survival of *E. coli* in the environment (Gordon, Bauer et al. 2002), this indicator organism has the advantage that it is known to correlate with a probability of gastrointestinal illness and is used for human health risk assessments. BST of *E. coli*, therefore, has the advantages of direct regulatory significance and availability of standardized culturing techniques for water samples, such as EPA's Method 1603 (USEPA 2005).

Description of Methods

There have been many different technical approaches to bacterial source tracking (Scott, Rose et al. 2002; Simpson, Santo Domingo et al. 2002; Meays, Broersma et al. 2004), but there is currently no consensus on a single method for field application. Genotypic (molecular) tools appear to hold promise for BST, providing the most conclusive characterization and level of discrimination for isolates. Of the molecular tools available, ribosomal ribonucleic acid (RNA), genetic fingerprinting (Ribotyping), repetitive element polymerase chain reaction (rep-PCR), and pulsed-field gel electrophoresis (PFGE) are emerging as a few of the versatile and feasible BST techniques. Antibiotic resistance analysis (ARA), a phenotypic characterization method, also has the potential to identify the human or animal origin of isolates, and variations of this technique have been applied in several BST studies. Carbon source utilization (CSU) is another phenotypic method that has been used in Texas, as well as other states, as a source tracking method.

Four years ago, a review of BST methods was performed before initiating the BST study for Lake Waco and Belton Lake. At that time, and still today, there is no consensus among BST experts as to the best approach or technique. The enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), RiboPrinting, pulsed field gel electrophoresis (PFGE) and Kirby-Bauer antibiotic resistance analysis (KB-ARA) were chosen for the Texas study because they appeared promising from previous BST and other scientific studies, and cover the spectrum in cost, ease of use, and discriminatory ability. This allowed researchers to not only evaluate the practical application of these methods for the identification of human and animal sources of fecal pollution, but also to perform a comprehensive comparison of the methods and composite data sets for further consideration and use in future studies. Additionally, CSU has been used in Texas in conjunction with KB-ARA. Each of these five methods has its strengths and weaknesses, which are described below. A disadvantage of each of these techniques is that reference libraries of genetic or phenotypic fingerprints for *E. coli* isolated from known sources (i.e., domestic sewage, livestock and non-domestic animals) are needed to identify the sources of bacteria isolated from environmental water samples. Thus, the

development of an identification library can be a time consuming and expensive component of a BST study. Libraries of isolates from various parts of Texas have been developed using these methods following the same protocols for comparability.

ERIC-PCR

Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), a type of rep-PCR, has moderately high ability to resolve different closely related bacterial strains (Versalovic, Schneider et al. 1994). Consumable costs for ERIC-PCR are inexpensive and labor costs for sample processing and data analyses are moderate. ERIC-PCR is a genetic fingerprinting method used in previous BST studies (i.e., McLellan, Daniels et al. 2003; Leung, Mackereth et al. 2004; Casarez, Pillai et al. 2006) as well as many microbial ecology and epidemiological studies. ERIC elements are repeat DNA sequences found in varying numbers and locations in the genomes of different bacteria such as *E. coli*. The PCR is used to amplify the DNA regions between adjacent ERIC elements. This generates a DNA banding pattern or fingerprint which looks similar to a barcode pattern. Different strains of *E. coli* bacteria have different numbers and locations of ERIC elements in their bacterial genomes, and therefore, have different ERIC-PCR fingerprints. ERIC-PCR is useful as a screening technique for library development because of its moderate cost and moderately high ability to resolve different strains of the same species of bacteria. Though rep-PCR banding patterns for isolates tend to be generally stable, differences in fingerprint image processing and PCR protocols between laboratories may result in reduced between-laboratory reproducibility and pose a challenge to generating a composite library in multiple laboratories. Rigorous quality control and quality assurance, standardized protocols for PCR and image processing, and adequate training of personnel is crucial for generation of comparable data. Two analytical strategies that enhance data comparability between laboratories are the use of horizontal fluorophore-enhanced rep-PCR (HFERP; Johnson et al., 2004), or a commercially packaged product such as the DiversiLab system (<http://www.bacbarcodes.com/>).

Ribotyping

Ribotyping is a genetic fingerprinting method used in previous BST studies (i.e., Parveen, Portier et al. 1999; Scott, Parveen et al. 2003; Moore, Harwood et al. 2005), as well as many microbial ecology and epidemiological studies (i.e., Verduin, Kools-Sijmons et al. 2000; Clark, Kruk et al. 2003), although there is not a consensus as to the best protocol. Ribotyping has a moderate ability to resolve different strains of the same bacteria species. An automated ribotyping system (DuPont Qualicon RiboPrinter) is available, which saves labor costs and requires little training, but the initial investment and the consumable cost per isolate are expensive. The RiboPrinter was originally developed for use in identification and BST of microbial isolates for the food industry. An endonuclease enzyme (i.e., *Hind*III) selectively cuts *E. coli* DNA wherever it recognizes a specific DNA sequence. The resulting DNA fragments are separated by size and probed for fragments containing particular conserved ribosomal RNA gene sequences, which results in DNA banding patterns or fingerprints that look similar to barcode patterns. Different strains of *E. coli* bacteria have differences in their DNA sequences and different numbers and locations of enzyme cutting sites, and therefore have different ribotyping fingerprints. By automating the process, the RiboPrinter System can analyze up to 32 samples per day, whereas manual ribotyping methods, which require highly trained and experienced personnel, may require up to several days to complete. All bacteria isolate sample processing is automated using standardized reagents and a robotic workstation, providing a high level of reproducibility. Since the system employs standardized methods and reagents, results obtained from other laboratories using the system are directly comparable.

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE is another leading genetic fingerprinting method used in BST. PFGE has very high resolution and can discriminate between closely related bacteria strains. The entire bacterial genome is fragmented using an infrequent cutting restriction endonuclease enzyme (i.e., *Xba* I) which cuts DNA wherever it recognizes a specific rare sequence. All the DNA fragments are separated by size and stained to visualize the resulting genetic fingerprint that resembles a barcode. Different strains of *E. coli* bacteria have differences

in their DNA sequences and different numbers and locations of enzyme cutting sites and therefore, have different PFGE fingerprints. PFGE is currently being used by the Centers for Disease Control and Prevention (CDC) to track foodborne *E. coli* O157:H7 and *Salmonella* isolates. CDC currently uses this standardized protocol as the basis of its “PulseNet” outbreak surveillance network that allows public health laboratories nationwide to quickly compare their PFGE fingerprints to the CDC central reference library. Although it requires more training and cost, PFGE has very high resolution and can discriminate between closely related strains. While this level of resolution allows higher confidence in the matches made, fewer identifications of water isolates can be made, and an unrealistically large (and costly) library would be needed for field application. In addition, some bacterial strains have genomic DNA in configurations that do not permit effective restriction endonuclease digestions and cannot be analyzed by PFGE.

Kirby-Bauer Antibiotic Resistance Analysis (KB-ARA)

Antibiotic Resistance Analysis was one of the first approaches developed for BST and has been used for many TMDL studies in the United States. It relies on the principle that bacteria from intestinal tract of different animals have been exposed to different antibiotics and will exhibit different profiles of resistance to a panel of antibiotics. Several methods have been used for antibiotic resistance analysis; however all rely on the same principle and the data are usually statistically analyzed using discriminant analysis to categorize isolates. The KB-ARA technique follows procedures used in the clinical laboratory for evaluating the antibiotic resistance of bacteria isolates and has the advantage of strong quality assurance/quality control requirements. The method involves measuring the diameter of the zone of inhibition of bacterial growth around a filter disk impregnated with a specific antibiotic. By comparison to resistant and susceptible control strains, the response of the *E. coli* isolates can be determined. In the procedure used in several studies in Texas (Mott) to further standardize and automate the assay, an image analysis system is used to measure the zones of inhibition and provide electronic archival of data. The KB-ARA profile for an isolate consists of the measurements of the zones of inhibition in response to 20 antibiotics, each at a standard single concentration. Among

ERIC-PCR, RiboPrinting, PFGE and KB-ARA, the KB-ARA method has the lowest ability to discriminate closely related bacteria strains. However, it also has the lowest initial and per sample cost and takes the least time and training.

Carbon Source Utilization (CSU)

CSU is another phenotypic method and is less widely used than ARA. It is based on the principle that bacteria from different animals have been exposed to a range of carbon sources (dietary differences between animals). It is most commonly conducted using the Biolog Microbial Identification System (MIS) (Biolog, Inc., 3939 Trust Way, Hayward, CA 94545). Bacteria are grown on specific media and then transferred to 96 well microplates, with each well containing a different carbon source and an indicator that changes color if the source is utilized. The plates are incubated and read using an automated plate reader, which can provide color intensity data for each well. The profiles of utilization of the carbon sources of unknown source isolates are compared to the profiles of known source isolates (the library) and discriminant analysis is used to categorize isolates. This approach has been used both in Texas, in conjunction with KB-ARA, and in other states as a stand-alone method. In Texas, CSU profiles of *E. coli* were used in conjunction with ARA of the same isolates for a study of sources of contamination in White Oak and Buffalo Bayous (Mott and Lehman, with Dr. Rifai, University of Houston). Combining both profiles provided increased accuracy in terms of rates of correct classification for the library.

Table 2 compares several BST techniques.

Table 2. Relative comparison of several bacterial source tracking techniques

Technique	Acronym	Target organism(s)	Basis of characterization	Previously Used or in Progress in Texas	Used in other states	Accuracy of source identification	Size of library needed for water isolate IDs	Capital cost	Cost per sample (reagents and consumables only)	Ease of use	Hands on processing time for 32*** isolates	Time required to complete processing 32 isolates
Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction	ERIC-PCR	<i>Escherichia coli</i> (<i>E. coli</i>) and <i>Enterococcus</i> spp.	DNA fingerprint	Yes (Di Giovanni)	Yes	Moderate	Moderate	\$20,000 (\$15,000 BioNumerics software, \$5,000 equipment)	\$8	Moderate	3 h	24 h**
Automated ribotyping (RiboPrinting)†	RP	<i>E. coli</i> and <i>Enterococcus</i> spp.	DNA fingerprint	Yes (Di Giovanni)	Yes	Moderate	Moderate	\$115,000 (\$100K RiboPrinter, \$15K BioNumerics software)	\$40	Easy	1 h	24 h
Pulsed field gel electrophoresis	PFGE	<i>E. coli</i> and <i>Enterococcus</i> spp.	DNA fingerprint	Yes (Pillai and Lehman)	Yes	High	Large	\$30,000	\$40	Difficult	10 h	5 days
Kirby-Bauer antibiotic resistance analysis‡	KB-ARA	<i>E. coli</i> and <i>Enterococcus</i> spp.	Phenotypic fingerprint	Yes (Mott)	Yes	Moderate*	Moderate	\$35,000	\$15	Easy	3 h	24 h**
Carbon source utilization	CSU	<i>E. coli</i> and <i>Enterococcus</i> spp.	Phenotypic fingerprint	Yes (Mott)	Yes	Moderate	Moderate	\$15,000	\$10	Easy	4 h	24 h**
<i>Bacteroidales</i> polymerase chain reaction	<i>Bacteroidales</i> PCR	<i>Bacteroidales</i> species	Genetic marker presence or absence (not quantitative)	Yes (Di Giovanni)	Yes	Moderate to high for <u>only</u> human, ruminant, horse, and pig sources	Not applicable	\$5,000	\$8	Easy to moderate	3 h	8 h**
<i>Enterococcus faecium</i> surface protein polymerase chain reaction or colony hyb.	<i>E. faecium</i> esp marker	<i>E. faecium</i>	Genetic marker presence or absence (not quantitative)	Yes (Di Giovanni)	Yes	High for <u>only</u> human	Not applicable	\$8,000	\$8 to \$12	Easy to moderate	3 to 6 h	8 to 24 h**
ERIC and RP 2-method composite	ERIC-RP	<i>E. coli</i>	DNA fingerprints	Yes (Di Giovanni)	No	Moderate to high	Moderate	\$120,000	\$48	Moderate	4 h	24 h
ERIC and KB-ARA 2-method composite	ERIC-ARA	<i>E. coli</i>	DNA and phenotypic fingerprints	Yes (Di Giovanni)	No	Moderate to high	Moderate	\$55,000	\$23	Moderate	6 h	24 h
KB-ARA and CSU 2-method composite	ARA-CSU	<i>E. coli</i> and <i>Enterococcus</i> spp.	Phenotypic fingerprints	Yes (Mott)	Yes	Moderate to high	Moderate	\$50,000	\$23	Easy to moderate	7 h	24 h

†A manual ribotyping version is also used by some investigators (i.e. Dr. M. Samadpour with IEH Laboratories and Consulting Group in Seattle), but no detailed information is available for comparison.

‡A variation of this technique using replica plating and +/- scoring of growth on media with different concentrations of antibiotics, called ARA, has been used extensively in Virginia for TMDLs.

*This technique is better for distinguishing broader groups of pollution sources. For example, “wildlife” and “livestock” as opposed to “avian wildlife”, “non-avian wildlife,” “cattle,” etc.

**With sufficient personnel, up to approximately 150 isolates can be analyzed in 24 h.

***Thirty two isolates selected for comparison because it is the maximum throughput per day of the RiboPrinter, which is the only automated system described.

Method Comparisons and Composite Data Sets in a Previous Texas Study

As mentioned above, a BST project was completed for Lake Waco and Belton Lake in which *E. coli* isolates were analyzed using RP, ERIC-PCR, PFGE and KB-ARA. For this study, BST analyses were performed using the individual techniques, as well as composite data sets (Casarez, Pillai et al. 2006). The congruence (concordance) between the groupings of isolates from known fecal sources by individual BST methods and different combinations of composite data sets was determined using the same statistical software (BioNumerics, Applied Maths, Austin, Texas) and Pearson's product moment correlation coefficient. The four-method composite library generated the most desirable BST results in regards to accuracy, blind quality control study results, library quality measures and ability to identify water isolates. However, as few as two methods in combination may be useful based on congruence measurements, library internal accuracy (i.e., rates of correct classification, RCCs), and comparison of water isolate identifications. In particular, the combinations of ERIC-PCR and RiboPrinting (ERIC-RP), or ERIC-PCR and Kirby-Bauer Antibiotic Resistance Analysis (ERIC-ARA) appeared promising. These two-method composite data sets were found to have 90.7% and 87.2% congruence, respectively, to the four-method composite data set. More importantly, based on the identification of water isolates, they identified the same leading sources of fecal pollution as the four-method composite library. ERIC-ARA has the lowest cost for consumables and has high sample throughput, but requires a considerable amount of hands-on sample and data processing. Due to the high cost of RiboPrinting consumables and instrumentation, ERIC-RP has a higher cost than ERIC-ARA. However, ERIC-RP has the advantage of automated sample processing and data preprocessing that the RiboPrinter system provides.

Regulatory Expectations and Capabilities of BST Methods

Regulatory agencies continue to have high hopes and expectations for BST in aiding them to address water quality issues. BST may not be needed, or may not be suitable for all TMDL studies. In some cases, more rigorous monitoring of water bodies and

determination of *E. coli* levels may identify the major sources of pollution. No BST method developed to date can provide 100% accuracy of pollution source identification in field studies. In many cases, the reduced accuracy often does not justify the high cost of some of the analyses.

In addition, the issue of indicator bacteria regrowth in the environment has been raised. There are many uncertainties surrounding this issue and there is a lack of scientific evidence that demonstrates regrowth occurs naturally in subtropical and temperate environments. However, because of the obvious implications for regulatory efforts (particularly for *E. coli*), the survival and regrowth of fecal indicator organisms in the aquatic environment, sediments and soils will need to be addressed not only for source tracking, but the concept of bacterial fecal indicators. While no BST method has specifically been developed to identify environmentally adapted indicator organisms, *ex post facto* analysis of BST data for environmental water isolates may provide some insight into this issue.

