DEPARTMENT OF THE AIR FORCE
HEADQUARTERS 72D AIR BASE WING (AFMC)
TINKER AIR FORCE BASE OKLAHOMA

24 Aug 2006

MEMORANDUM FOR DIANE SMITH
ENVIRONMENTAL PROTECTION SPECIALIST
WATER QUALITY PROTECTION DIVISION
ENVIRONMENTAL PROTECTION AGENCY REGION 6
1445 ROSS AVE
DALLAS, TEXAS 75202-2733

FROM: 72 ABW/JAV
7460 Arnold Street, SE Wing
Tinker AFB, OK 73145-9002

SUBJECT: Comments, Turkey Creek Total Maximum Daily Load

1. This memorandum constitutes Tinker Air Force Base's (Tinker AFB) initial comments on the Turkey Creek total maximum daily load (TMDL), including all of the tributaries of Turkey Creek. For the reasons set forth below, Tinker is opposed to the Turkey Creek TMDL, the use anywhere in Oklahoma of the load duration curve as the basis, in whole or in part, for setting bacteria reduction levels, and to the process and procedures EPA is using in setting the Turkey Creek TMDL.

2. Although the Turkey Creek TMDL does not directly impact Tinker, we are very concerned due to the method used to develop it and the process EPA is using to establish the Turkey Creek TMDL.

3. The Oklahoma Department of Environmental Quality (ODEQ) prepared a report titled "TMDL Development for Turkey Creek HUC 11 Watersheds, Draft Report" (the Turkey Creek Report). The Turkey Creek Report uses the load duration curve method ("LDC method") as the principle basis for establishing TMDL reduction calculations. Parsons and ACOG – the Area Council of Governments for central Oklahoma, used the **same LDC method** to prepare the TMDL for the North Canadian River and Cruthco Creek, which does directly impact Tinker.

4. We are opposed to the Turkey Creek TMDL for the same reasons stated in the comment letter from Robert M. Gill, Department of Defense Regional Environmental Coordinator, to John Craig, ODEQ. Attached for the record is a copy of that letter and certain enclosures.

5. We recommend that EPA and/or ODEQ at a minimum take the same actions as recommended in the comment letter.

6. The LDC method as used in Oklahoma is based on estimates. The Turkey Creek Report is almost entirely based on estimates. Only one station on the entire watershed has actual stream gauging records. The LDC method does not consider background bacteria, does not differentiate between human and animal bacteria, and does not identify specific sources or link them to actual levels of pathogens found in the Turkey Creek, the Canadian River, the North Canadian River, or any other water body currently being studied for pathogen impairment. In short, it is not based on sound science at all.

7. There are methods available: to involve stakeholders in the study of pathogen loading; to obtain information from stakeholders, other state agencies, and interested individuals regarding watersheds, bacteria sources and control methods; to trace bacteria to their sources; to tell the difference between human and animal bacteria; to physically view watersheds and look for bacteria sources; and to develop management strategies that are actually designed to address identified sources of bacteria. The Turkey Creek TMDL does not use any of these methods, and is therefore not based on sound science or sound evidence.

8. Due to these very serious shortcomings of the LDC method, Tinker requests a moratorium on the development of all bacteria TMDLs in Oklahoma until a science-based, evidence-based method is used to develop TMDLs.

9. Process and Procedural Concerns. Tinker has substantive concerns regarding the process and procedures EPA is using in establishing TMDLs for the state of Oklahoma.

a. Documents and Reports not Publicly Available Prior to the Meeting. In conducting any public meeting under the Clean Water Act, EPA is subject to 40 CFR Part 25. Section 25.6 of Part 25 states:

> The requirements of § 25.5 (b) and (c) are applicable to public meetings, except that the agency holding the meeting may reduce the notice to not less than 30 days if there is good reason that longer notice cannot be provided.

Section 25.5 (b) states, among other things, that:

> Reports, documents and data relevant to the discussion at the public hearing [and public meetings under Section 25.6] shall be available to the public at least 30 days before the hearing [or meeting under Section 25.6].

Tinker is unaware of any documents related to the Turkey Creek TMDL that were made available to the public prior to 11 Aug 2006. On 11 Aug 2006, the Federal Register notice for this public meeting was published, and the Web site link listed in the Federal Register appeared on EPA's TMDL web site. This link is the first time Tinker is aware that the Turkey Creek reports or documents were available to the public. In order to comply with 40 CFR § 25.5 (b) and 25.6, EPA was required to make the Turkey Creek Report and any other documents or data available to the public on or about 25 Jul 2006. Because the reports, documents and data were not provided to the public at least 30 days

prior to the meeting, EPA has not complied with 40 CFR sections 25.5 (b) and 25.6. A highlighted copy of the cited sections is attached.

b. Lack of Adequate Notice. Tinker is concerned about the lack of public notice. EPA's Federal Register notice for the Turkey Creek TMDL is defective. It did not mention the meeting agenda, time, or location of the public meeting. It did not mention that a public meeting would take place at all. Further, there was no mention on EPA's TMDL web site for the Turkey Creek TMDL that any public meeting would take place. Tinker has had inadequate time to comment on the proposed TMDL or to prepare for this public meeting. Please note in this regard that 40 CFR § 25.5 (b), which applies to public meetings such as this (see 40 CFR § 25.6), requires notice to the public at least 45 days prior to the meeting. Forty-five days prior to today was July 9th, 2006. Further, EPA has made no showing of any good reason why forty-five (45) days notice could not be provided. This forty-five day advance notice requirement is the same as that contained in Oklahoma's Continuing Process Planning (the CPP). Page 200 of that document requires that the state (or EPA in this case) provide public notice of the meeting agenda, time and location at least forty-five (45) days prior to the meeting. So, in addition to not complying with the forty-five day public notice requirements of 40 CFR § 25.6, EPA is not in compliance with Oklahoma's CPP. A copy of page 200 of the CPP is attached.

c. Public Comment Period too Short. Tinker is concerned that the public comment period is too short. Oklahoma's CPP requires that formal written and oral comments be accepted for a period of thirty (30) days following the public meeting. Therefore, Tinker requests that EPA extend the deadline for comments to be submitted from the current deadline of September 11, 2006, to September 22, 2006. This extension is required to comply with Oklahoma's CPP.

d. EPA Interference in Oklahoma's Delegated Program. EPA has delegated the responsibility of issuing permits and enforcing the Clean Water Act and Oklahoma water quality standards to Oklahoma, specifically, the Oklahoma Department of Environmental Quality. This delegation includes studying and establishing TMDLs. Despite this delegation, EPA has taken over the process of establishing the Turkey Creek TMDL in place of the state of Oklahoma. The sole basis for EPA taking this action is that a deadline internal to EPA would not be met. This is not a legally sufficient basis for EPA to conduct the public participation or establish the Turkey Creek TMDL. The public is entitled to have Oklahoma decision makers, who answer to the residents of the state of Oklahoma, to make the decision to establish this TMDL, not EPA.

10. Conclusion. Tinker objects to this TMDL. It is not based on sound science or evidence. Tinker reserves the right to oppose any TMDL established using the load duration curve method when stakeholders have not been involved in the process, when no ground reconnaissance has been performed to identify sources, when estimates are used in place of actual data, when sources of bacteria have not been actually identified and linked to the pathogen levels found in the water body, when background pathogen sources have not been considered, or when human bacteria have not been differentiated from animal bacteria or animal bacteria sources. Tinker requests that no TMDLs be

established in Oklahoma until these requirements for a sound, science and evidence-based TMDL are met. Tinker further objects to EPA establishing this TMDL in place of ODEQ for the reasons stated. Tinker reserves the right to make additional comments within the comment period. Tinker also reserves the right to continue to object to any TMDL based in whole or in part on the load duration curve method.

MEL A. MCFARLAND
Attorney-Advisor

3 Attachments:
1. Letter from Robert Gill with enclosures
2. Pertinent sections of 40 CFR Part 25
3. Page 200, CPP

DEPARTMENT OF THE AIR FORCE
AIR FORCE CENTER FOR ENVIRONMENTAL EXCELLENCE
REGIONAL ENVIRONMENTAL OFFICE
525 SOUTH GRIFFIN SUITE 505
DALLAS TEXAS 75202-5023

July 20, 2006

John Craig
Director, Water Quality Division
Oklahoma Department of Environmental Quality
P.O. Box 1677
Oklahoma City, Oklahoma 73101-1677

VIA electronic and regular mail

Dear Mr. Craig:

I am the Department of Defense (DOD) Regional Environmental Coordinator for the states located within Environmental Protection Agency Region 6. In this capacity, my staff and I have worked well with Oklahoma environmental and natural resource agencies to exchange environmental topics, to coordinate related projects and initiatives, to avoid conflicts where possible, and to help resolve issues efficiently.

On behalf of DOD, I am respectfully providing comments regarding the Oklahoma Department of Environmental Quality (ODEQ) North Canadian River Bacteria Total Maximum Daily Load proposal(the Bacteria TMDL), dated 22 March 2006, the North Canadian River Pathogens Total Maximum Daily Load report by ACOG (the ACOG Report), and the proposed Appendix F, dated 22 June 2006.

Our concerns with ODEQ's currently proposed actions to meet water quality standards in the North Canadian River (NCR) watershed are five-fold:

(1) The public record reflects that the Total Maximum Daily Load (TMDL) and Best Management Practices (BMPs) are being implemented without adequate background source analysis and/or monitoring of the current MS4 discharges;

(2) The lack of adequate analysis may result in a reduction goal for pathogens that is unattainable due to naturally occurring background source contamination, thus causing continuous violations by DOD facilities regardless of our efforts to comply;

(3) ODEQ's current proposal, which overlooks nonpoint source discharges and focuses exclusively on BMPs for MS4 permit holders, may result in unnecessary and increasingly expensive BMPs being continuously imposed on DOD facilities without regard to hardship or likelihood of success;

(4) Unless mandated BMPs and reduction goals account for background levels and consider the actual levels of pathogens discharged by a DOD facility, our permit holders will in

effect be required to fund the additional clean-up of pathogens from sources over which they do not exercise any control; and,

(5) The current proposal does not appear to be in compliance with Oklahoma statutory requirements and the Federal Water Pollution Control Act.