Ideally, agencies and stakeholders would prefer identification of pollution sources to the level of individual animal species. Performing a three-way split of pollution sources into domestic sewage, livestock and non-domestic animals source classes would likely be more scientifically justified with the current levels of accuracy and confidence of BST techniques in general. The division of host sources into the seven classes in the Lake Waco study was a compromise between the capabilities of the *E. coli* BST techniques and their practical application. Rates of correct classification (RCCs, measurements of library internal accuracy) are calculated using jackknife statistical analyses in which a single known source isolate is removed from the identification library, treated as an unknown, and is tested against the remaining library isolates to determine the percent of correct source identification. Comparisons of the RCCs for two- or three-way splits of host sources show high accuracy in distinguishing human from animal sources (83% and 95% RCCs, respectively); and distinguishing domestic sewage, livestock and non-domestic animals (83%, 72% and 73% RCCs, respectively). The RCCs for the seven-way split of source classes ranged from 22% to 83%, although those classes of particular

interest (domestic sewage and cattle) were relatively high (83% and 60%, respectively). It is important to note that these RCCs for each of the source classes were three- to seven-fold greater than random chance based on library composition. Identifying pollution loading down to the sampling station level would require collecting hundreds of water samples per sampling site to analyze a statistically significant number of *E. coli*, making this approach not feasible. More realistic for BST is identification of fecal pollution sources at the watershed level. Alternatively, a higher number of *E. coli* isolates (i.e., 50) can be analyzed from fewer water samples to identify statistically significant differences in pollution sources. However, this will only provide pollution source identification on limited time scales, and will not provide an overall assessment of the water body.

Although quantitative allocation of fecal contamination to source categories is a goal of most TMDL projects, uncertainty in classification limits our capacity for absolute quantification. In some cases, library-dependent methods may enable identification of a source that contributes more fecal contamination than other sources, or identification of sources for which there is no credible evidence of substantial contamination. The results of library-dependent classification are conservatively seen as semi-quantitative and suitable for sample-level classification of sources as “contribution not detected” or “contribution detected” with possible refinement to “contribution detected greater than (alternative source).” This information may not be suitable for incorporation into quantitative water-quality models. However, most library-independent methods are even less quantitative than library-dependent methods, and the correlation between the fecal pollution indicators targeted by these methods and regulated contaminants (i.e., *E. coli*) is uncertain.

Another issue is that BST typically only identifies the major sources of pollution for a water body, not the entry pathways of the fecal pollution. This can be an important issue when developing implementation actions for different watershed stakeholders. This emphasizes the importance of sampling station selection and upstream/downstream targeted sampling of suspected pollution sources.

Future Direction

As described above for the EPA CWA §319(h) funded study for Lake Waco and Belton Lake, a cost-effective alternative to using multiple BST methods for each study is to use the two-method combination of either ERIC-PCR and RiboPrinting (ERIC-RP), or ERIC-PCR and Kirby-Bauer antibiotic resistance analysis (ERIC-ARA). Based on other studies in Texas, CSU and KB-ARA may also be a suitable combination. It is important to note that in nearly all cases, no single BST method should be solely relied upon. This is particularly true with the phenotypic methods, such as KB-ARA, which alone cannot provide the resolution needed for most BST studies. A factor to be considered when choosing the methods includes the level of resolution needed for the particular study. In some cases, it may be critical to determine specific animal groups, while in other cases broad categories may provide the level of resolution needed for management decisions. Cost constraints, equipment needs and expertise available are other factors that should be considered in the decision.

Since the Lake Waco study was initiated, there have been significant developments in library-independent BST methods, including bacterial genetic markers specific to different animal sources and humans (i.e., Bernhard and Field 2000; Dick, Bernhard et al. 2005; Scott, Jenkins et al. 2005; Hamilton, Yan et al. 2006). Library-independent methods are cost-effective, rapid and potentially more specific and accurate than library-dependent methods. Concerns with many of the recently developed library-independent approaches include uncertainties regarding geographical stability of markers and the difficulty of interpreting results in relation to regulatory water quality standards and microbial risk, since some target microorganisms are not regulated. More importantly, these library-independent methods can only detect a limited range of pollution sources and are currently not quantitative. For example, the *Bacteroidales* PCR (Bernhard and Field 2000; Dick, Bernhard et al. 2005) can detect fecal pollution from ruminants, humans, dogs, horses and pigs; but no further discrimination is possible. This method involves the concentration of 100 ml water samples, extraction of bacterial DNA, and detection of animal group-specific *Bacteroidales* bacteria genetic markers using the PCR.

This method is qualitative (presence/absence), and does not identify sources of *E. coli*, but rather the potential sources of fecal pollution that may contribute *E. coli*. Despite these limitations, this method may be useful for a rapid assessment of the possible sources of fecal pollution for a water body and are currently being applied to BST studies in Texas. Another example is the detection of optical brighteners used in laundry detergent using fluorometry. This approach can be used in real-time for the detection of leaking septic systems and sewage releases, is ideal for lakes and beach waters but obviously cannot identify other sources of fecal pollution.

Identification libraries consisting of thousands of isolates from different geographical regions in Texas have already been established for ERIC-PCR, PFGE, RiboPrinting, CSU and KB-ARA patterns. In addition, several thousand more *E. coli* isolates from source samples have been archived and are available to researchers. Library development is one of the most costly components of BST studies. It would be most economical to build upon the libraries already established in Texas. It is recommended that agencies use contractors that use BST methods that will strengthen and expand the current Texas library and follow previously approved standard operating procedures (SOPs) for performing analyses. However, further research is needed to determine if a regional library built from different projects using the same protocols may be useful for the identification of water isolates from other watersheds. That is, geographic and temporal stability of the existing *E. coli* library will need to be explored. Currently, the Di Giovanni laboratory at the El Paso Agricultural Research and Extension Center is cross-validating the libraries generated in the Lake Waco study and the San Antonio watershed study in an attempt to explore issues of geographical and temporal stability of BST libraries, refine library isolate selection and determine accuracy of water isolate identification. By selecting *E. coli* source isolates that are correctly identified in both watershed libraries, we hope to find more geographically universal and host-specific isolates, resulting in more accurate source tracking.

The infrastructure for BST work in Texas needs to be expanded for both library dependent as well as library independent methods. More laboratories with trained

personnel and equipment are needed. This could be facilitated by the use of automated systems for BST analyses. In addition to the RiboPrinter, an automated system for repetitive element PCR (repPCR) using BOX primers (Diversilab, Athens, Georgia) has been developed and may be an alternative to ERIC-PCR. For just the *E. coli* isolates selected through cross-validation of existing libraries, funding is needed to develop a complete data set using BST techniques selected for use in Texas.

For future TMDL studies, an assessment phase using a “toolbox” approach is recommended. The assessment phase should include targeted monitoring of suspected pollution sources, use of library-independent methods to identify the presence of domestic sewage pollution and screening of water isolates from the new watershed against the existing library to determine the need for collection of local source samples and expansion of the library.

Recommended Approach for Bacteria TMDL and Implementation Plan Development

With the background provided by the preceding sections on BST and modeling, as well as extensive input from the expert advisory group, the task force recommends that TCEQ and TSSWCB implement the following integrated approach to the TMDL and I-Plan development.

The task force recognizes that no single process is appropriate for all bacteria impairments, given the variations in: (1) the severity of the bacteria impairments, (2) the complexity of watershed land uses and development, and (3) the interests of stakeholders and the regulated community affected. For these reasons, we propose a tiered approach that identifies alternate procedures to accommodate the site-specific factors that will emerge in each assessment. The three-tiered approach to developing bacteria TMDLs and I-Plans described in this section incorporates adaptive management, phased TMDLs and phased implementation to the extent allowable by EPA. The objectives of Tiers 1 and 2 are to ensure that each TMDL is developed using a scientifically credible, cost-effective process with strong stakeholder involvement. Tier 3 is designed to develop a feasible I-Plan, and, for some complex TMDLs, expands the information available for TMDL development. Figure 5 summarizes the three-tier approach.

Tier 1 should be required of all bacteria TMDLs to provide the minimum level of technical analysis and stakeholder input needed to make sound decisions regarding load reductions and allocations. Tier 2 provides a more in-depth and extensive assessment of the water body in question. We anticipate that it will provide sufficient scientific content and stakeholder involvement to develop most bacteria TMDLs. Tier 3 should provide additional information regarding bacteria sources and impacts of best management practices. We anticipate that for most contaminated water bodies Tier 3 activities will be limited to I-Plan development, though in some cases Tier 3 may be required for TMDL development.

TMDLs requiring Tier 3 could include: (1) those requiring large reductions in bacteria loads (e.g. >75 %), (2) those with highly uncertain and conflicting results, (3) those with large implementation costs or (4) those that have not obtained stakeholder acceptance. The decision to move to Tier 3 is strictly at the discretion of the TCEQ and TSSWCB, but with adequate scientific and stakeholder input.

The tiered framework presented here is intended to be flexible to ensure it best fits the complexity of the watershed sources, availability of data, degree of impairment and level of accuracy required.

TIER 1

Tier 1 is a one-year analysis required for all TMDLs. It provides for early stakeholder involvement, development of a comprehensive geographic information system of watershed information, surveys of potential bacteria sources, development of a load duration curve and analysis of the assembled information by agency staff, agency consultants and stakeholders.

1.1 - Stakeholder Involvement

TCEQ, with the TSSWCB and other key agencies, have been developing TMDLs in consultation with watershed-based stakeholder groups. The process includes extensive efforts to identify and involve diverse and sometimes competing interests. The task force believes it is essential to preserve and enhance these processes. Leaders within the watershed should be enlisted to participate at the outset of the project (after listing on the 303(d) list) and collaborate on all aspects of the TMDL development. County commissioners courts; soil and water conservation districts; community leaders; non-governmental environmental, agricultural and civic organizations; TPDES permit holders; cities and water districts (especially with MS4 permits); watershed landowners; and other potential contributors should form the basis for the stakeholder group. In most cases, the TCEQ should initiate a TMDL project with a planning meeting of state and local entities (such as TSSWCB, Texas Department of Agriculture [TDA], Texas Parks and Wildlife Department [TPWD], Texas Cooperative Extension, key municipalities, county commissioners and soil and water conservation districts) and the appropriate river

authority to develop an initial outreach strategy whereby agency representatives will solicit the commitment and involvement at the local level of key leaders.

Figure 5: Recommended Three-Tier Approach for Bacteria TMDL Development

Tier 1 Analysis (T1) (one-year)

Required for all bacteria TMDLs.

1. Form TMDL stakeholder advisory group.
2. Develop comprehensive GIS inventory for watershed.
3. Implement source survey for watershed.
4. Calculate load duration curves (LDCs).
5. Analyze Tier 1 data with stakeholder advisory group.

Decision 1 (D1) Are data and analysis adequate?

Yes → Go to D2.

No → Go to T2.

Decision 2 (D2) Are needed load reductions socially and economically attainable?

Yes → Complete and submit draft TMDL for agency approval.

No → Complete and submit a draft TMDL that includes a recommended change in designated use (i.e. Use Attainability Analysis).

Tier 2 Analysis (T2) (one-to-two years)

Implemented for most bacteria TMDLs. May be adequate for I-Plan development for non-controversial TMDLs.

1. Implement targeted monitoring to fill data gaps.
2. Perform library-independent BST and limited library-dependent BST analysis.
3. Develop simple LDC, GIS and/or Mass Balance Models.
4. Analyze Tier 2 data with stakeholder advisory group.

Decision 3 (D3) Are data and analysis adequate?

Yes → Go to D4.

No → Initiate a “phased TMDL” and go to T3.

Decision 4 (D4) Are needed load reductions socially and economically attainable?

Yes → Complete and submit draft TMDL (or I-Plan) for agency approval.

No → Complete and submit a draft TMDL that includes a recommended change in designated use (i.e. Use Attainability Analysis).

Tier 3 Analysis (T3) (two-to-three years)

Normally used for I-Plan development. May be required for development of complex “phased TMDLs.”

1. Assure extensive stakeholder involvement.
2. Implement extensive targeted monitoring.
3. Perform extensive library-dependent BST analysis.
4. Complete mechanistic modeling.
5. Analyze Tier 3 data with stakeholder advisory group.

Decision 5 (D5) Are needed load reductions socially and economically attainable?

Yes → Complete and submit draft I-Plan (or revise “phased TMDL”) for agency approval.

No → Complete and submit a draft TMDL that includes a recommended change in designated use (i.e. Use Attainability Analysis).

The identified stakeholder participants and agencies should be invited by TCEQ (or, when appropriate, TSSWCB and other participating organizations) for a kick-off meeting. The meeting will discuss the project goals, the existing water quality, relevant water quality standard(s) not met, the time line and process for TMDL development and what implementation strategies are typical for addressing bacteria impairments. Stakeholder participants should understand the complexity of the watershed, the uncertainty associated with bacteria monitoring and analysis and what may be required of the regulated community during implementation, as well as the typical voluntary or incentive-based strategies needed for implementation. Either at a kick-off meeting or soon after, the TCEQ (and TSSWCB) should discuss with stakeholders and seek consensus on the overall project's design, including the tools, models and monitoring that would be used in Tier 1 of the TMDL assessment. Attentive efforts to receive broad input, involvement and consensus should pay off in the end, particularly with groups representing entities that are not regulated point sources, to improve the water quality. Stakeholders can provide insight into sources and solutions that may not be apparent to those living outside the watershed. Since stakeholders will ultimately be responsible for helping achieve TMDL goals, it is appropriate that they be involved throughout TMDL development.

1.2 - Comprehensive Geographic Information System

A comprehensive geographic information system should be developed for the watershed, including available land use, elevation, soil, stream network, reservoir, road, municipality and satellite or aerial photographic information. In addition, locations of other relevant information (such as wetlands, sewage treatment plants, subdivisions, confined animal feeding operations, etc.) should be included for use by scientists, agencies and other stakeholders. In most cases, almost all data layers required for Tier 1 analysis can be obtained from readily available public sources. Locations of potential bacteria sources (identified in 1.3 below) should be incorporated into the GIS.

1.3 - Surveys of Potential Bacteria Sources

Conducting a potential source survey is an important Tier 1 activity. Texas and other states have conducted these surveys to better characterize the pollutant sources that contribute loadings that cause impairments. As with many efforts of this nature, there is a substantial range of costs and investigative activities that could occur, dependent on the project design, complexity of land uses and geographic size of the target watershed. For instance, in the Buffalo and White Oak Bayous in the Houston area, the TCEQ and its contractors spent several years and substantial funds to assure the watershed (with its intensive land use, dense population and hundreds of point sources) was adequately characterized.

The potential source survey should be representative of warm and cool seasons and, if possible, low and high flow conditions. The survey should be conducted by an integrated team including stakeholders, TPWD biologists, TDA/TCE personnel, Texas Department of State Health Services, TCEQ Regional Office staff, local governments and SWCD and TSSWCB personnel in order to identify all potential contamination sources. Point sources like wastewater treatment plants and industrial outfalls, nonpoint sources, stream sediment sources (especially those below wastewater outfalls) and other possible bacteria sources should be evaluated. According to a study by Harris County *E. coli* in fully disinfected WWTP effluent can regrow a thousand-fold over the course of four days. It is possible that in many effluent-dominated streams, regrowth is a major source of *E. coli*. To complete this evaluation, it will be important to examine permit compliance issues, residences and businesses on central sewage collection systems, septic systems and livestock and non-domestic animal concentrations.

1.4 - Data Analysis / Load Duration Curves

Note: For bacteria impairments in reservoirs or estuaries, the load duration curve method is not usually applicable.

The fourth component of Tier 1 includes analysis of ambient bacteria concentrations using statistical methods and LDCs. LDC development at this point in the process will use existing data and provide stakeholders insight into when exceedences occur, potential reductions needed, and potential sources. This will help stakeholders develop monitoring methodologies needed for the next tier. LDCs should be developed (using the methods described earlier in the document) for all sites having at least 24 bacteria data points (including at least three high flow points). For those sites with large data sets, only the most recent five years of data should be used. As additional data become available following targeted monitoring in Tier 2, this initial LDC should be updated. The analysis of the data should include an assessment of the degree of impairment, expressed as a percentage exceedance of the standard. This will help identify the course of action needed to address the impairment. In addition, this will provide some indication of the need for a UAA, particularly in areas where large exceedances of the criteria are unlikely to be resolved by revised criteria.