Thank you for the opportunity to submit comments regarding this matter. The enclosed comments were jointly prepared by the U.S. Army and Air Force for your consideration.

Please do not hesitate to contact me at (214)767-4653 if you have any questions or need additional information.

Sincerely,

Robert M. Gill

Robert M. Gill
Department of Defense
Regional Environmental Coordinator
Region VI

Enclosures

cc: Mark Derichsweiler
ODEQ, Water Quality Division

Jeffrey Short
Chair, Water Quality Management Advisory Council

Lowell Hobbs
Vice-Chair, Water Quality Management Advisory Council

The Honorable Miguel Flores
Director, Water Quality Protection Division
EPA, Region 6

The Honorable W. A. Drew Edmondson
Oklahoma Attorney General

COMMENTS OF THE DEPARTMENT OF DEFENSE
REGIONAL ENVIRONMENTAL COORDINATOR FOR REGION VI
REGARDING THE OKLAHOMA DEPARTMENT OF ENVIRONMENTAL QUALITY'S NORTH CANADIAN RIVER TMDL PROPOSAL, THE NORTH CANADIAN RIVER PATHOGENS TMDL REPORT BY ACOG,
AND THE PROPOSED APPENDIX F.

The current proposals do not adequately analyze the following: (1) background source contamination and the impact of nonpoint sources; (2) the propriety of the proposed reduction levels; (3) the adequacy and appropriateness of the proposed BMP program; (4) the costs involved; and (5) statutory requirements under Oklahoma and federal law. The proposal also does not significantly consider alternative methods used by other states to create TMDLs. Accordingly, we respectfully recommend that neither the bacteria TMDL nor Appendix F should be adopted at this time.

Concerns with Oklahoma DEQ's Current Approach

The Load Duration Curve (LDC) method used in the ACOG Report does not provide significant and sufficient scientific or regulatory support for either the Bacteria TMDL reduction goals or Appendix F. Although it is expedient (uses existing data, and provides some general information on the total pathogen loading to the North Canadian River (NCR)), the LDC method and the ACOG Report have fatal shortcomings when used to support regulatory action.

First of all, the LDC method does not differentiate between background levels of pathogen loading and pathogens attributable to point and non-point sources. Furthermore, the current method not only fails to differentiate between human and animal sources of pathogens, but it also does not differentiate between pathogens that have recently been introduced into a water body and pathogens flushed from colonies that have regrown in the sediment or in other locations.

Most seriously, the LDC method and the ACOG Report, with few exceptions, do not identify any actual sources of pathogens. Nevertheless, the Bacteria TMDL sets pathogen reduction goals of up to 99.7%. Although a reduction of this level may be warranted and ultimately required, the probability and practicality of achieving such a goal solely by using BMPs for municipal separate storm sewer systems (MS4s) is problematic in the absence of additional research and testing of background levels, as well as monitoring of the actual pathogenic contributions of the currently permitted MS4s. Moreover, if the proposed plan was implemented without additional studies, the achievement of the proposed reduction would require the elimination of background pathogens, animal pathogens, and pathogens from sources over which MS4s have no control, in addition to the implementation of BMPs for pathogens actually contributed by MS4s.

As a result, regulated entities such as MS4s, point source dischargers, and entities not directly regulated by DEQ have no means of determining: (1) whether they are a

source of pathogen loading; (2) how much, if any, they are contributing to pathogen loading; (3) whether any particular BMP will be even minimally effective in reducing pathogen loading; or, (4) whether the percentage reductions proposed in the bacteria TMDL are even appropriate.

Specifically with respect to Tinker Air Force Base (TAFB), the appropriate water quality standard is secondary body contact recreation (SBCR). According to the Oklahoma Administrative Code, Crutcho Creek, upstream from SE15th Street, has a designated beneficial use of SBCR. *See* Table 1, 785 OAC §45 at 62. The unnamed tributaries of Soldier Creek and Kuhlman Creek, at the point where they leave Tinker AFB, are not listed, and therefore have the default beneficial use of SBCR. *See generally*, 785 OAC §45-5-4.

Waters with a designated beneficial use of SBCR "shall be maintained to be free from human pathogens in numbers which may produce adverse health effects in humans." 785 OAC §45-5-17(d). This is a narrative criterion only. Neither the ACOG report nor DEQ have shown that TAFB has violated this narrative standard for the water body segments listed above, or that TAFB is likely to contribute to a violation by virtue of pathogen loading allowed under the SBCR standard.

Alternative Approaches for Consideration

Washington

Other states have taken much different approaches to the development of bacteria TMDLs, with good results. For example, the State of Washington used DNA ribotyping to locate sources of pathogens. (*See* Attachment 1). The Institute of Environmental Health in that state conducted the DNA analyses. The total analytical cost for 690 isolates was approximately $55,000, which amount is within a reasonable range.

This type of source identification dramatically changed source identification and loading. The ribotyping provided evidence that the most significant bacterial loading was from rodents and ducks, which results ultimately changed the development of the TMDL.