1.5 - Analysis of Tier 1 Information

Components 1.1 through 1.4 should be implemented simultaneously during the first year of TMDL development. As information from components 1.1 through 1.4 is collected, it should be shared with interested stakeholders, scientists and agency personnel. In addition to organizational meetings at the beginning of Year 1, at least one meeting with stakeholders should be held in the watershed approximately halfway through Year 1 to share information collected to that point. As the end of Year 1 approaches, accumulated data and recommendations of agency staff and consultants should be collected and shared with stakeholders. A meeting should be held in the watershed to discuss the data and recommendations of experts, and consensus should be sought regarding future activities.

At the meeting, consensus should be sought concerning whether data and analysis are adequate to develop a draft TMDL, including target reductions in bacteria loads and waste loads. If data and analysis from Tier 1 are not adequate, Tier 2, a one- to two-year period of additional data collection and analysis, should begin. If Tier 1 data and analysis

are sufficient, the group must consider the question of whether the required reductions in bacteria waste loads and loads are socially and economically attainable. If they are attainable, a draft TMDL can be completed and submitted to the agencies for approval. However, in some cases, reaching the required waste load and load reductions may not be socially and economically attainable. In those cases, the group may reach consensus that the agencies should consider changing the designated use of the water body in order to meet alternative standards (see Figure 5).

A provisional TMDL should be drafted when a UAA is under way. A final TMDL may be required to comply with the new standard. In this case, the provisional TMDL should be adopted by the TCEQ and TSSWCB to meet the water quality standard in effect at that time and remain in place while standards issues are addressed. However, I-Plan development and implementation for these TMDLs should not proceed until the standards evaluation is completed. Once the standards are resolved, the provisional TMDL would be replaced by the final TMDL and an I-Plan developed.

TIER 2

If Tier 1 does not produce a consensus that data and analysis are adequate, the process should move to Tier 2, a one- to two-year effort to develop sufficient information to implement the needed TMDL or change the designated use of the water body. We recommend that Tier 2 include targeted monitoring to complement data collected in Tier 1, library-independent bacterial source tracking, statistical and mass balance modeling and development of a consensus draft TMDL.

2.1 - Targeted Monitoring

Agency personnel, expert consultants and stakeholders should develop a targeted monitoring plan to fill data gaps (for example, provide additional high flow samples or sample additional locations in the water body), evaluate the magnitude and impacts of possible waste loads or loads identified in Tier 1, and/or evaluate bacteria fate and

transport within the water body. For example, point sources could be sampled at their outfalls. Additional sampling points more representative of the watershed or stream segment than public access points could be sought. Measurements of waste loads and loads could be made and substituted for literature values or estimates used in Tier 1. More intensive (biweekly or monthly) monitoring could be implemented for stream segments at greatest risk for fecal pollution.

2.2 - Library-Independent BST

To further assess bacteria sources, BST analysis should be conducted in conjunction with the targeted monitoring to determine if livestock, humans and/or non-domestic animals are contributing bacteria to the water body. Fifty to 100 samples should be analyzed using the library-independent PCR genetic test for the *Bacteroidales* markers for human, ruminants, horse and swine sources.

If sufficient funds are available, *E. coli* isolates from 50 to 100 different water samples should be analyzed using the BST methods described in the Tier 3 BST discussion and compared with isolates from the previously developed Texas *E. coli* source library to determine the need for development of a local source library (see component 3.2 below). These analyses are needed to confirm that the sources of *E. coli* and *Bacteroidales* are comparable. If these *E. coli* analyses are not done in Tier 2, they should be included in subsequent Tier 3 activities.

2.3 - Spatially Explicit Statistical and Mass Balance Modeling

Spatially explicit statistical models and/or mass balance models should be used in combination with the LDC developed in Tier 1 to provide multiple lines of evidence for bacteria sources. This report does not recommend specific spatially explicit statistical models or mass balance models. We consider that the quality of model calibration, input data quality and the experience of the modeling team are as important as the choice of model used. Therefore, we recommend that agency staff and their expert consultants

evaluate the characteristics of the water body, its watershed and the data available for analysis, and then select the most appropriate model and modeling team.

The main value of LDC and mass balance models such as BLEST and BIT is in determining load reductions required to meet the water quality standard. Spatially explicit models such as Arc Hydro, SELECT or SPARROW should be used to estimate contaminant loads from various sources in a watershed.

Models should be run using the highest quality, readily available input data for the watershed. Every effort should be made to use measured loadings from wastewater treatment plants and other point and nonpoint sources. Data collected through the targeted monitoring in Tier 2.3 should be used to update the LDC as well as for providing the data needed to initialize, calibrate and validate the model(s) used. Updated LDCs should be developed (using the methods described earlier in the document) for all sites having at least 24 bacteria data points (including at least three high-flow points). At each step, confidence intervals for model inputs and outputs should be estimated and reported.

The principal reason for recommending that simpler spatially explicit statistical models and mass balance models be used in Tier 2 rather than more complex hydrologic/water quality models is that the simpler models should be more economical to implement. This may not be the case in the future as baseline simulations are developed by USDA with SWAT. If such baseline, partially calibrated models are available, agency personnel may choose to implement them for Tier 2 analyses.

2.4 - TMDL Development

Components 2.1 through 2.3 should be implemented simultaneously. As during Tier 1, at least one meeting with stakeholders should be held in the watershed approximately halfway through Tier 2 activities to share information about progress to date. As the end of Tier 2 approaches, a meeting should be held in the watershed to discuss the data and recommendations of experts, and consensus should be sought regarding future activities.

We anticipate that for most impacted segments, consensus will be reached by the end of Tier 2 to complete development a draft TMDL, including target reductions in bacteria loads and waste loads. If consensus is reached that Tier 2 data and analysis are sufficient, the group must consider whether the required reductions in bacteria waste loads and loads are socially and economically attainable. If the required reductions are attainable, a draft TMDL can be completed and submitted to the agencies for approval. However, if the required waste load and load reductions are not socially and economically attainable the agencies should consider conducting a Use Attainability Analysis (UAA) and changing the designated use of the water body in order to meet alternative standards (see Figure 5).

A provisional TMDL should be drafted when a UAA is under way. A final TMDL may be required to comply with the new standard. In this case, the provisional TMDL should be adopted by the TCEQ and TSSWCB to meet the water quality standard in effect at that time and remain in place while standards issues are addressed. However, I-Plan development and implementation for these TMDLs should not proceed until the standards evaluation is completed. Once the standards are resolved, the provisional TMDL would be replaced by the final TMDL and an I-Plan developed.

If Tier 2 data and analysis are not sufficient to reach consensus, a “phased TMDL” should be completed. TCEQ staff (with TSSWCB, as appropriate) will be responsible for deciding when consensus cannot be achieved.

The phased TMDL should consist of draft “phase 1” TMDL based on data gathered during Tiers 1 and 2. In addition, the draft “phase 1” TMDL should include a commitment to collect and analyze additional data during Tier 3 I-Plan analysis. This approach allows implementation of the TMDL (beginning with BMPs most socially and economically acceptable) while additional data are collected to reduce uncertainty. The TMDL may be reopened to consider new information that suggests that the WLA or LA should be modified. If standards are not met by target dates established by the stakeholders, increasingly stringent measures should be implemented in a phased manner over a period of years.

TIER 3

Tier 3 analyses will normally be required for development of I-Plans and, if necessary, phased TMDLs. Tier 3 should normally consist of a two- to three-year effort consisting of extensive watershed monitoring, library-dependent bacteria source tracking, mechanistic hydrologic modeling and I-Plan or phased TMDL development.

We recommend the use of “adaptive management” for the I-Plan process. During the multi-year course of development and execution of an I-Plan, changes in water quality, infrastructure and agency policies can be expected. As a result, the I-Plan or TMDL should be subject to revision as conditions change. Agency personnel and stakeholders should also remain sensitive to the need for identifying and implementing cost-effective BMPs. Costly BMPs can have adverse economic impacts on stakeholders and taxpayers, and they should be avoided if possible, especially when good scientific evidence of their effectiveness is lacking.

3.1 - Watershed Monitoring

Extensive watershed monitoring should be initiated to identify bacteria sources, quantify loading and provide the data needed for calibration and validation of watershed scale mechanistic models. Watershed monitoring should be targeted to fill information gaps identified in Tiers 1 and 2. At a minimum, monthly sampling for one year should be conducted, although greater frequency (weekly) and duration (two years) is preferred. Monitoring should include measurement of bacteria levels and flows from all wastewater treatment plants and other point sources that are suspected of contributing significant bacteria loads. Sampling sites should be carefully selected, and samples should represent spatial variability, seasonal fluctuations and typical (low, medium and high) flows. A minimum of 100–200 isolates from approximately 40 water samples should be collected in conjunction with the watershed monitoring effort for library-dependent BST analysis. These samples should be collected at carefully selected and representative sites, seasons and flow conditions (see 3.2 below).

3.2 - Library Dependent Bacteria Source Tracking

The isolates collected during the watershed monitoring described in component 2.2 (above) should first be analyzed using ERIC-RP, ERIC-ARA or CSU-ARA combination methods. Isolates should first be identified using the Texas Source Library. If the Texas Source Library does not provide 80% identification then a local library should be developed using *E. coli* isolates from known sewage and animal sources. The sampling design should be determined using data from the component 2.2 assessment.

At least 300 *E. coli* colonies from approximately 100 known fecal samples should be characterized by the selected combination method (i.e. ERIC-RP, ERIC-ARA, CSU-ARA). The *E. coli* isolates obtained from ambient water samples should be characterized using the selected method. DNA patterns of those isolates should be compared to the individual watershed *E. coli* source library as well as Texas library and identified to cattle, other livestock, avian and non-avian non-domestic animals, domestic sewage and pet sources. Identified sources and unknown sources should be expressed as percentages of total isolates with appropriate confidence intervals.

3.3 - Mechanistic Hydrologic / WQ Modeling

For Tier 3 analyses, a mechanistic hydrologic water quality model or combination of models (such as HSPF, SWAT, SWMM and/or WASP) should be used to model watershed hydrology and fate and transport of *E. coli* within the watershed. The main value of these detailed models is that they allow for spatial and temporal analysis of the effectiveness of different best management practices in improving in-stream water quality. The selected model(s) should be run using the highest quality, readily available input data for the watershed.

The model(s) should be calibrated and validated for flows using long-term weather and stream flow gauges within (or near) the targeted water body. Once the model(s) are calibrated and validated for flow, baseline *E. coli* loads and concentrations should be

calibrated and validated using monitoring data previously collected from the impacted segment. Finally, recommended best management practices (BMPs) identified by the stakeholder committee and/or cooperating agencies should be evaluated for their impacts on bacteria loads. At each step, confidence intervals for model inputs and outputs should be estimated and reported.

Every effort should be made to use measured loadings from wastewater treatment plants and other point and nonpoint sources for model calibration. This caution is particularly important if the effectiveness of BMPs is not well understood or monitored. Where effluent limits are specified as BMPs rather than loads that can be verified (as in the case of NPDES-regulated municipal and small construction storm water discharges), monitoring or other scientifically acceptable BMP performance data should be obtained to assess if the expected load reductions attributed to the BMP are achieved. Otherwise, adaptive management will be limited by our understanding of BMP effectiveness.

3.4 - IP-Plan and Phased TMDL Development

We anticipate that in most cases Tier 3 data collection and analysis will be used for IP-Plan development. However, for some complex and/or controversial water bodies, Tier 3 assessments will be required for phased TMDL development.

As pointed out above, we recommend the use of “adaptive management” for the I-Plan process. During the multi-year course of development and execution of an I-Plan, changes in water quality, infrastructure and agency policies can be expected. As a result, the I-Plan should be subject to revision as conditions change. We recommend that TCEQ identify a watershed coordinator to facilitate the communication, education and coordination needed during I-Plan development, revision and execution.

Adaptive management of I-Plans should facilitate progress toward achieving water quality goals while using new data and information to reduce uncertainty and adjust implementation activities. Adaptive management can include immediate implementation

of BMPs, success monitoring, use of improved monitoring data and modeling, new experimental results and revision of the implementation plan.

Phased TMDLs are an example of the adaptive implementation approach because each new phase utilizes new information to reevaluate the original TMDL. However, even for TMDLs where there is little uncertainty regarding the loading capacity of the water body and the necessary load reductions, an adaptive implementation approach can be a useful tool. Implementation of TMDLs can take many years, and uncertainty about the effectiveness of implementation activities usually exists. Follow-up monitoring addresses uncertainty in the efficacy of implementation actions and can provide assurance that implementation measures are succeeding in attaining water quality standards, as well as in inform the ongoing TMDL implementation strategy. If adaptive implementation activities reveal that TMDL loading capacity needs to be changed, the revision may require EPA approval. In most cases, however, adaptive implementation is not anticipated to lead to the re-opening of a TMDL. Instead, it is a tool used to improve implementation strategies.

The I-Plan describes the actions that the TCEQ, TSSWCB and the project's stakeholders will undertake to achieve restoration of the water body's use. Implementation strategies specify actions to meet the load allocations assigned to all point sources and nonpoint sources identified in the TMDL report. Action strategies may be selected from a menu of possible measures based on an evaluation of feasibility, costs, support, timing and other factors. Activities may be implemented in phases with evaluation of progress and success before proceeding to a subsequent phase.

In addition to the actions taken to reduce pollutant loads from sources, an I-Plan includes provisions to track the progress of the plan using both implementation and water quality indicators. The I-Plan identifies in-stream monitoring at specific locations and targets constituents that will be used to evaluate whether the water quality criterion is achieved. It includes tracking using a schedule identified in the I-Plan for evaluating whether administrative actions undertaken to improve water quality actually occurred.

An I-Plan includes a review strategy that will consider when and if the I-Plan needs to be revised, or as mentioned earlier, whether a subsequent phase of implementation is needed. The review strategy implements adaptive management into the planning process by providing decision points in the process for such consideration. The review strategy accounts for unexpected changes in implementation of controls. In some special circumstances, the I-Plan may include strategies to evaluate the underlying water quality standard and its appropriateness as a goal driving implementation. Recommended changes to a standard could drive the need to re-evaluate and modify the TMDL itself.

Communication is necessary to ensure stakeholders understand the I-Plan and the progress that is being made to restore water quality conditions. The TCEQ disseminates information derived from tracking I-Plan activities to watershed stakeholders and others.

Agency personnel and stakeholders should also remain sensitive to the need for identifying and implementing cost-effective BMPs the effectiveness of which can be verified. Costly BMPs can have adverse economic impacts on stakeholders and taxpayers, and should be avoided if possible, especially when good scientific evidence of their effectiveness is lacking.

Research and Development Needs

This section, coordinated by Drs. Larry Hauck and George Ward, summarizes research and development needed in the next three to five years to improve the tools and methods available to TCEQ and TSSWCB for bacteria TMDL and I-Plan development. These research and development needs may be generally considered to be motivated by the need to quantify and reduce uncertainties in the measurement, identification and modeling of bacterial contaminants.

In previous sections, the diversity of bacteria sources within watersheds and the complexity of their proliferation and movement on the landscape and in receiving waters have been discussed. There are unavoidable inaccuracies in the depiction of the origin, fate and transport of environmental indicator bacteria within fate and transport models, and these represent common sources of uncertainties associated with the load allocation process of a TMDL and its subsequent I-Plan development. Broadly, uncertainties may be reduced by efforts along two parallel paths: (1) refinement of kinetic- and transport-type input parameters used in the preferred models to be applied in Texas and (2) reformulation of kinetic processes in these same models to better represent the present state of understanding of bacteria fate and transport. Laboratory and field studies in combination with model enhancements will be needed in these efforts to reduce uncertainties. BST methods can strongly support the TMDL process, and additional research and development in this arena is also anticipated to be important in reducing uncertainties and providing for improved tools and methods.