Virginia

These results are similar to those for Four Mile Run in the State of Virginia. (*See* Attachment 2). Using DNA fingerprinting (specifically, pulsed–field gel electrophoresis), Virginia was able to determine that deer, raccoons and waterfowl accounted for 62% of the identified isolates. The Four Mile Run results also indicated that bacterial re-growth was occurring. Once the source of a large percentage of the pathogens was determined, Virginia was able to accurately assess the methods most likely to result in a reduction of contamination. Some of the methods proposed for Four Mile Run watershed included the modification of storm drains to dissuade raccoon usage, management of waterfowl, the cleanout of catchbasins to remove areas where bacteria re-growth was occurring, and encouraging bacterial predation by other microbes such as rotifers and paramecium.

Texas

The State of Texas has used watershed advisory groups to both provide insight into potential sources of pathogen loading and to help establish appropriate TMDLs. Additionally, Texas has utilized bacterial source tracing for the Lake Waco and Belton Lake watersheds, and for the San Antonio River, Salado and Peach creeks. (*See* Attachment 3).

Limitations to the Current Regulatory Process

Oklahoma Statutory Requirements

Effective rule making must start at the basic level of data and knowledge in order to create an appropriate and scientifically defensible program. Experience has shown that municipalities, other state agencies and interested stakeholders can be valuable sources of information regarding potential sources, problem areas and practical approaches to problems that are identified at any point in the process. Additionally, background and source identification have proven to be key components in developing scientifically-defensible bacteria TMDLs.

Unfortunately, under the current proposal, it appears as though DEQ's approach of proposing bacteria reduction goals and the implementation of BMPs prior to utilizing background source identification and individual source monitoring has placed the proverbial cart before the horse. Instead, an achievable goal should be based on inputs from other agencies and interested stakeholders, reconnaissance of the affected areas, defensible, source-specific pathogen information, and practical BMPs designed to address the actual findings. Both EPA and DEQ guidance and regulations support this latter approach.

In addition, the Bacteria TMDL and Appendix F are rules, but have not been promulgated as such. 252 OAC Section 690-1-7, states:

> The WQMP [Water Quality Management Plan] must be updated in accordance with the planning, approval and public participation procedures described in the CPP [Continuing Planning Process] whenever a facility seeks to increase pollutant loading, relocate a discharge point, **or when a TMDL is adopted**. (emphasis added).

Under the heading of "Rulemaking Activities of the Oklahoma Department of Environmental Quality and Other State and Federal Agencies" at pages 199-200, the 2002 CPP states:

> All requirements relating to **water quality management plans**, pollution abatement, wastewater treatment and disposition, permitting, approval of remediation plans, **enforcement of Oklahoma WQS** [Water Quality Standards],

administrative proceedings, natural resource damage assessments, and similar requirements **shall be contained in appropriate Chapters of the DEQ's rule**s. (emphasis added).

Because the Bacteria TMDL and Appendix F are both requirements related to the Water Quality Management Plan and are related to enforcement of Oklahoma Water Quality Standards, the provisions cited above appear to require the proposed actions to be adopted using normal rulemaking procedures. Moreover, the same result would be reached by applying the Oklahoma Administrative Procedures Act (OAPA) since the Bacteria TMDL and Appendix F both qualify as rules, and should therefore be promulgated as rules under the OAPA. We have searched and can find no evidence that either the Bacteria TMDL or Appendix F have been so promulgated. If they have not been promulgated as required by statute, then we respectfully submit that they would be unenforceable guidelines.

Federal Water Pollution Control Act Requirements

Under Section 313 of the Federal Water Pollution Control Act (33 U.S.C. § 1323, the Clean Water Act or CWA), sovereign immunity for federal facilities is only waived for substantive or procedural "requirements, administrative authority, and process and sanctions" that have been legally implemented. If the Bacteria TMDL and Appendix F are not legally implemented, the United States will not have waived sovereign immunity, which could create an impediment to the implementation of the BMPs by affected DOD facilities within Oklahoma.

Additionally, Sections 1329 (a) and (b) of the CWA require a state to identify categories and subcategories of nonpoint sources or particular nonpoint sources which add significant pollution to navigable waters in amounts which contribute to a navigable water within the state, such as the NCR, not meeting water quality standards. However, it does not appear from our review of the record that there has been any source identification for the pathogen loading to the NCR, or identification of the amounts of pathogens contributed by any source, nonpoint or otherwise, located in the NCR watershed. Also, the current approach, which focuses exclusively on BMPs for MS4s, appears to overlook the requirement in Section 1329 (b)(2)(A) to identify and implement BMPs for each category, subcategory or particular nonpoint source designated under (1)(B) of Section 1329.

The CWA also limits the ability of DEQ to revise effluent limitations based on a TMDL. In situations where the water quality standards are not being met, effluent limitations, such as the Appendix F BMPs and the proposed percentage reductions in pathogen loading, "may be revised only if . . . the cumulative effect of all such revised effluent limitations based on such total maximum daily load ... will assure the attainment of such water quality standard, or (ii) the designated beneficial use which is not being attained is removed . . . ." 33 U.S.C. §1313(d)(4)(A). Since there does not appear to be any information on background pathogens, or on the actual contribution of point and nonpoint sources to pathogen levels in the NCR, DEQ cannot demonstrate that the

cumulative effects of the effluent limitations will assure the attainment of the required water quality standard, and are thus prohibited by the CWA from revising these effluent limitations based on a TMDL or waste load allocation.