Finally, it is recommended that TCEQ and TSSWCB form a work group, in addition to the Task Force, whose focus would be to create a blueprint for a successful stakeholder process. Such a blueprint could be used by staff of both agencies statewide in TMDL, I-plan and watershed protection plan processes. This work group would address stakeholder group membership, attendance at meetings and communication to stakeholders. In addition, it would evaluate the inclusion of state and local agencies on

stakeholder groups in order to ensure TMDL and other regulatory issues are addressed in a manner appropriate to the relevant authority.

The specific research and development needs reported herein were accumulated based on the collective expertise of Task Force members and Expert Advisors, recommendations from the Texas A&M Bacteria Roundtable Discussion of August 10, 2006, studies reported in Rifai et al. (2005), and an overview report on bacteria fate and transport modeling (Benham et al., 2006).

Characterization of Sources

Often a high level of uncertainty exists in the specification of bacteria sources within fate and transport models. Because of the numerous sources of fecal bacteria and the diversity of delivery mechanisms of the bacteria to receiving waters, a broad spectrum of research items will provide useful information.

- Studies to quantify species-specific bacteria production in feces and to measure the variability of this production. There are numerous mammalian and avian species (human, pet, livestock and non-domestic animals), all of which shed fecal bacteria. Within these proposed studies, focus is suggested on dominant and relevant species. While bacteria content of feces has been reported in literature for some species and has been summarized in some reports used in TMDL development, often this information has not been the focus of the reported research and therefore has not undergone thorough peer review. Further, some indications exist that fecal bacteria content may be related to latitude, elevation, diet and water source, which increases the anticipated variability of the data.
- Studies to quantify behavioral patterns of important mammalian and avian species, such as feral hogs. These studies would entail investigations for important species of the fraction of time spent in the water, preferential defecation locations (herding and flocking behavior), and other factors that impact location of feces deposition.

- Studies to improve methods of estimating animal densities in watersheds. Estimations of livestock and non-domestic animal densities in a watershed are often based on limited data and county-level data, and potentially there may be no existing data for some important sources of fecal bacteria (e.g., feral hogs, coyote, rodents and migratory birds). Improved methods are needed to optimize use of existing data to quantify source densities and to provide means of estimating pet, livestock, and non-domestic animals densities for species for which there is no or limited information. These improved methods could include integration of GIS land use and land cover with various types of census data, enhanced stakeholder involvement and detailed sanitary surveys, and other methods that optimize use of existing data and watershed specific resources and knowledge.
- Studies to improve characterization of bacteria loadings from WWTPs and sanitary sewer collection systems. WWTPs are required to disinfect their effluents using chlorination or other equivalent methods (e.g., ultraviolet radiation), and under conditions of proper operation bacteria loads are typically low from such facilities. However, under less than optimal conditions, WWTPs can directly discharge significant bacterial loads directly into receiving waters. Less than optimal conditions may occur because of wet weather, upsets in operating conditions, etc. Regrowth of bacteria relative to the completeness of disinfection is another area of focus worthy of more study. Regrowth and reactivation of bacteria after the disinfection process of WWTPs effluent appears to be a research topic of some concern within Texas and, in particular, Harris County where existing research is occurring on this subject in effluent dominated receiving streams. Quantification of fecal bacteria concentrations in the effluent of WWTPs will require sampling of facilities of various permitted capacities, different operational conditions and varying ages over a wide range of environmental and operational conditions. A related facet of bacteria loading is associated with sanitary sewer collection systems. Studies to quantify frequency of and conditions causing overflows (e.g., wet weather, grease and rags) from sanitary sewer systems and associated bacteria loadings can provide important information to models of urban areas.

- Studies to quantify failure rates of on-site sewage facilities (OSSFs). Failing OSSFs and even straight pipe discharges can be an important contribution of bacteria loading to some receiving waters. Local information at the county- and city-level will often be important in estimating failed OSSFs in a watershed. However, state- or regional-level statistical analyses relating failure rates to income level, education, age of communities, soil types, rainfall and physiographic region will provide additional support and defense of estimates regarding failure rates and bacteria loadings from OSSFs. As a starting point of these studies, a 2001 study funded by the Texas On-Site Wastewater Treatment Research Council (Reed, Stowe & Yanke, LLC, 2001) provides regional information on reasons for and magnitude of malfunctioning OSSFs.
- Studies to quantify bacteria (especially indicator bacteria such as *E. coli*) production/shedding from non-animal sources, such as mulch, gardens or other organic-rich environments.
- Studies to determine sources of the high concentration of indicator bacteria in rainfall runoff from a spectrum of land uses that might be anticipated to contain low concentrations of bacteria, (e.g., parking lots, streets, building roofs).
- Studies on pathogen sources and forms in stormwater. Research is needed on the sources of pathogenic bacteria in stormwater, the relationship of pathogenic bacteria to indicator bacteria in stormwater as compared to the relationship in such sources as human sewage and the human health significance of the pathogenic bacteria forms found in stormwater.
- Studies to better define indicators and disease risk for water bodies without focused areas of frequent swimming and contact recreation. The original EPA studies to identify indicator bacteria criteria to protect contact recreation use were conducted in lakes with designated swimming areas and nearby wastewater discharge points (EPA, 1986). Criteria development is needed for water bodies without focused or designated areas where swimming and contact recreation use are infrequent. Such water bodies would more likely involve recreational use with incidental contact during boating and wading activities.

Characterization of Kinetic Rates and Transport Mechanisms

Quantification of production, mobilization and movement of fecal bacteria from the landscape to receiving waters and the subsequent fate and transport of these bacteria in receiving waters is addressed by some comprehensive hydrologic/water-quality models as well as simpler *ad hoc* models (e.g., mass balance methods). In many instances, the scientific understanding of relevant processes concerning fate and transport on the landscape and in receiving waters is primitive, and in other instances, this scientific understanding exceeds the representation of those processes in existing models. Note that buildup (i.e., accumulation and proliferation) and mobilization of bacteria on the landscape will also be considered under this loose definition of fate and transport. Within this subsection, the focus will be upon possible studies to enhance the understanding of fate and transport processes, and within the next subsection, potential enhancements to hydrologic/water-quality models will be discussed. This artificial separation, however, should not be taken to mean that the empirical data collection and analyses designed to better understand physical, chemical and biological processes important to landscape and receiving water fate and transport of fecal bacteria can occur in the absence of interaction with model enhancement efforts. On the contrary, such interactions are encouraged and are necessary to the overall success of improving our TMDLs and I-Plans.

- Studies to quantify buildup and mobilization of fecal bacteria from the landscape. The release of fecal bacteria during rainfall-runoff events is a function of many processes antecedent to the event (e.g., rates and timing of manure and feces deposition, *in situ* die-off rates, growth, etc.) and activities during the event (e.g., rainfall duration and intensity and transport processes). Studies that increase understanding of the processes and factors determining bacteria buildup and mobilization and that provide quantification of those processes and factors are needed to enhance our understanding of mechanisms delivering bacteria to receiving waters. These studies can include different land uses (e.g., low-density residential and surface applied manure application fields, to name but two) and could also include investigations of important transport mechanisms such as

surface mobilization and runoff and subsurface movements, including preferential pathways.

- Studies to identify dominant environmental factors that affect bacterial transport in landscapes (e.g., vegetative cover, soil type, temperature, etc.) and persistence and survival of bacteria in soil.
- Studies to quantify bacteria die-off phases in receiving waters. While bacteria die-off is generally assumed to follow Chick's law, experimental data indicate that fecal bacteria often do not follow this law and that die-off occurs in various phases. Further, die-off (and possibly regrowth) during these phases is further complicated by such factors as 1) whether the bacteria are planktonic, attached to suspended sediment, or re-suspended from bed sediments and 2) water temperature, sunlight intensity and levels of nutrients and suspended solids present. Further research to identify and define bacteria die-off phases and to mathematically describe die-off under a variety of conditions will improve our knowledge base on bacteria persistence in receiving waters. Instream regrowth of bacteria under various sediment, nutrient, occurrences of WWTP effluent and water temperature conditions is an additional important consideration under the broad category of bacteria die-off. Harris County is involved in a study on die-off and regrowth of bacteria from WWTP effluents, and any additional studies in this area should be coordinated with that ongoing study.
- Studies to identify dominant environmental factors that affect bacterial transport in streams (e.g., physical and chemical composition of stream waters [pH, total suspended solids, total dissolved solids, nutrients, etc.], temperature, etc.).
- Studies to provide better understanding of spatial and temporal distribution of instream bacteria conditions under various environmental conditions. The State's existing monitoring programs provide insufficient information to quantify spatial and temporal distributions of fecal bacteria at the time scales typically applicable in TMDLs. These time scales may in some instances be less than a day. Intensive spatial and temporal sampling of selected streams under various conditions (e.g.,

rainfall-runoff and dry conditions, high and low water temperatures) could enhance our basic understanding of bacteria issues within the state.

- Studies to quantify suspended bacteria in stream water column and deposited bacteria in stream sediments. Resuspension of bacteria from sediments to water column during peak flows and streambed erosion should be studied as well as, studies to improve understanding of sediment contributions to water-column bacteria levels. Settling of bacteria to sediments during periods of low turbulence and re-suspension of sediments and associated bacteria during conditions of high turbulence are recognized fate and transport processes. Studies are needed to better understand and quantify the interactions of the water column and sediments from the perspective of bacteria fate and transport dynamics in Texas streams and tidal systems.
- Determination of acceptable default inputs which can be used in model development and application. An initial set of inputs representing reasonable estimates of these processes needs to be proposed and agreed upon by the participating agencies. Initially these values should be taken from available literature and as time progresses may be changed to reflect recent research developments.

Enhancements to Bacteria Fate and Transport Models

Hydrologic/water quality models and some of the simpler models that partially incorporate mechanistic processes could benefit from improvements to their fate and transport processes.

- Enhancements to allow better representation of bacteria life cycle and processes. With support from the previously mentioned experimental studies on characterization of bacteria fate and transport, existing models can benefit from improvements and modifications in their representation of bacteria life cycles and processes.

- Enhancements to allow better representation of fate and transport processes. Again, with support from experimental studies, benefits to selected bacteria models would result from improvements in their representations of 1) sorption and bacteria release processes on the landscape and in receiving waters, 2) die-off and regrowth, 3) other fate and transport processes, and 4) effects of various environmental factors (e.g., temperature, light intensity, pH, etc.) on growth and die-off.
- Development of a spatially-explicit tool that will assess bacterial sources, distribute estimated loads to the land as a function of land use and source type, and generate bacterial load input parameters for watershed-scale simulation. This tool can build upon existing and under-development tools, such as SELECT, SPARROW, BLEST and BIT, among others previously mentioned in the Bacteria Fate and Transport Model section.
- Studies to improve linkages of BST and allocation modeling. A stand-alone spatially explicit load allocation tool's results can be validated with BST data or vice versa. Research is needed to determine benefits of linking BST and allocation modeling. At least two questions need to be addressed by these studies. Can BST and modeling be linked in a manner the benefits either TMDL load allocation or I-Plan development? If there were benefits from such linkage, how would that linkage occur?

Bacteria Source Tracking

Within the section on BST, a few areas of research and development were mentioned, and these are repeated below with some additional research items.

- Determine a reasonable expectation for the level of source identification by BST. For example, can BST methods reliably identify to the individual animal species level or is a coarser cut all that is reasonable (e.g., separation into categories of sewage, livestock and non-domestic animals)?

- Investigation and refinement of library-independent BST methods to assist in the TMDL process, and determine which library-independent BST method or methods are best suited for Texas TMDL development and implementation.
- Continue investigations into the most promising library-dependent BST methods, and continue research into promising combinations of such methods.
- Investigate the usefulness of a regional known-source library for BST library-dependent methods. Geographic and temporal stability of BST libraries is not well understood. Additional studies are needed to address these issues and to make library-dependent BST techniques better tools. These studies should build upon and not duplicate ongoing studies in Texas that are cross-validating the libraries generated in the Lake Waco study and the San Antonio watershed study in an attempt to explore issues of geographical and temporal stability of BST libraries, refine library isolate selection, and determine accuracy of water isolate identification.
- Define appropriate ambient water sampling protocol to provide desired statistical confidence with BST findings. For example, in some past studies, 10 to 12 monitoring events are conducted at each station; for each ambient water monitoring event five water samples are collected at a station with one to two minutes between collection of each sample; and two randomly selected bacteria isolates from each cultured sample are subsequently identified using BST methods (e.g., TIAER et al., 2005). Is this level of sampling adequate for the subsequent statistical characterization of sources and determination environmental conditions influencing source contributions (often wet versus dry weather conditions)?
- What is an appropriately sized watershed or sub-watershed for BST sampling design? Beyond what size drainage area do BST results tend to show a wide mix of contributions from all species in a library? Are there factors such as the degree of land development or anthropogenic activity that determine an appropriate drainage size to optimize source discrimination?

These listed items can be developed into a series of studies to advance BST methods and the utility of these methods for Texas bacteria TMDLs.

Determination of Effectiveness of Control Measures

Another research area directly supporting bacteria TMDL and I-Plan development entails determination of effectiveness of various control practices and best management practices to remove and reduce bacteria loadings and concentrations from sources. Success of an I-Plan and willingness of individuals and entities to adopt various control practices and best management practices may be greatly enhanced in the presence of scientifically conducted studies showing efficacy of various practices under a variety of conditions (e.g., rainfall patterns, landscape position, etc.).

- Studies to determine efficiency of agricultural best management practices. Prominent BMPs for bacteria load reduction from agriculture should be evaluated to determine their efficiencies under a variety of environmental conditions. Specifically, those conditions that exceed the ability of these measures to function should be identified.
- Studies to determine efficiency of urban control practices. Prominent control practices for achieving bacteria load reduction from urban and suburban areas should be evaluated to determine their efficiencies under a variety of environmental conditions. Specifically, those conditions that exceed the ability of these measures to function should be identified.

Quantification of Uncertainty and Communication of Risk

Because of the nature of the pollutant, bacteria TMDLs and I-Plans, while using best available information and applying accepted methods of determination, will contain uncertainties. Even if sources of error in field sampling, kinetics modeling and numerical implementation could be eliminated, there is a core uncertainty associated with the “noise” in the bacterial determination methodologies themselves, as indicated by

imprecision in replicate measurements. The spectrum of those involved in bacteria TMDLs (including, to name a few, the regulated community, environmental regulatory agencies, engineers and scientists performing technical evaluations and those providing assistance to unregulated sources) are cognizant of the degree of uncertainty in bacteria TMDL development. Efforts to reduce this uncertainty and to provide heightened defensibility of the process are both worthy and necessary goals, and the research and development studies enumerated above will provide significant advances toward realization of those goals. However, the brutal reality is that over the near future uncertainties — that are sometimes quite large compared to other water-quality parameters — will exist in bacteria TMDL and I-Plan development. Hence, the need exists for one other potential area of research — quantification of uncertainty and the associated communication of risk resulting from the uncertainty associated with TMDLs and I-Plans.

- Studies to quantify uncertainty. A body of literature exists on uncertainty and how to incorporate uncertainty analysis, typically via Monte Carlo techniques, into environmental models. In fact, the latest version of SWAT contains a feature allowing the performance of uncertainty analysis. Some other models employed in TMDL load allocation development do not contain this feature. Knowledge of how to incorporate uncertainty into the TMDL and implementation process can be obtained from existing literature. That knowledge can be transferred to the unique characteristics of the Texas TMDL process for purposes of developing methods to allow quantification of uncertainty.
- Development of proper means to communicate the risks arising from uncertainties in the TMDL process. This communication can assist in overcoming roadblocks that can dampen stakeholder enthusiasm or become excuses for no action in reducing bacteria loadings. Again, a body of literature (e.g., Morgan and Henrion, 1990) and experience exists on risk communication that can be exploited for application to the Texas situation. Successful risk communication can assist in overcoming the uncertainties within bacteria TMDLs and I-Plans and assist in moving the process toward the desired outcome of restored and improved water quality. Areas of study could review lessons learned, examples in the United

States of successful TMDL implementation in spite of uncertainty, and identifying positive paths forward in areas of known difficulties in present and recent bacteria TMDLs that include uncertainty. Results could be implemented to enhance interactions with stakeholders with a focus on the development of best mechanisms of risk communication.

Prioritization of Research and Development Activities

The activities briefly described above are not exhaustive, but these activities do provide indications of major areas where research and development advancements can benefit TMDL and I-Plan development. Additionally, this listing of needs helps to inform the decision makers at the TCEQ and TSSWCB of the current state of the science and techniques. These needs encompass a breadth of activities that most certainly will exceed the resources available for the successful performance of all potential studies. Purposefully, within this section no attempt has been made to prioritize these research and development activities. The selected activities need to be focused to support management decisions, which provide a broad direction to the TMDL and I-Plan processes. For example, such broad direction could include which models are to be supported in the near term for TMDL development. Also, known technical needs can define and inform prioritization of research and development needs that will, over the next several years, improve the tools and methods available to TCEQ and TSSWCB for bacteria TMDL and I-Plan development. Review of existing literature will be an important first step in further defining some of the possible research and development activities enumerated above under several research headings: characterization of sources, characterization of kinetic rates and transport mechanisms, enhancements to bacteria fate and transport models, BST, determination of effectiveness of control measures, and quantification of uncertainty and risk communication.

Some Expert Advisory members voiced certain research preferences during the conference call process used to refine and develop this final report. Those research topics preferentially voiced by certain Expert Advisory members include the following: 1) the

issue of regrowth of bacteria in WWTP effluents and in effluent dominated streams, 2) better quantification of species-specific bacteria production and loadings that used as input to many bacteria fate and transport models, 3) integration of fate and transport models and BST methods within the TMDL and I-Plan process, and 4) improved understanding of the accuracy and precision of BST methods. This list of preferred research topics is included within the more refined lists provided previously within this report section. It should be noted that these four research topics only reflect the views of certain Expert Advisory members and do not necessarily reflect the views of all Expert Advisory members and the Task Force members.

References

Bacteria Fate and Transport Models

- Alexander, R. B., R. A. Smith and G. E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403:758–761.
- . 2001. Atmospheric nitrogen flux from the watersheds of major estuaries of the United States: An application of the SPARROW watershed model, in *Nitrogen Loading in Coastal Water Bodies: An Atmospheric Perspective*, Coastal Estuarine Study, edited by R. Valigura et al., 57: 119–170. Washington, D. C.: AGU.
- Arnold J. G. and N. Fohrer, 2005. SWAT2000: current capabilities and research opportunities in applied watershed modeling. *Hydrological Processes*, 19(3): 563-572.
- Bonta, J.V. March 2002. Framework for Estimating TMDLs with Minimal Data. ASAE Proceedings of the Watershed Management to Meet Emerging TMDL Regulations Conference. Fort Worth, TX. pp. 6-12.
- Borah D. K. and M. Bera, 2004. Watershed-scale hydrologic and nonpoint-source pollution models: Review of applications. *Transactions of ASAE*, 47(3): 789-803.
- Borah D. K. and M. Bera, 2003. Watershed-scale hydrologic and nonpoint-source pollution models: Review of mathematical bases. *Transactions of ASAE*, 46(6): 1553-1566.
- Cleland, B.R. 2002. TMDL Development from the “bottom up” – Part II: Using Duration Curves to connect the Pieces. America’s Clean Water Foundation, Washington DC.
- Cleland, B. 2003. TMDL Development from the “bottom up” – Part III: Duration Curves and wet-weather assessments. America’s Clean Water Foundation, Washington, DC. Nevada Division of Environmental Protection, 2003. Load Duration Curve methodology for assessment and TMDL development. <http://ndep.nv.gov/bwqp/loadcurv.pdf>

- Gassman, P.W., M. Reyes and J.G. Arnold. "SWAT Peer-Reviewed Literature: A Review." 3rd International SWAT Conference. Zurich, Switzerland. 13 July 2005a.
- Gassman, P.W., S. Secchi, M. Jha and C.L. King 2005b. Nonpoint source needs assessment for Iowa: Part III – Economic and environmental outcomes. Proceedings of the Third Conference on Watershed Management to Meet Water Quality Standards and Emerging TMDL, ASAE Publication Number 701P010.
- Gibson, C. J, B.S., D. R. Maidment and M. J. Kirisits, 2006. Bacterial loadings watershed model in Copano Bay. Unpublished online report, <http://www.crwr.utexas.edu/reports/2006/rpt06-6.shtml>
- Petersen, T. M. "Spatial and Temporal Modeling of Escherichia coli Sources and Load Allocations in an Urban Watershed." Ph.D. diss. University of Houston. 2006.
- Preston, S. D. and J. W. Brakebill, 1999. Application of Spatially Referenced Regression Modeling for the Evaluation of Total Nitrogen Loading in the Chesapeake Bay Watershed. USGS Water-Resources Investigations Report 99-4054:12, U.S. Geological Survey.
- Schwarz, G.E., A.B. Hoos, R.B. Alexander and R.A. Smith. 2006. "The SPARROW Surface Water-Quality Model: Theory, Application and User Documentation," <http://pubs.usgs.gov/tm/2006/tm6b3>
- Smith, R.A., Schwarz, G.E. and Alexander, R.B. 1997. Regional interpretation of water-quality monitoring data: *Water Resources Research* 33(12): 2781–2798.
- Steinhardt, U. and M. Volk, 2003. Meso-scale landscape analysis based on landscape balance investigations: problems and hierarchical approaches for their resolution. *Ecological Modeling*, 168 (3) 251-265.
- Stiles, T.C. March 2001. A Simple Method to Define Bacteria TMDLs in Kansas. ASIWPCA / ACWF / WEF TMDL Science Issues Conference: On-site Program. St. Louis, MO. 375–378.
- Teague, A., M. Babbar-Sebens, R. Karthikeyan, R. Srinivasan, J. Jacobs, M. McFarland, N. Dictson and D. Boellstorff. 2007a. "Spatially Explicit Load Enrichment Calculation Tool (SELECT) and Load Duration Curves for Identification of E. coli Sources in Plum Creek Watershed, TX". Conference Proceedings. ASABE

- Watershed Management to Meet Water Quality and TMDLs (Total Maximum Daily Load) Issues: Solutions and Impediments to Watershed Management and TMDLs, March 2007, San Antonio, TX (to be presented).
- Teague, A., M. Babbar-Sebens, R. Karthikeyan, R. Srinivasan, J. Jacobs, M. McFarland, N. Dictson and D. Boellstorff. 2007b. "Spatially Explicit Load Enrichment Calculation Tool (SELECT) and Cluster Analysis for Identification of E. coli Sources in Plum Creek Watershed, TX". International ASABE Conference, June 2007, Minneapolis (to be presented).
- Ward, G. H. and Benaman, J. 1999. "Models for TMDL Application in Texas Watercourses: Screening and Model Review." Center for Research in Water Resources, University of Texas, Austin, Texas, CRWR-99-7, xx-pp.

Bacteria Source Tracking

- Bernhard, A. E. and K. G. Field. 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. *Journal of Applied and Environmental Microbiology* 66 (10): 4571–4574.
- Cesarez, E. A., S. D. Pillai, J. Mott, M. Vargas, K. Dean and G. D. DiGiovanni. 2006. Direct comparison of four bacterial source tracking methods and a novel use of composite data sets. *Journal of Applied and Environmental Microbiology*.
- Clark, C. G., T. M. Kruk, L. Bryden, Y. Hirvi, R. Ahmed, and F. G. Rodgers. 2003. Subtyping of *Salmonella enterica* serotype enteritidis strains by manual and automated PstI-SphI ribotyping. *J Clin Microbiol* 41:27-33.
- Dick, L. K., A. E. Bernhard, T. J. Brodeur, J. W. Santo Domingo, J.M. Simpson, S. P. Walters and K. G. Field, 2005. Host distributions of uncultivated fecal Bacteroidales bacteria reveal genetic markers for fecal source identification. *Journal of Applied and Environmental Microbiology* 71 (6): 3184–3191.
- Dombek, P. E., L. K. Johnson, S. T. Zimmerley, M. J. Sadowsky, 2000. Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human

- and animal sources. *Journal of Applied and Environmental Microbiology* 66 (6): 2572–7.
- Gordon, D. M., S. Bauer, J. R. Johnson. 2002. The genetic structure of *Escherichia coli* populations in primary and secondary habitats. *Journal of Microbiology and Environmental Quality* 148 (5): 1513–1522.
- Graves, A. K., C. Hagedorn, A. Teetor, M. Mahal, A. M. Booth and R. B. Reneau, 2002. Antibiotic Resistance Profiles to Determine Sources of Fecal Contamination in a Rural Virginia Watershed. *Journal of Microbiology and Environmental Quality* 31 (4): 1300–1308.
- Griffith, J. F., S. B. Weisberg, and C. D. McGee 2003. Evaluation of microbial source tracking methods using mixed fecal sources in aqueous test samples. *Journal of Water and Health* 1 (4): 141–51.
- Hamilton, M. J., T. Yan and M. J. Sandowsky. 2006. Development of goose- and duck-specific DNA markers to determine sources of *Escherichia coli* in waterways. *Journal of Applied and Environmental Microbiology* 72 (6): 4012–9.
- Hartel, P. G., J. D. Summer and W. I. Segars, 2003. Deer diet affects ribotype diversity of *Escherichia coli* for bacterial source tracking. *Water Research* 37 (13): 3263–8.
- Johnson, L. K., M. B. Brown, E. A. Carruthers, J. A. Ferguson, P. E. Dombek and M. J. Sadowsky, 2004. Sample size, library composition, and genotypic diversity among natural populations of *Escherichia coli* from different animals influence accuracy of determining sources of fecal pollution. *Applied and Environmental Microbiology* 70 (8):4478-85.
- Kuntz, R. L., P. G. Hartel, D. G. Godfrey, J. L. McDonald, K. W. Gates and W. I. Segars, 2003. Targeted sampling protocol as prelude to bacterial source tracking with *Enterococcus faecalis*. *Journal of Microbiology and Environmental Quality* 32 (6): 2311–2318.
- Leung, K. T., R. Mackereth, Y. Tien and E. Topp, 2004. A comparison of AFLP and ERIC-PCR analyses for discriminating *Escherichia coli* from cattle, pig and human sources. *FEMS Microbiology Ecology* 47 (1): 111–119.
- McLellan, S. L., A. D. Daniels and A. K. Salmore, 2003. Genetic characterization of *Escherichia coli* populations from host sources of fecal pollution by using DNA

- fingerprinting. *Journal of Applied and Environmental Microbiology* 69 (5): 2587–2594.
- Meays, C. L., K. Broersma, R. Nordin and A. Mazumder, 2004. Source tracking fecal bacteria in water: a critical review of current methods. *Journal of Applied and Environmental Microbiology* 73 (1): 71–79.
- Moore, D. F., V. J. Harwood, D. M. Ferguson, J. Lukasik, P. Hannah, M. Getrich and M. Brownell, 2005. Evaluation of antibiotic resistance analysis and ribotyping for identification of fecal pollution sources in an urban watershed. *Journal of Applied and Environmental Microbiology* 99 (3): 618–28.
- Parveen, S., K. M. Portier, K. Robinson, L. Edmiston, and M. L. Tamplin, 1999. Discriminant Analysis of Ribotype Profiles of *Escherichia coli* for Differentiating Human and Nonhuman Sources of Fecal Pollution. *Journal of Applied and Environmental Microbiology* 65 (7): 3142–3147.
- Scott, T. M., T. M. Jenkins, J. Lukasik, and J.B. Rose, 2005. Potential use of a host associated molecular marker in *Enterococcus faecium* as an index of human fecal pollution. *Environment Science Technology* 39 (1): 283–7.
- Scott, T. M., S. Parveen, D.E. Chestnut, D.A. Graves, M.D. Sobsey and G.I. Scott, 2003. Geographical Variation in Ribotype Profiles of *Escherichia coli* Isolates from Humans, Swine, Poultry, Beef, and Dairy Cattle in Florida. *Journal of Applied and Environmental Microbiology* 69 (2): 1089–1092.
- Scott, T. M., J. B. Rose, T.M. Jenkins, S. R. Farrah and J. Lukasik, 2002. Microbial Source Tracking: Current Methodology and Future Directions. *Journal of Applied and Environmental Microbiology* 68 (12): 5796–5803.
- Simpson, J. M., J. W. Santo Domingo and D. J. Reasoner, 2002. Microbial source tracking: state of the science. *Environmental Science & Technology* 36 (24): 5279–88.
- Stoeckel, D. M., M. V. Mathes, K. E. Hyer, C. Hagedorn, H. Kator, J. Lukasik, T. L. O'Brien, T. W. Fenger, M. Samadpour, K. M. Strickler and B. A. Wiggins, 2004. Comparison of seven protocols to identify fecal contamination sources using *Escherichia coli*. *Environmental Science & Technology* 38 (22): 6109–6117.

- USEPA. 2005. *Method 1603: Escherichia coli (E. coli) in water by membrane filtration using modified membrane-thermotolerant Escherichia coli agar (Modified mTEC)*. Washington, DC, Office of Research and Development, Government Printing Office.
- . *Microbial Source Tracking Guide Document*. Cincinnati, OH, Office of Research and Development.
- Verduin, C. M., M. Kools-Sijmons, J. van der Plas, J. Vlooswijk, M. Tromp, H. van Dijk, J. Banks, H. Verbrugh, and A. van Belkum. 2000. Complement-resistant *Moraxella catarrhalis* forms a genetically distinct lineage within the species. *FEMS Microbiol Lett* 184:1-8.
- Versalovic, J., M. Schneider, F. J. de Brulin, and J. R. Lupski., 1994. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods in Molecular and Cellular Biology* 5: 25–40.

Research and Development Needs

- Benham, B. 2006. Modeling bacteria fate and transport in watersheds to support TMDLs. *Transcript of the ASABE* 49 (4): 987–1002.
- EPA (U.S. Environmental Protection Agency). 1986. Quality Criteria for Water 1986. EPA 440/5-86-001.
- Morgan, M.G. and M. Henrion. 1990. *Uncertainty – a guide to dealing with uncertainty in quantitative risk and policy analysis*. Cambridge University Press, New York, N.Y.
- Reed, Stowe & Yanke, LLC (2001). *Study to Determine the Magnitude of, and Reasons for, Chronically Malfunctioning On-Site Sewage Facility Systems in Texas*. Funded by: Texas On-Site Wastewater Treatment Research Council.
- Rifai, K. et al. 2005. Total maximum daily loads for fecal pathogens in Buffalo Bayou and White Oak Bayou. Prepared for: Texas Commission on Environmental Quality, Report by University of Houston, PBS&J, Texas A&M–Corpus Christi.
- TIAER (Texas Institute for Applied Environmental Research), Institute for Environmental Health, and Parsons. 2005. *Monitoring Report for Bacterial Source*

Tracking Upper Oyster Creek (Segment 1245) Bacteria TMDL. Prepared for:
Texas Commission on Environmental Quality.

Appendix 1: Bacteria TMDL Task Force Members and Expert Advisors

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Appendix 2: Models Used in Bacteria TMDLs as Described in EPA Publications

HSPF: Hydrological Simulation Program—Fortran

HSPF is a comprehensive watershed-scale model developed by EPA. The model uses continuous simulation of water balance and pollutant buildup and washoff processes to generate time series of runoff flow rates, as well as pollutant concentration at any given point in the watershed. Runoff from both urban and rural areas can be simulated using HSPF; however, simulation of CSOs is not possible. Because of the comprehensive nature of the model, data requirements for HSPF are extensive and using this model requires highly trained personnel (EPA 2002b).