The BMPs in Appendix F, combined with the percentage reductions currently proposed for the NCR, will likely require affected DOD installations in Oklahoma to clean up pathogens from sources other than their facilities. Moreover, because there has not been sufficient monitoring accomplished to isolate the source of pathogens and determine what, if any, pathogens TAFB may be contributing to the NCR, TAFB will be forced to clean up to a higher standard and at a greater cost in order to account for unknown background pathogens (such as may be contributed by deer, raccoons, beaver and waterfowl). Not only does the lack of background analysis and absence of specific standards based on actual discharges from TAFB lead to uncertainty for the permit holder, but it also will lead to unnecessary and uneconomical decisions concerning required BMPs. The problem could be further exacerbated by federal fiscal law prohibitions concerning the unauthorized expenditure of appropriated funds for certain activities.

Recommendations

For the above reasons, we respectfully request that DEQ take the following steps prior to implementing its proposed TMDL and BMP program:

(1) Form a watershed advisory group including interested stakeholders and any state or federal agency that may provide technical, enforcement, regulatory, nonregulatory, financial, educational, training or technology assistance;

(2) Perform an actual reconnaissance of the NCR watershed to identify potential and/or actual point and nonpoint sources of pathogen loading, taking into consideration the potential for bacterial re-growth in storm drains, culverts and sediments, combined sewer overflows, sanitary sewer overflows, leaking septic systems, ranch and agricultural sources, sanitary sewer connections to stormwater drains (this can be done by tracking optical brighteners and other chemicals), sanitary sewer sludge land application sites, domestic animals and wildlife in addition to MS4s;

(3) Locate actual sources of pathogen loading using sound science, including, but not limited to, bacterial source tracing;

(4) Propose methods to control or limit pathogen loading based on the source(s) of the loading;

(5) Promulgate (under the OAPA) bacteria TMDLs and best management practices in consultation with the watershed advisory group and state and federal agencies;

(6) Tailor BMPs to the sources of the pathogen loading, and implement them as necessary and practical to achieve water quality standards; and,

(7) Only require MS4s to implement BMPs to the extent necessary to control the discharge of pathogens over which they have actual responsibility and control.

Lake Waco / Belton Lake BST Project

An Agricultural-initiated Cooperative Effort to Determine Bacterial Sources in Municipal Drinking Water

Presented by:
Ned Meister and Kevin Wagner

Upper Watershed Statistics

Bosque River

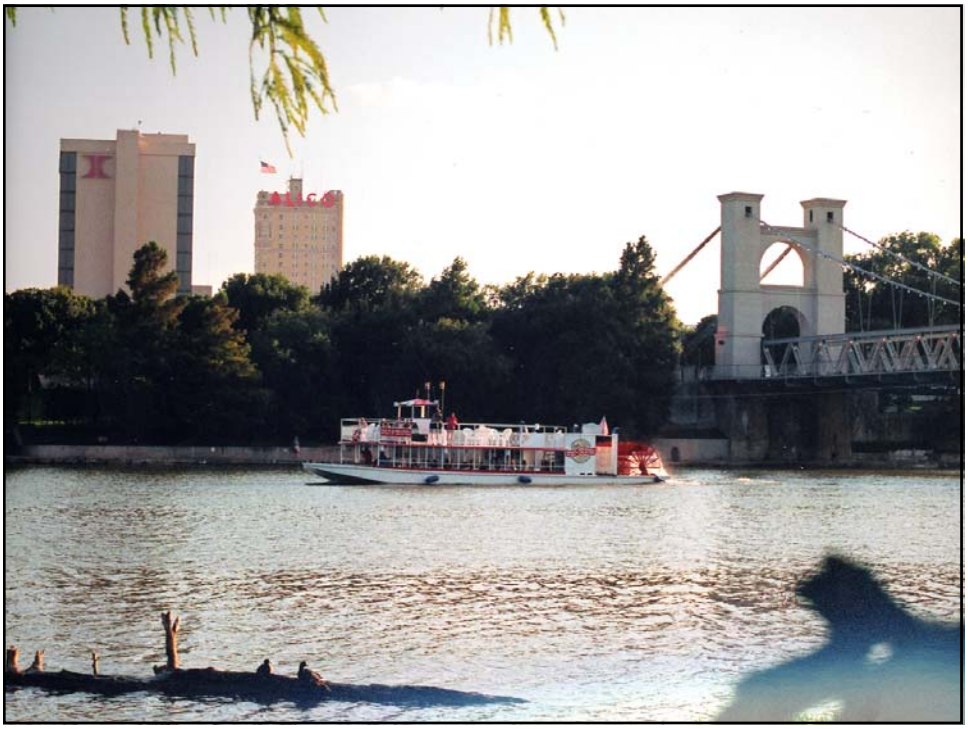
- 1200 sq. miles
- 76 dairies
- 55,000 cows