SWMM: Storm Water Management Model

SWMM is a comprehensive watershed-scale model developed by EPA. It can be used to model several types of pollutants on either a continuous or storm event basis. Simulation of mixed land uses is possible using SWMM, but the model's capabilities are limited for rural areas. SWMM can simulate loadings from CSOs. The model requires both intensive data input and a special effort for validation and calibration. The output of the model is time series of flow, storage and contaminant concentrations at any point in the watershed (EPA 2002b).

STORM: Storage, Treatment, Overflow, Runoff Model

STORM is a watershed-loading model developed by the U.S. Army Corps of Engineers for continuous simulation of runoff quantity and quality. The model was primarily designed for modeling storm water runoff from urban areas, but it also can simulate combined sewer systems. It requires relatively moderate to high calibration and input data. The simulation output is hourly hydrographs and pollutographs (EPA 2002b).

CE-QUAL-RIV1: Hydrodynamic and Water Quality Model for Streams

CE-QUAL-RIV1 is a dynamic, one-dimensional model for rivers and estuaries consisting of two codes — one for hydraulic routing and another for dynamic water quality simulation. CE-QUAL-RIV1 allows simulation of unsteady flow of branched river systems. The input data requirements include the river geometry, boundary conditions, initial in-stream and inflow boundary water quality concentrations and meteorological data. The model predicts time-varying concentrations of water quality constituents (EPA 2002b).

Predicting the response of lakes and estuaries to pathogen loading requires an understanding of the hydrodynamic processes. Shallow lakes can be simulated as a simplified, completely mixed system with an inflow stream and an outflow stream. However, simulating deep lakes or estuaries with multiple inflows and outflows that are affected by tidal cycles is not a simple task. Pathogen concentration prediction is dominated by the processes of advection and dispersion, and these processes are affected by the tidal flow. The size of the lake or the estuary, the net freshwater flow, and wind conditions are some of the factors that determine the applicability of the models. The lake and estuary models are briefly described below (EPA 2002b).

WASP5: Water Quality Analysis Simulation Program

WASP5 is a general-purpose modeling system for assessing the fate and transport of pollutants in surface water. The model can be applied in one, two or three dimensions and can be linked to other hydrodynamic models. WASP5 simulates the time-varying processes of advection and dispersion while considering point and nonpoint source loadings and boundary exchange. The water body to be simulated is divided into a series of completely mixed segments, and the loads, boundary concentrations, and initial concentrations must be specified for each state variable (EPA 2002b).

CE-QUAL-ICM: A Three-Dimensional Time-Variable Integrated-Compartment Eutrophication Model

CE-QUAL-ICM is a dynamic water quality model that can be applied to most water bodies in one, two or three dimensions. The model can be coupled with three-dimensional hydrodynamic and benthic-sediment model components.

CE-QUAL-ICM predicts time-varying concentrations of water quality constituents. The input requirements for the model include 140 parameters to specify the kinetic interactions, initial and boundary conditions, and geometric data to define the water body to be simulated. Model use might require significant expertise in aquatic biology and chemistry (EPA 2002b).

EFDC: Environmental Fluid Dynamics Computer Code

EFDC is a general three-dimensional hydrodynamic model developed by Hamrick (1992). EFDC is applicable to rivers, lakes, reservoirs, estuaries, wetlands and coastal regions where complex water circulation, mixing and transport conditions are present. EFDC must be linked to a water quality model to predict the receiving water quality conditions. HEM-3D is a three-dimensional hydrodynamic eutrophication model that was developed by integrating EFDC with a water quality model. Considerable technical expertise in hydrodynamics and eutrophication processes is required to use the EFDC model (EPA 2002b).

CE-QUAL-W2: A Two-Dimensional, Laterally Averaged Hydrodynamic and Water Quality Model

CE-QUAL-W2 is a hydrodynamic water quality model that can be applied to most water bodies in one dimension or laterally averaged in two dimensions. The model is suited for simulating long, narrow water bodies like reservoirs and long estuaries, where stratification might occur. The model application is flexible because the constituents are arranged in four levels of complexity. Also, the water quality and hydrodynamic routines

are directly coupled, allowing for more frequent updating of the water quality routines. This feature can reduce the computational burden for complex systems. The input requirements for CE-QUAL-W2 include geometric data to define the water body, specific initial boundary conditions and specification of approximately 60 coefficients for the simulation of water quality (EPA 2002b).

QUAL2E: The Enhanced Stream Water Quality Model

QUAL2E is a steady-state receiving water model. The basic equation used in QUAL2E is the one-dimensional advective-dispersive mass transport equation. Although the model assumes a steady-state flow, it allows simulation of diurnal variations in meteorological inputs. The input requirements of QUAL2E include the stream reach physical representation and the chemical and biological properties for each reach (EPA 2002b).

TPM: Tidal Prism Model

TPM is a steady-state receiving water quality model applicable only to small coastal basins. In such locations, the tidal cycles dominate the mixing and transport of pollutants. The model assumes that the tide rises and falls simultaneously throughout the water body and that the system is in hydrodynamic equilibrium. Two types of input data are required to run TPM. The geometric data that define the system being simulated are the returning ratio, initial concentration and boundary conditions. The physical data required are the water temperature, reaction rate, point and nonpoint sources and initial boundary conditions for water quality parameters modeled (EPA 2002b).

BASINS: Better Assessment Science Integrating Point and Nonpoint Sources

BASINS system Version 2.0, with the Nonpoint Source Model (NPSM), can be used to predict the significance of fecal coliform sources and fecal coliform levels watersheds. BASINS is a multipurpose environmental analysis system for use in performing watershed and water quality-based studies. A geographic information system (GIS)

provides the integrating framework for BASINS and allows for the display and analysis of a wide variety of landscape information (e.g., land uses, monitoring stations, point source discharges). The NPSM model within BASINS simulates nonpoint source runoff from selected watersheds, as well as the transport and flow of the pollutants through stream reaches. Through calibration of model parameters and representation of watershed sources, the transport and delivery of bacteria to watershed streams and the resulting in-stream response and concentrations were simulated (EPA 2002a).

*Models Used in Bacteria Source Tracking as
Described in EPA Publications References*

EPA (Environmental Protection Agency). 2002a. *Protocols for Developing Pathogen TMDLs*. EPA 841-R-00-002.

EPA (Environmental Protection Agency). 2002b. *National Beach Guidance and Required Performance Criteria for Grants*. June 2002.

Appendix 3: EPA Bacteria TMDL Guidelines

This section provides an overview of several EPA guidance documents related to the use of models and BST to develop bacteria TMDLs. Components of a TMDL include (1) Problem Statement, (2) Numeric Targets, (3) Source Assessment, (4) Linkage Analysis, (5) Allocations and (6) Monitoring/Evaluation Plan (for phased TMDLs). Because BST and modeling are primarily used to assist with source assessment, linkage analysis and allocations, this chapter will focus primarily on these components of the TMDL (EPA 2002a).

Overall, EPA allows a great deal of flexibility in bacteria TMDL development as long as the method selected adequately identifies the load reductions or other actions needed to restore the designated uses of the water body in question. There are trade-offs associated with using either simple or detailed approaches. These trade-offs, along with site-specific factors, should always be taken into account and an appropriate balance struck between cost and time issues and the benefits of additional analyses (EPA 2002a).

Source Assessment

Source Assessment involves characterizing the type, magnitude and location of pollutant sources of fecal indicator loading. Source assessments also consider the conditions under which a particular source may have the most influence. For example, nonpoint sources typically predominate during high flow events while point sources predominate under low flows. For this reason, data collection efforts to support source assessment must focus on specific conditions. Monitoring data should be used to estimate the magnitude of loads from the major sources when available. In the absence of such data, a combination of literature values, best professional judgment, BST and empirical techniques/models is necessary. In general, EPA (2002a) recommends the use of the simplest approach that provides meaningful predictions.

EPA (2002a) encourages starting with the assumption that models are not required. If it is determined that models are required, then the following factors should be considered:

- Availability of data and/or funds to support data collection
- Availability of staff
- Familiarity of staff with potential models or other analytical tools
- Level of accuracy required

Depending on the complexity of the sources in the watershed, load estimation might be as simple as conducting a literature search or as complex as using a combination of long-term monitoring and modeling. Analysis of pollutant waste loads from point sources are generally recommended to be based on the effluent monitoring required for the National Pollutant Discharge Elimination System (NPDES) permit or based upon the permit's effluent limitations (EPA 2002a). However, many NPDES (and TPDES permits in Texas) require monitoring of chlorine residual to verify the effectiveness of chlorination but do not include either report or effluent limits for bacteria.

Nonpoint source loads are typically separated into urban and rural categories since runoff processes differ between these environments. Pathogen loads in urban stormwater can be estimated using a variety of techniques, ranging in complexity from simple loading rate assumptions and constant concentration estimates, to statistical estimates, to highly complex computer simulation (EPA 2002a). Examples of techniques for estimating pathogen loads in urban storm water include the FecaLOAD model, constant concentration estimates, statistical or regression approaches and stormwater models, such as SWMM and HSPF.

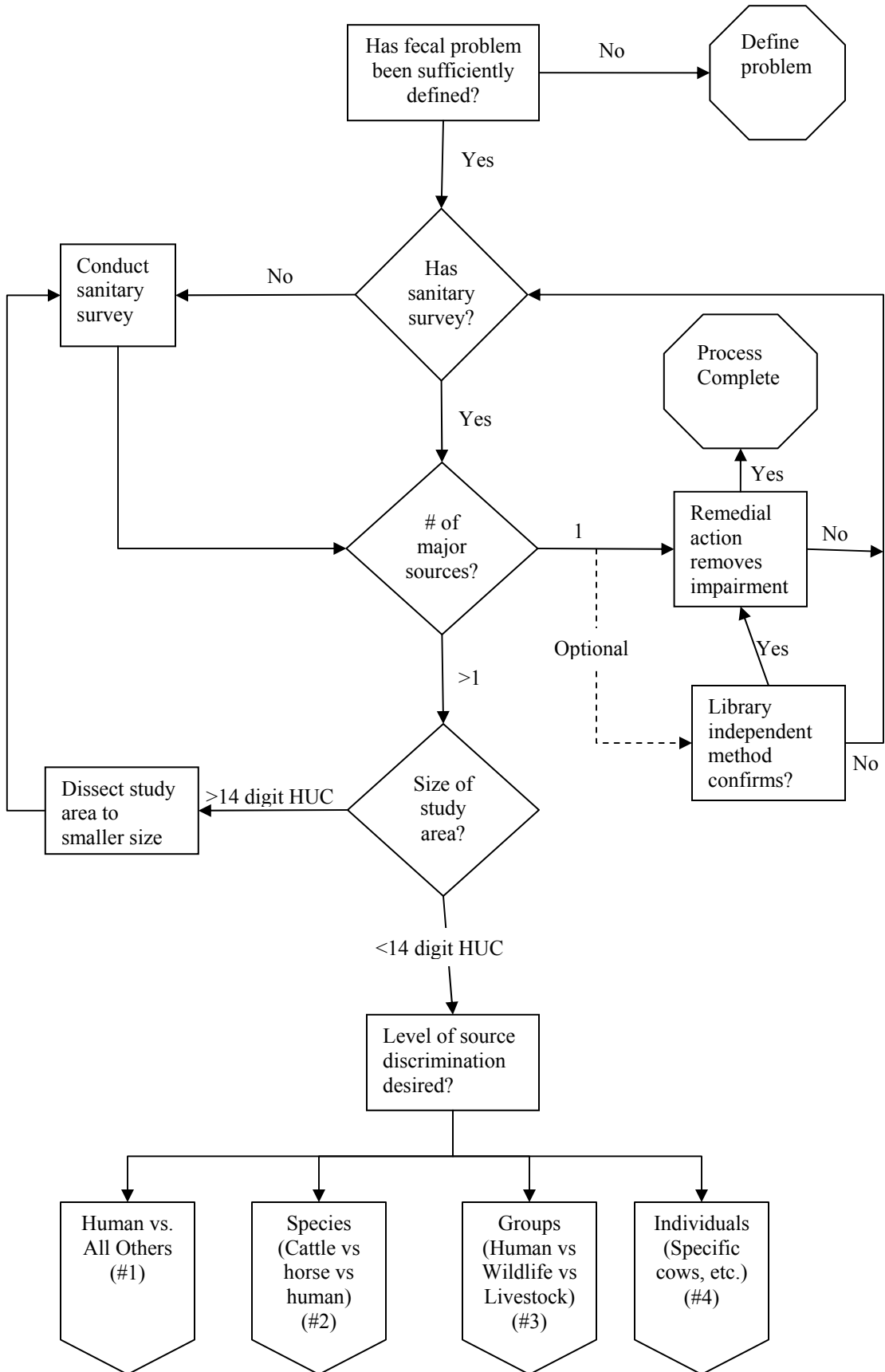
Rural nonpoint source loads may also be estimated using a variety of techniques, ranging from simple loading function estimates to use of complex simulation models. Techniques, such as the loading function approach, site-specific analysis, estimates of time series of loading and detailed models, such as AGNPS (Agricultural Nonpoint Source), may be used (EPA 2002a). Models are discussed in greater depth in the Linkage Analysis section.

DNA fingerprinting may also provide information for Source Assessments (EPA 2002a). There are many BST methods available and more are under development. Overall, molecular BST methods may offer the most precise identification of specific types of sources, but are limited by high costs and detailed, time-consuming procedures (EPA 2002c). Costs vary however, based on:

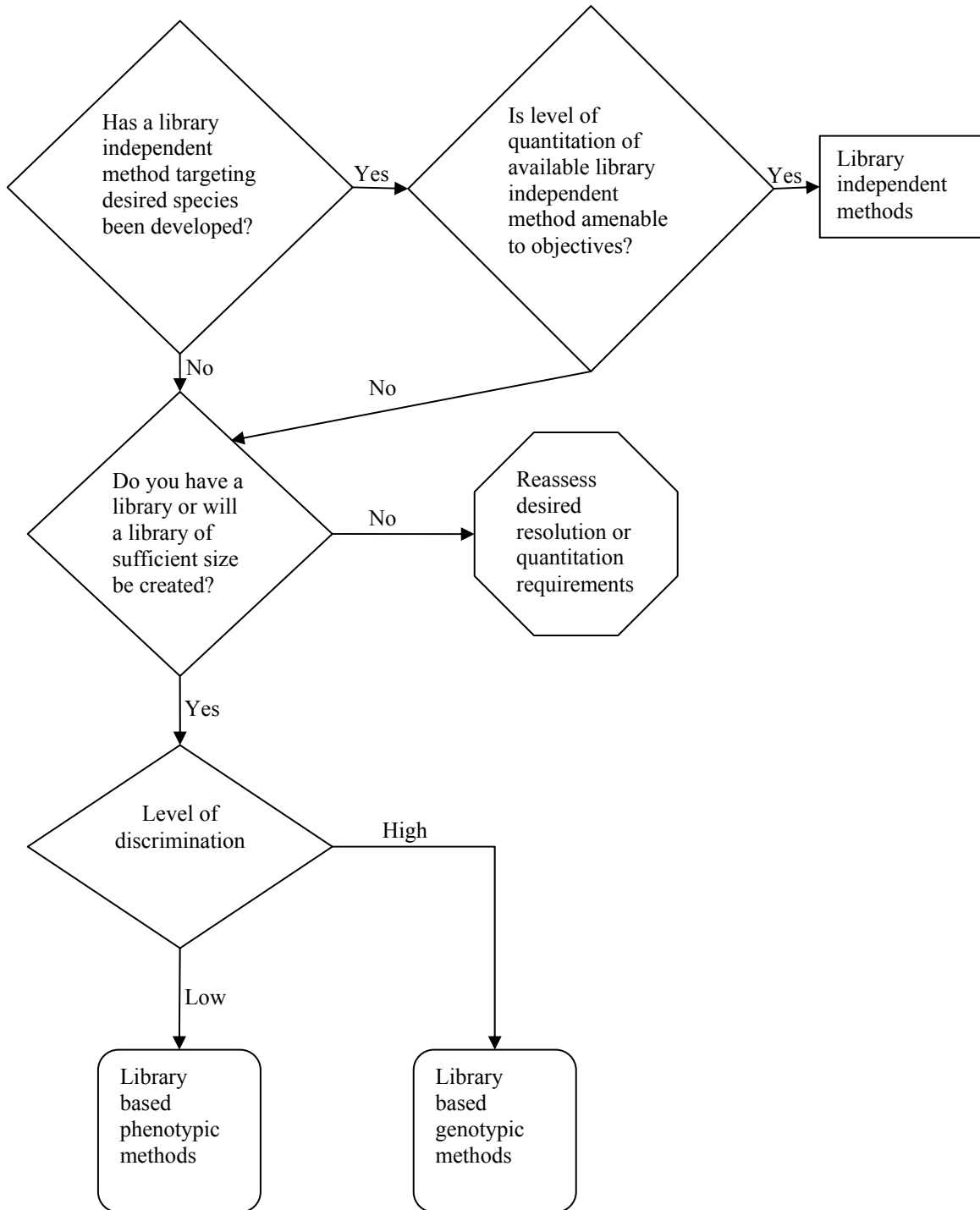
- Analytical method used
- Size of the database needed
- Number of environmental isolates analyzed
- Level of accuracy needed
- Number of subwatersheds and geographical size of the area under study

Comparison studies have shown that no single method is clearly superior to the others. Thus, the decision on which method to use depends on the unique set of circumstances associated with the area in question, the results of sanitary surveys, and budgetary and time constraints. A decision tree was created by EPA to assist in deciding whether BST methods are necessary to determine the sources of fecal pollution in a particular watershed and, if so, which group of methods might be most appropriate (EPA 2005). The decision tree included on the next four pages consists of five steps:

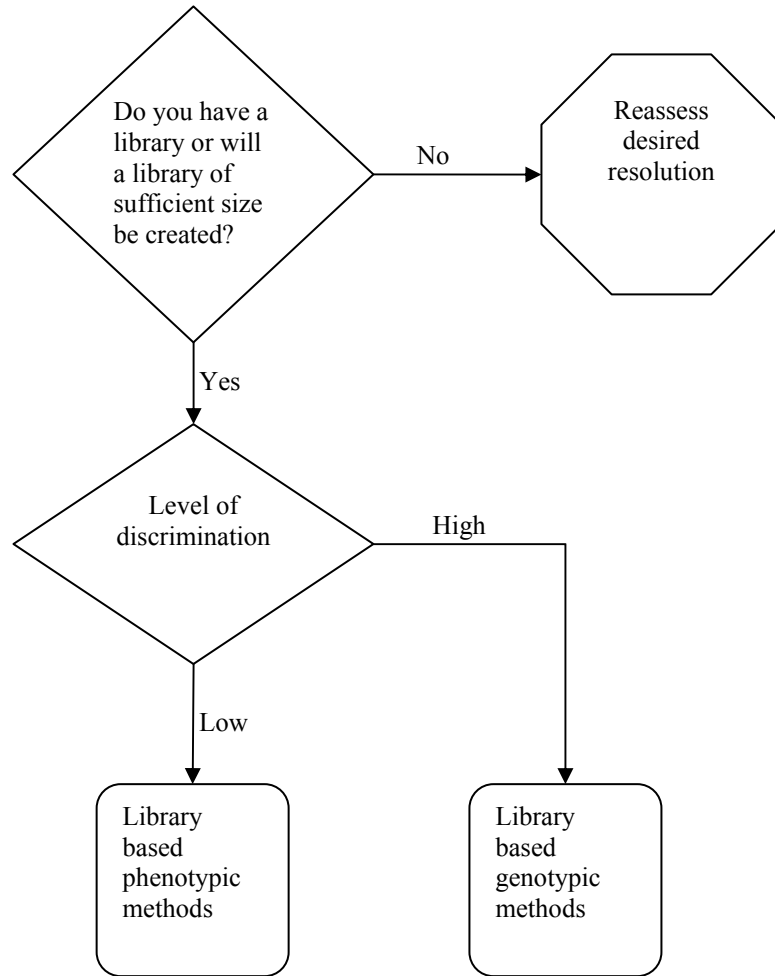
- Adequately defining the problem
- Conducting a sanitary survey
- Determining the potential number of major sources
- Ensuring the watershed/study area is of manageable size
- Determining the desired level of discrimination



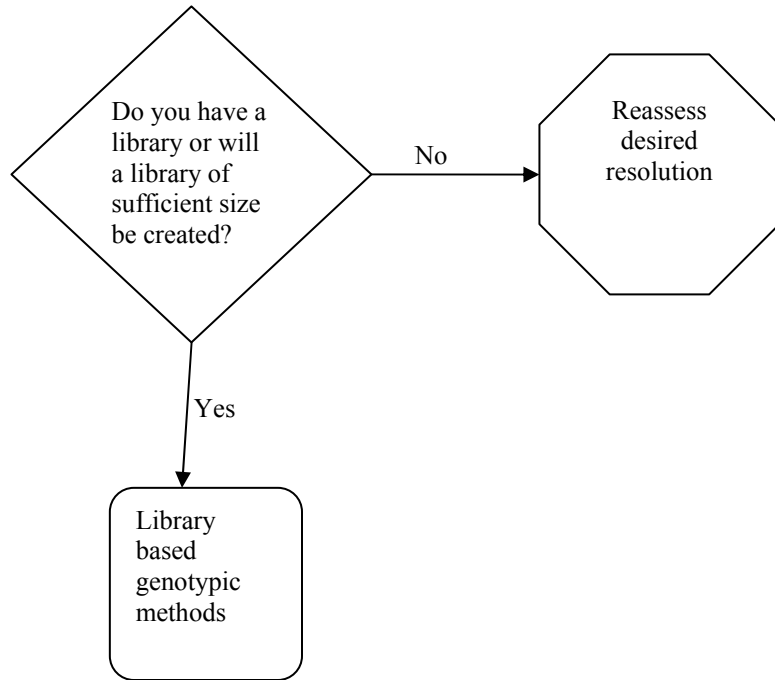
Human only #1 and Species Specificity #2 (EPA 2005)



Define by Groups #3 (EPA 2005)



Define to specific sources by type and location #4 (EPA 2005)



Linkage Analysis and Allocations

EPA (2003) has identified three analytical methods appropriate for calculating loads and linking water quality targets and sources:

- Empirical Approaches – When sufficient observations are available, existing data can be used to determine linkage between sources and water quality targets (e.g., regression approach).
- Simple Approaches – When permitted sources are sole source of bacteria, simple dilution calculations and/or compliance monitoring are adequate.
- Detailed Modeling – When sources of bacteria are complex, a water quality modeling approach (e.g. dynamic or steady-state modeling) is typically used. When detailed modeling is used, different types of models are required for accurate simulation for rivers and streams as compared to lakes and estuaries because the response is specific to the water body.

Steady-state modeling uses constant inputs for effluent flow and concentration, receiving water flow and meteorological conditions and is generally used where insufficient data exists for developing a dynamic model. Steady-state modeling provides very conservative results when applied to wet weather sources. If a state elects to use a steady state model, EPA recommends a dual design approach (e.g., load duration curve) where the loadings for intermittent or episodic sources are calculated using a flow duration approach and the loadings for continuous sources are calculated based on a low flow statistic (EPA 2003).

Dynamic modeling considers time-dependent variation of inputs and applies to the entire record of flows and loadings. In certain situations, EPA (2003) recommends the use of dynamic modeling to calculate loads. The three dynamic modeling techniques recommended are:

- Continuous simulation
- Monte Carlo simulation
- Log-normal probability modeling

Specific models recommended by EPA can be divided into two categories — watershed loading models and pathogen concentration prediction models. Loading models provide estimates of either the total pollutant loading or a time series loadings. The key watershed loading models suited for pathogens include HSPF, SWMM and STORM (EPA 2002b). These are briefly described in Appendix 3 and the previous section about models.

Prediction of pathogen concentration in rivers and streams is dominated by advection and dispersion processes and bacteria die-off. One-, two- and three-dimensional models have been developed to describe these processes. Water body type and data availability are the two most important factors that determine model applicability. For most small and shallow rivers, one-dimensional models are sufficient. However, for large and deep rivers and streams, two- or three-dimensional models that integrate the hydrodynamics of the system should be used (EPA 2002b). The river and stream models are briefly described in Appendix 3 and include the following:

- HSPF: Hydrological Simulation Program–FORTRAN
- CE-QUAL-RIV1: Hydrodynamic and Water Quality Model for Streams
- WASP5: Water Quality Analysis Simulation Program
- CE-QUAL-ICM: A Three-Dimensional Time-Variable Integrated-Compartment Eutrophication Model
- EFDC: Environmental Fluid Dynamics Computer Code
- CE-QUAL-W2: A Two-Dimensional, Laterally Averaged Hydrodynamic and Water Quality Model
- QUAL2E: The Enhanced Stream Water Quality Model
- TPM: Tidal Prism Model

In closing, EPA (2002a) recommends that when developing linkages between water quality targets and sources, states should:

- Use all available and relevant data (specifically monitoring data for associating water body responses with flow and loading conditions).
- Perform a scoping analysis using empirical analysis and/or steady-state modeling to review and analyze existing data prior to any complex modeling. The scoping

- analysis should include identifying targets, quantifying sources, locating critical points, identifying critical conditions, and evaluating the need for more complex analysis.
- Use the simplest technique that adequately addresses all relevant factors when selecting a technique to establish a relationship between sources and water quality response.

EPA Bacteria TMDL Guidelines References

- EPA (Environmental Protection Agency). 2002a. Protocols for Developing Pathogen TMDLs. EPA 841-R-00-002.
- EPA (Environmental Protection Agency). 2002b. National Beach Guidance and Required Performance Criteria for Grants. June 2002.
- EPA (Environmental Protection Agency). 2002c. Wastewater Technology Fact Sheet – Bacterial Source Tracking. EPA 832-F-02-010.
- EPA (Environmental Protection Agency). 2003. Implementation Guidance for Ambient Water Quality Criteria for Bacteria. DRAFT Document.
- EPA (Environmental Protection Agency). 2005. Microbial Source Tracking Guide Document. EPA/600-R-05-064.

Appendix 4: State Approaches to Bacteria TMDLs

This section provides a brief overview of approaches other states are using to develop TMDLs for bacteria and related issues. EPA has allowed much flexibility in developing pathogen TMDLs, as outlined in the agency’s 2002 publication “Protocols for Developing Pathogen TMDLs” and 2003 DRAFT publication “Implementation Guidance for Ambient Water Quality Criteria for Bacteria.” As a result, states have taken a variety of approaches to developing bacteria TMDLs. A large number of bacteria TMDLs have been approved by EPA since 1996 (Table 1). Most of these have been approved in states in which EPA is under court order or agreed consent decree to establish TMDLs.

Table 1. EPA Approved Pathogen TMDLs in Regions 3, 4, 6 and 7.

EPA Region	State	EPA Approved TMDLs					Court Order	Litigation Dismissed	
		FC	Pathogen	Bacteria	Fecal	TC			<i>E. coli</i>
3	Delaware			25				Yes	No
3	DC	22	9					Yes	No
3	Maryland	57	1					No	Yes
3	Pennsylvania	1	100					Yes	No
3	Virginia	186	94		1			Yes	No
3	W. Virginia	196						Yes	No
4	Georgia	534						Yes	No
4	Florida	21			48	45		Yes	No
4	Kentucky	23						No	No
4	Alabama	26						Yes	No
4	Mississippi	172						Yes	No
4	N. Carolina	38			1			No	Yes
4	Tennessee	62					191	Yes	No
4	S. Carolina	270						No	No
6	Arkansas	2						Yes	No
6	Louisiana	27						Yes	No
6	New Mexico	20						Yes	No
6	Oklahoma	0						No	Yes
6	Texas	0						No	No
7	Kansas	471						Yes	No
7	Nebraska	11					20	No	No
7	Missouri	3						Yes	No
7	Iowa		1					Yes	No

TMDLs that are prepared hurriedly to meet court-mandated deadlines may not be sufficiently specific or achieve adequate stakeholder acceptance to support development of comprehensive I-plans, particularly where multiple sources and types of sources are involved. Accordingly, the approval of a large number of bacteria TMDLs does not necessarily represent a significant improvement in water quality.

A brief overview of the TMDL methods used by states in EPA Regions 4 and 6, along with a few examples from select states in other regions is presented here. No examples were found of any state that has successfully improved water quality by implementing a bacterial TMDL. EPA Region 4 was initially targeted by this survey because environmental conditions (e.g. climate, rainfall, temperature) are most like the eastern portion of Texas where a majority of the 303(d) listings occur. This was later expanded to other regions. Much of the information for this summary was acquired from EPA's TMDL Web site at <http://www.epa.gov/owow/tmdl/> and the Web sites of the individual states referenced.

EPA Region 6

In EPA Region 6, a total of 49 fecal coliform TMDLs are reported to have been approved since January 1, 1996. The load duration curve model was the primary model used to develop the approved TMDLs to date. The only exceptions are the two TMDLs developed in Arkansas, which used empirical methods.

Although no bacteria TMDLs are currently approved in Texas, a number of bacteria TMDLs and watershed protection plans are under way. Texas has taken the approach of spending more time in developing TMDLs that are designed to achieve stakeholder buy-in and result in meaningful implementation of water quality improvements. Texas has primarily used the HSPF and load duration curve models for a majority of the TMDLs under development to date (Table 2).

Table 2. Bacteria TMDLs Under Development in Texas.

Project	HSPF	Load Duration	Other Models	Bacteria Source Tracking Method
Upper San Antonio River	①			ERIC-PCR and RiboPrinting
Leon River	①			ERIC-PCR and RiboPrinting
Peach Creek	①			ERIC-PCR and RiboPrinting
Adams and Cow Bayous	①		RMA2/ACE	No BST
White Oak and Buffalo Bayous	①			ARA and CSU
Lower San Antonio River		①		ERIC-PCR and RiboPrinting
Atascosa River		①		No BST
Elm and Sandies Creeks		①		No BST
Upper Trinity River		①		Ribotyping (Institute for Environmental Health, Inc., Seattle, WA)
Guadalupe River above Canyon Lake		①		Ribotyping (Source Molecular Corporation, Inc., Miami, FL)
Upper Oyster Creek		①		Ribotyping (Institute for Environmental Health, Inc., Seattle, WA)
Copano Bay and Mission and Aransas Rivers			ArcHydro\Monte Carlo Simulation	ARP and PFGE
Oso Bay and Oso Creek			ArcHydro\SWAT	No BST
Gilleland Creek		①		No BST
Clear Creek		①		
Metropolitan Houston (Brays, Greens, Halls and other Bayous)	①			ARA and CSU
WPP – Lake Granbury				
WPP – Buck Creek			TBD	<i>E. faecium</i> , ERIC-PCR, RP
WPP – Bastrop Bayou				
WPP – Plum Creek		①	SELECT, SPARROW, SWAT	No BST

Unlike other states in Region 6, Texas has supplemented the models utilizing BST. The primary BST methods that have been used include:

- ERIC-PCR conducted at TAES – El Paso
- RiboPrinting conducted at TAES – El Paso
- Antibiotic Resistance Analysis (ARA) conducted at the University of Houston
- Carbon Source Utilization (CSU) conducted at the University of Houston
- Ribotyping conducted at Institute for Environmental Health, Inc., Seattle, WA
- Ribotyping conducted at Source Molecular Corporation, Inc in Miami, Florida
- Antibiotic Resistance Profiling (ARP) conducted at Texas A&M University – Corpus Christi
- Pulse Field Gel Electrophoresis (PFGE) conducted at Texas A&M University- Corpus Christi and Texas A&M University – College Station

EPA Region 4

A total of 1,146 fecal coliform, 191 *E. coli*, 49 fecal and 45 total coliform TMDLs are reported to have been approved in EPA Region 4 since January 1, 1996. As in Region 3, litigation has driven much of the TMDL development. Only Kentucky and South Carolina have escaped litigation.