Leon River

- 1730 sq. miles
- 83 dairies
- 57,000 cows

Dairy Economics

- **Dairy economic <u>Contribution</u>**
- 307.5 mil


ALCO

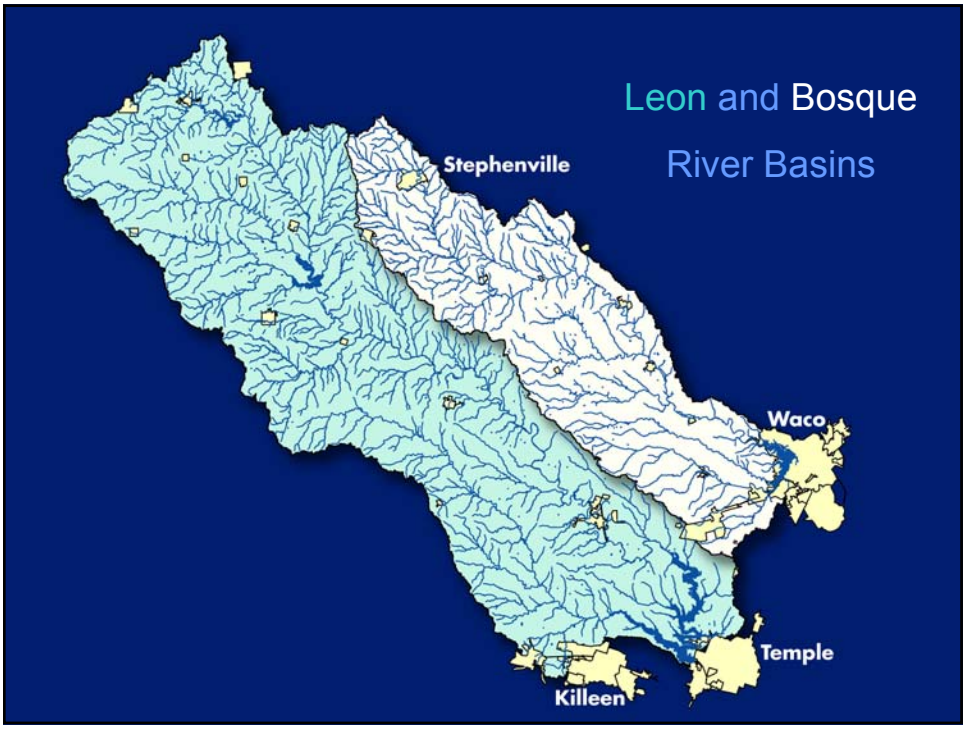
Waco Statistics

- **Waco Area**
  Population – 213,617
- Water Source – Lake Waco
- Lake Waco - Sole Source Water Supply
- Lake Waco – Shallow, mud bottom
- Main Water Source – Bosque River

- **Belton Area**
  Population – 312,952
- Main Water Source – Lake Belton
- Main Water Source – Leon River

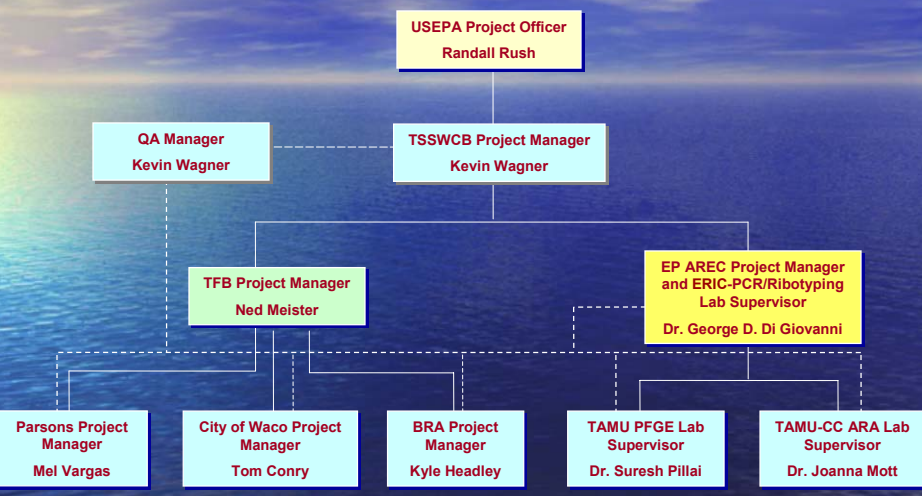
Waco Tribune Herald

- Wednesday, March 21, 2001
- "Scientists have found runoff from dairy waste responsible for excessive phosphorus that leads to massive algae blooms. The algae causes odor and taste problems in Lake Waco. City officials have also expressed concern over bacteria such as E. coli coming from that runoff."