Georgia has led the way in TMDL approval. EPA Region 4 completed a number of these (e.g., Chickasawatchee Creek) using the BASINS model (HSPF) for both source analysis and for linking sources to indicators. LDCs and the equivalent site approach (as described below) have also been used extensively in Georgia. Georgia has not explicitly published a guidance document describing its methodology for developing bacteria TMDLs; however, their “Analytical Approach” is outlined in TMDLs, such as the “TMDL Evaluation for the Chattahoochee River Basin (Fecal coliform)” which can be found at:

www.gadnr.org/epd/Files_PDF/techguide/wpb/TMDL/Chattahoochee/chatt_fc_tmdl.pdf.

For those segments in which sufficient water quality data was available to calculate at least one 30-day geometric mean that was above the regulatory standard, the load duration curve approach was used. The method involves comparing the “current” critical

load to summer and winter seasonal TMDL curves. For listed segments that do not have sufficient data to calculate the 30-day geometric mean fecal coliform concentrations, Georgia used an equivalent site approach to estimate the “current” and TMDL loads. This approach involves calculating loads based on the relationship of the stream segments that lack sufficient data to equivalent site(s) that have data. This method provides estimates that can be refined as additional data are collected. The WLA loads are calculated based on the permitted or design flows and average monthly permitted fecal coliform concentrations or a fecal coliform concentration of 200 counts/ 100 mL as a 30-day geometric mean. The LA is calculated as the remaining portion of the TMDL load available after allocating the WLA and the MOS.

The Florida Department of Environmental Protection has developed and published their methodology in a document titled “TMDL Protocol” in June 2006. Source identification in Florida begins with evaluating existing data and developing GIS layers to map probable sources. If the sources cannot be determined with existing data and field inspections, then simple and inexpensive chemical and biological tracers are used for identifying sources during dry and wet weather conditions. If the simple methods do not provide the level of source identification needed, then more complex and expensive BST methods are used. Modeling tools are then used to evaluate flows and loads, define allowable loads, and evaluate BMPs. No watershed models are specifically identified for developing bacteria TMDLs; however, a number of receiving water models are identified (e.g. AESOP, CE-QUAL-R1, CE-QUAL-RIV1, EFDC, EPDRiv1, HEC-5Q, HSPF-RCHRES, SWMM, TWQM, and WASP). A document such as this would be very beneficial in Texas: (http://www.dep.state.fl.us/water/tmdl/docs/TMDL_Protocol.pdf).

Kentucky is one of only two states in EPA Region 4 not under litigation or consent decree to complete TMDLs. Like Georgia, Kentucky has not published specific guidance on development of TMDLs; however, a review of its approved TMDLs shows that, mass balance and load duration curves have been used to develop a large number of their bacteria TMDLs. Their approach to using LDCs is typically outlined under the “Data Analysis” section of their TMDLs, such as the Brush Creek and Crooked Creek TMDL:

http://www.water.ky.gov/NR/rdonlyres/B2EE6B7D-A658-4FA6-A0BA-67C10EB77369/0/TMDLBrush_Crooked.pdf

In this TMDL, where a LDC was used, the allowable loading curves was divided into five zones representing high flows (0-10%), moist conditions (10-40%), median or mid-range flows (40-60%), dry conditions (60-90%), and low flows (90-100%). If more than five sample points plot above the allowable load line, a trendline can be drawn through the data violations for predicting the load at other duration intervals and the correlation factor (r^2) determined. If insufficient numbers of samples are available or r^2 is not high, then no line is drawn. Existing loads are expressed as a range based on the zones where the violations occur (e.g. median flow, moist conditions, etc.). When multiple violations occur within a zone, the existing load is represented as 90th percentile value. The percent reduction required to meet the criterion is calculated based on the 90th percentile of coliform concentrations collected during the recreation season that violate the fecal coliform target and calculated as follows:

$$\text{Percent Reduction (\%)} = (\text{existing concentration} - \text{target}) / \text{existing concentration} \times 100$$

Alabama, like most other states, has not published a protocol for developing bacteria TMDLs. A review of approved TMDLs in Alabama on EPA's TMDL website reveals that a variety of approaches have been used including:

- Empirical models
- Loading Simulation Program in C++ (LSPC), Environmental Fluid Dynamics Code (EFDC), Water Quality Analysis Simulation Program (WASP)
- BASINS Watershed Characterization System (WCS) and Nonpoint Source Model (a modified version of HSPF)
- Mass balance
- Load duration curves (LDCs)

A review of approved TMDLs in Mississippi reveals that state has primarily utilized empirical linear regression models, BASINS NPSM, and mass balance. The BASINS NPS Model (NPSM), a modified version of HSPF, was used in the Pearl River TMDL, ([http://www.deq.state.ms.us/MDEQ.nsf/pdf/TWB_PearlRivMarionDc00/\\$File/PearlRBPearlRiv\(Marion\)Dc00.pdf?OpenElement](http://www.deq.state.ms.us/MDEQ.nsf/pdf/TWB_PearlRivMarionDc00/$File/PearlRBPearlRiv(Marion)Dc00.pdf?OpenElement)) for estimating current conditions. The key reason for using BASINS as the modeling

framework was its ability to integrate both point and nonpoint sources in the simulation, as well as its ability to assess instream water quality response.

North Carolina has used a number of models including BASINS HSPF, LDCs and Watershed Analysis Risk Framework (WARMF). Load duration curves are based on the cumulative frequency distribution of flow conditions in the watershed. Allowable loads are average loads over the recurrence interval between the 95th and 10th percentile flow record (excludes extreme drought (>95th percentile) and floods (<10th percentile). Percent reductions are expressed as the average value between existing loads (typically calculated using an equation to fit a curve through actual water quality violations) and the allowable load at each percent flow exceeded.

Tennessee utilizes a variety of models including the BASINS Watershed Characterization System and NPS Model (NPSM); Loading Simulation Program in C++ (LSPC) / Hydrologic Simulation Program –FORTRAN (HSPF) / Watershed Characterization System (WCS) model combination, LDCs and mass balance.

South Carolina has primarily used LDCs. In limited circumstances, they have also used empirical methods, mass balance or the BASINS/HSPF/WSC combo. A “TMDL Talk” on TMDLS.NET titled *Watershed Characterization & Bacteria TMDL’s: South Carolina’s Approach* may indicate greater use of BASINS/HSPF/WSC in coming years. The use of the Watershed Characterization System (WSC) ensures adequate consideration of the wide array of sources and is a key component of the technical approach toward building bacteria TMDLs and describing allocation options. In evaluating pollutant sources, loads are characterized using the best available information (e.g. monitoring data, GIS data layers, literature values and local knowledge). Pollutant sources are then linked to water quality targets using analytical approaches including WCS and the Nonpoint source Model (NPSM), a modified version of HSPF. Estimates of loading rates are generated by fecal coliform spreadsheet tools included with WCS. These loading rate estimates are then used by NPSM to simulate the resulting water quality response. Allocation for point sources considers discharge-monitoring

information. NPS allocations for significant categories are identified at key points in the watershed from the model analyses. This approach was used for the Rocky Creek TMDL and others.

Other States

Connecticut and Delaware use the Cumulative Frequency Distribution Function Method, developed by the Connecticut Department of Environmental Protection, to develop TMDLs. The reduction in bacteria density from current levels needed to achieve compliance with state water quality standards is quantified by calculating the difference between the cumulative relative frequency of the sample data set (a minimum of 21 sampling dates during the recreational season) and the criteria adopted to support recreational use. Adopted water quality criteria for *E. coli* are represented by a statistical distribution of the geometric mean 126 and log standard deviation 0.4 for purposes of the TMDL calculations. TMDLs developed using this approach are expressed as the average percentage reduction from current conditions required to achieve consistency with criteria. The procedure partitions the TMDL into wet and dry weather allocations by quantifying the contribution of ambient monitoring data collected during periods of high stormwater influence and minimal stormwater influence to the current condition.

In EPA Region 7, a total of 485 fecal coliform, 20 *E. coli* and 1 pathogen TMDLs are reported to have been approved since October 1, 1995. Development in Kansas, Missouri, and Iowa has been driven by court orders. Much like EPA Region 6, LDCs appear to be the method of choice for developing bacteria TMDLs. Bacteria TMDLs approved in Kansas, Missouri and Nebraska primarily used LDCs. Kansas has lead the way in the use of LDCs. Kansas TMDL Curve Methodology can be found at the following website: (<http://www.kdheks.gov/tmdl/basic.htm#data>).

Use of LDCs in Nebraska is described in the document entitled “Nebraska’s Approach for Developing TMDLs for Streams Using the Load Duration Curve Methodology.” Only one pathogen TMDL (*E. coli*) has been approved in Iowa. Iowa used the Soil and Water Assessment Tool (SWAT) model to estimate daily flow into Beeds Lake. The SWAT

flow estimates were then used to create a load duration curve. Use of EPA's bacterial indicator tool was used to identify the significance of bacteria sources in the watershed.

Indiana also uses LDCs and provides very good "Duration Curve Information" on their website (<http://www.in.gov/idem/programs/water/tmdl/documents.html>) and includes helpful tutorials, spreadsheets and publications on developing LDCs.

Nevada Division of Environmental Protection uses Load Duration Curve Methodology for Assessment and TMDL Development and has developed guidance on their website (<http://www.ndep.nv.gov/bwqp/loadcurv.pdf>) for developing LDCs.

New Jersey's policy is published in a fact sheet titled "Fecal Coliform TMDLs" (<http://www.nj.gov/dep/watershedmgt/DOCS/tmdlfactsheet2.pdf>). Nonpoint sources and stormwater point sources were identified as the primary contributors to all listed streams while Publicly Owned Treatment Works (POTWs) were considered insignificant and not allocated any loading reductions. The percent reduction was calculated empirically; however, when data was available, LDCs were used. Sources are identified using river assessments, visual surveys, stakeholder input and aerial photography.

New York used a statistical rollback method to estimate reductions needed and the Watershed Treatment Model (WTM) for characterizing the water bodies. The method for determining the geometric rollback factor follows:

$$F_{\text{rollback}} = (\text{Observed geometric} - \text{water quality standard}) / (\text{Observed geometric})$$

The same method is applied for the 90th percentile values and standards:

$$F_{\text{rollback}} = (\text{Observed 90}^{\text{th}} \text{ percentile} - \text{water quality standard}) / (\text{Observed 90}^{\text{th}} \text{ percentile})$$

The most restrictive of the two (i.e., the greatest percent reduction required) is chosen as the target reduction. More information on the WTM is included in section 6 of the "Final Report for Peconic Bay TMDL" (<http://www.dec.state.ny.us/website/dow/pecpart56.pdf>).

Virginia's approach is outlined through a series of Guidance Memos. "HSPF Model Calibration and Verification for Bacteria TMDLs" is outlined in Guidance Memo No. 03-

2012 (<http://www.deq.virginia.gov/waterguidance/pdf/032012.pdf>). Guidance Memo No. 03-2015 (<http://www.deq.virginia.gov/waterguidance/pdf/032015.pdf>) describes the “Method for Representing WLAs in Bacteria TMDLs”. These memos were released to achieve greater consistency in their TMDL development. Even though the state is under court order, it is similar to Texas in many respects. Virginia develops bacteria TMDLs primarily using either LDCs or the HSPF model (or a modified version – NPSM); however, in a number of TMDLs, BST has been utilized in conjunction with simplified modeling approaches. One such example is the Little Wicomico River Watershed TMDL and Coan River Watershed TMDL, where Virginia DEQ utilized its point source inventory, a shoreline survey, and antibiotic resistance analysis to determine the potential sources of bacteria and quantify source loadings from humans, livestock and non-domestic animals. In addition, a simplified modeling approach (Tidal Volumetric Model) was used. The BST data was used to determine the relative sources of fecal coliform violations and ambient water quality data used to determine the load reductions needed to attain the applicable criteria. The most recent 30 months of data coinciding with the end of the TMDL study were reviewed to determine the loading to the water body. The geometric mean loading is based on the most recent 30-month geometric mean of fecal coliform. The load is also quantified for the 90th percentile of the 30-month grouping.

The geometric mean load is determined by multiplying the geometric mean concentration based on the most recent 30-month period of record by the volume of the water. The acceptable load is then determined by multiplying the geometric mean criteria by the volume of the water. The load reductions needed for the attainment of the geometric mean are then determined by subtracting the acceptable load from the geometric mean load.

Example: (Geometric Mean Value MPN/100ml) x (volume) = Existing Load
 (Criteria Value 14 MPN/100ml) x (volume) = Allowable Load
 Existing Load – Allowable Load = Load Reduction

The 90th percentile load is determined by multiplying the 90th percentile concentration, based on the most appropriate 30-month period of record, by the volume of the water. The acceptable load is determined by multiplying the 90th percentile criteria by the volume of the water. The load reductions needed for the attainment of the 90th percentile criteria are determined by subtracting the acceptable load from the 90th percentile load. The more stringent reductions between the two methods (i.e. 90th percentile load or geometric mean load) are used for the TMDL. The more stringent method is combined with the results of the BST to allocate source contributions and establish load reduction targets among the various contributing sources.

The BST data determines the percent loading for each of the major source categories and is used to determine where load reductions are needed. Since one BST sample per month is collected for a period of one year for each TMDL, the percent loading per source is averaged over the 12-month period if there are no seasonal differences between sources. The percent loading by source is multiplied by the more stringent method (i.e. 90th percentile load or geometric mean load) to determine the load by source. The percent reduction needed to attain the water quality standard or criteria are allocated to each source category.

Washington primarily uses LDCs for calculating bacteria TMDLs. To identify nonpoint sources of bacteria, a yearlong (minimum) water quality study of possible source areas is conducted. Once the locations of the bacterial sources are narrowed down, the state works with local interests to identify sources of pollution. Two methods that can be used to identify bacteria sources: (1) pinpointing the location of the source and (2) identifying the types of sources contributing to the problem. One of the most economical methods pinpointing the locations of sources is to conduct intensive upstream-downstream water quality monitoring, including flow measurements, to identify specific stream reaches, land uses or tributaries that are a problem. Dye testing can also be used for pinpointing the locations of sources. BST can be used to determine the types of sources. Most BST techniques are quite costly; thus, it is important to pick the appropriate method and time to use BST. BST does not tell you how much each source contributes to bacterial

contamination, only the different kinds of sources. In addition, it is possible that not all source types will be identified or, with some techniques, that sources will be misidentified. Washington State Department of Ecology's Fact Sheet "Focus on Bacterial Source Tracking" provides an overview of their approach for identifying sources and using BST (<http://www.ecy.wa.gov/pubs/0310059.pdf>).

Other states using BST for TMDLs include New Hampshire and Maryland. Ribotyping has been used in New Hampshire to determine sources of bacteria for TMDLs. Maryland is using Antibiotic Resistance Analysis (ARA) BST methodology to determine the relative contribution of bacteria from various categories.

EPA Bacteria TMDL Guidelines References

EPA (Environmental Protection Agency). 2002. Protocols for Developing Pathogen TMDLs. EPA 841-R-00-002.

EPA (Environmental Protection Agency). 2003. Implementation Guidance for Ambient Water Quality Criteria for Bacteria. DRAFT Document.

EPA (Environmental Protection Agency). 2006. Total Maximum Daily Loads. <http://www.epa.gov/owow/tmdl/>

FDEP (Florida Department of Environmental Protection). 2006. TMDL Protocol. Task Assignment 003.03/05-003. Version 6.0.

TMDLs.NET. 2006. America's Clean Water Foundation and Association of State and Interstate Water Pollution Control Administrators.