Leon and Bosque
River Basins
Stephenville
Waco
Temple
Killeen

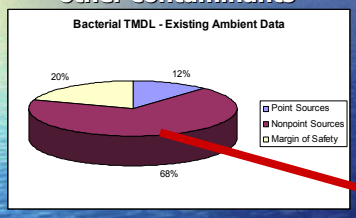
TEXAS FARM BUREAU

Project Organization

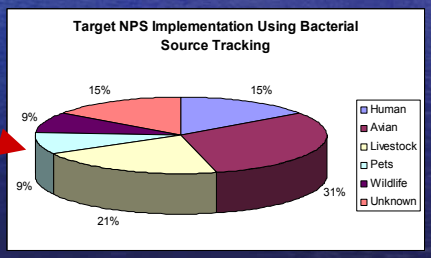


TMDLs and BST

- Total maximum daily load (TMDL) is the calculated maximum amount of pollution that a water body can receive & still meet water quality standards – developed for fecal bacteria, e.g. *E. coli,* and other contaminants



Typical bacterial TMDL – gross allocations into point sources and nonpoint sources based on land use



BST used to better define nonpoint sources of pollution and aid development of effective bacterial TMDLs

What the Heck is *E. coli*?

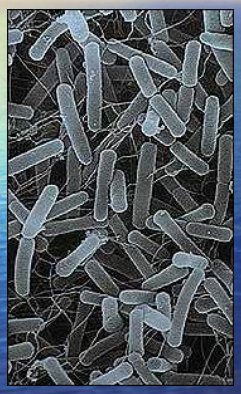
- *Escherichia coli* (*E. coli*) is a bacterium naturally found in the intestines and the feces of warm-blooded animals
- Many different types, most harmless, but some may cause illness (e.g. hamburger restaurant outbreaks)
- Laboratory tests can distinguish one strain from another
- Commonly used as an indicator of fecal pollution of water

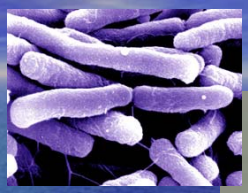
Texas Water Quality Criteria

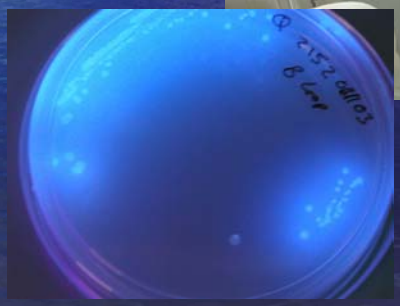
E. coli for contact recreation

- long-term geometric mean ≤ 126/ 100 mL
- single sample ≤ 394/100 mL

What Does *E. coli* Look Like?







There's *E. coli* in the Water, But Where Did it Come From?

- Bacterial Source Tracking (BST) uses laboratory tests to determine if *E. coli* (or other fecal bacteria) in water samples came from animal or human feces
- Most BST methods are Library Dependent, need database of reference bacteria from known animal and human sources to identify sources of water isolates
- "Local" watershed libraries of known source *E. coli* isolates currently considered most useful for identifying *E. coli* obtained from ambient water samples

Current BST Studies

- Texas Commission on Environmental Quality (TCEQ)
  - San Antonio River, Salado and Peach Creeks
  - Over 3,000 *E. coli* isolated from 750 known source samples and over 3,000 *E. coli* isolated from 850 ambient water samples
  - BST analyses underway
- Texas State Soil and Water Conservation Board (TSSWCB)
  - North Bosque and Leon River Watersheds – Lakes Waco and Belton
  - Sampling campaign Sept. 2003 – Aug. 2004

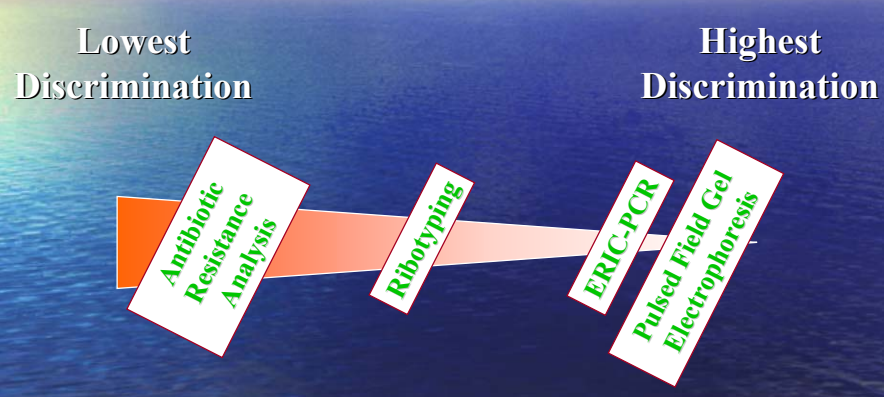
Objectives

Bacterial Source Tracking Projects

- Develop publicly available, genetic and phenotypic fingerprint libraries of *E. coli* isolates from known animal and human sources
- Determine the reproducibility and accuracy of the methods
- Determine the potential human or animal sources of *E. coli* contamination of water to aid development of TMDLs and watershed protection strategies
- Provide a foundation for additional watershed BST studies

Ability of Methods to Discriminate Differences Between Bacterial Strains

Bacterial Source Tracking Projects

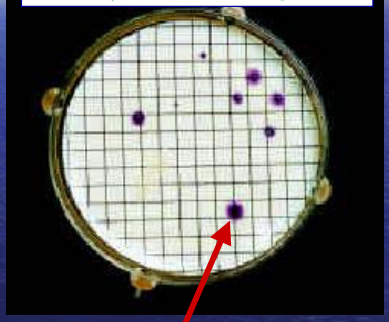


Which method or combination is best?

Isolation of *E. coli* From Feces & Water

Fecal Specimens

Water Sample Filtered and Filter Placed on Modified mTEC Medium (EPA Method 1603)

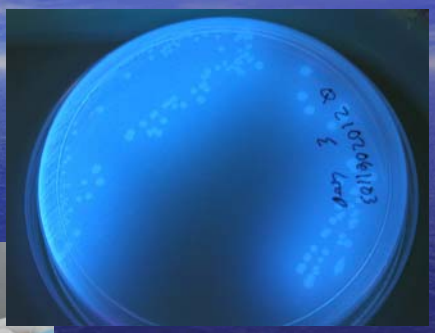


Modified mTEC Medium

E. coli Colonies

Each *E. coli* colony is called an "Isolate"

Purification & Confirmation of *E. coli*

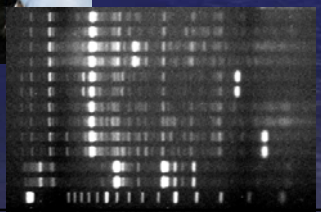


Archival (Freezing) of *E. coli* Isolates

- Isolates stored frozen at -80 °C (-112 °F)
- Remain alive for years
- Living library of isolates can be shared with other researchers

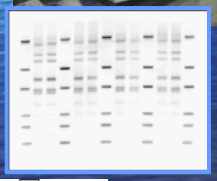
BST Technique 1
ERIC-PCR Fingerprinting

- Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR)
- Method of generating a "DNA Fingerprint" for each *E. coli* isolate
- Different strains of *E. coli* have different fingerprints



BST Technique 2
Automated Ribotyping

- Another DNA Fingerprinting Test
- Also Confirms Isolates as *E. coli*



Sample Number	Label	RiboGroup	Similarity to Selected 295-21-S-1	RiboPrint ® Pattern (1 kbp, 5, 10, 15, 50)
295-21-S-1	QC 101	HindII 295-21-S-1	1.00	
295-21-S-2	QC 101	HindII 295-21-S-1	0.98	
295-21-S-3	QC 101	HindII 295-19-S-1	0.95	
295-21-S-4	QC 101	HindII 295-21-S-1	0.97	
295-21-S-5	QC 101	HindII 295-21-S-1	0.97	
295-21-S-6	QC 101	HindII 295-21-S-1	0.96	
295-21-S-7	QC 101	HindII 295-21-S-1	0.93	
295-21-S-8	QC 101	HindII 295-21-S-1	0.94	

BST Technique 3
Pulsed Field Gel Electrophoresis (PFGE)

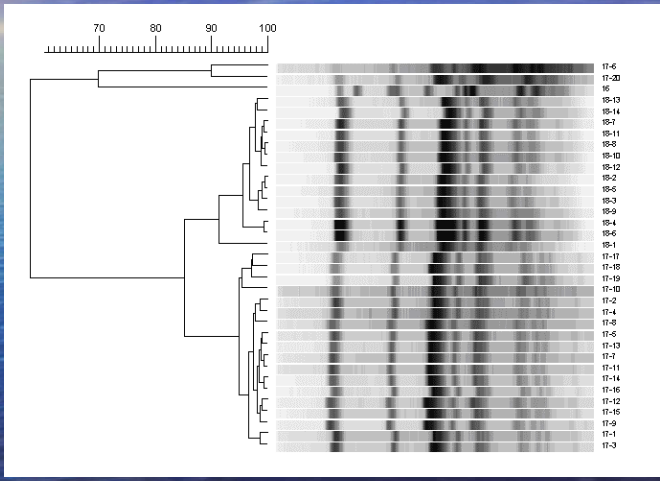


Photo Courtesy of Dr. S. D. Pillai

BST Technique 4
Antibiotic Resistance Analysis (ARA)

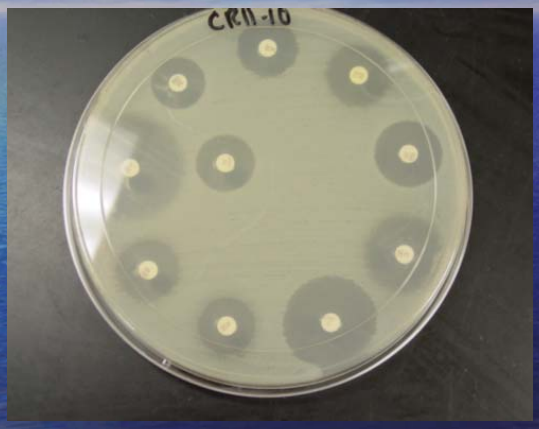


Photo Courtesy of Dr. J. Mott

Data Analysis

- DNA Fingerprints and antibiotic resistance patterns of isolates from water samples compared to *E. coli* reference library to identify likely human or animal sources of pollution
- Applied Maths BioNumerics software
  - Performs multiple statistical tests of each data set individually and in combination – Discriminant analysis, ARCC, dendrogram significance tools, congruence between data sets
- Developed *E. coli* libraries will provide a foundation for other BST studies in Texas

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