PRELIMINARY ENGINEERING REPORT

FOR

WASTEWATER COLLECTION SYSTEM TO SERVE STEWARTSVILLE

FOR THE

POSEY COUNTY REGIONAL SEWER DISTRICT

POSEY COUNTY, INDIANA

MEI PROJECT #2023019

Midwestern Engineers, Inc.

PRELIMINARY ENGINEERING REPORT
FOR
WASTEWATER COLLECTION SYSTEM
TO SERVE STEWARTSVILLE
FOR THE
POSEY COUNTY REGIONAL SEWER DISTRICT
POSEY COUNTY, INDIANA

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March 2024

TABLE OF CONTENTS

FIGURES

LIST OF TABLES

LIST OF APPENDICES

Chapter 1

Project Planning Area

A. Project Location and Background Information

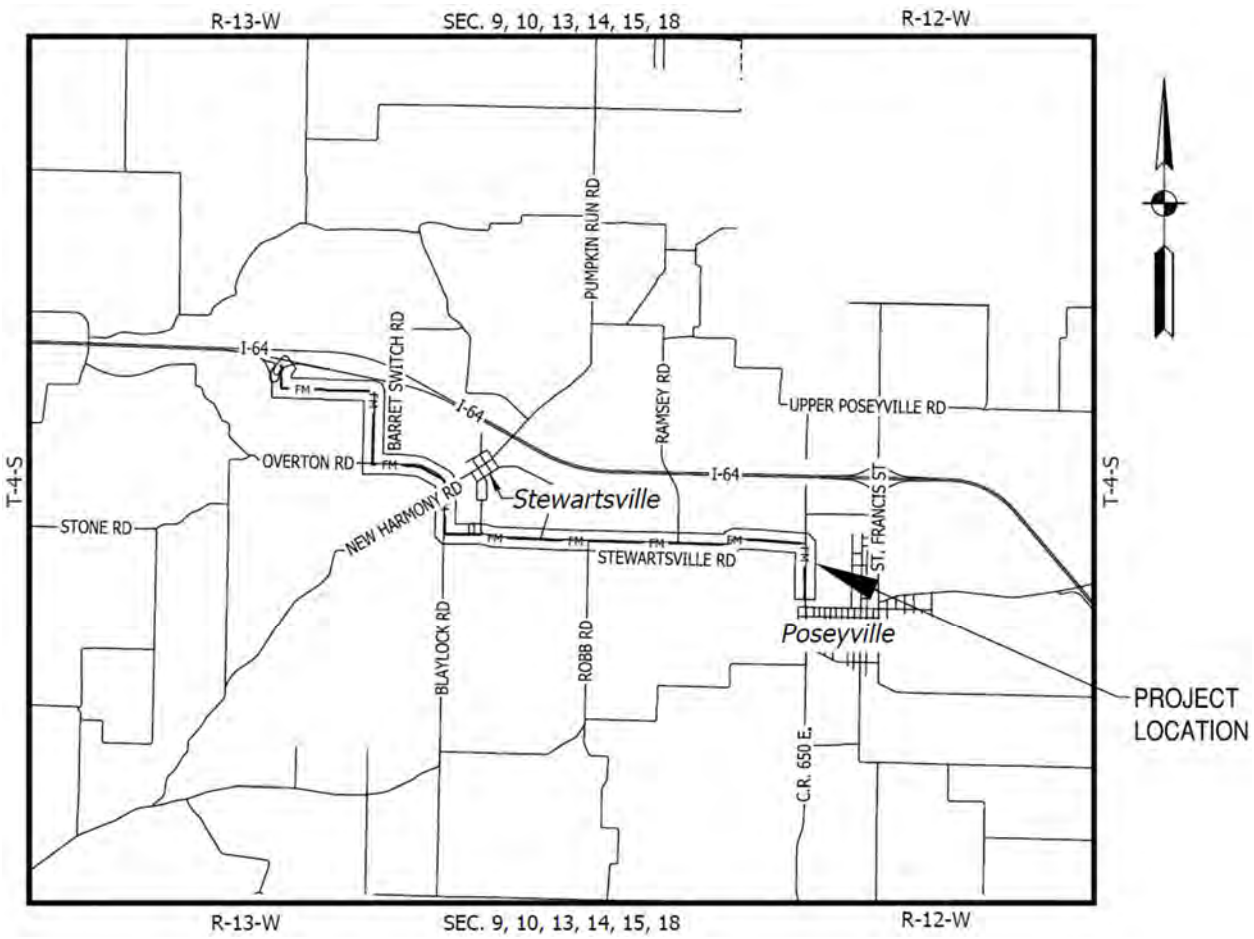
1. General Location Map

Posey County lies in the far southwest corner of the state of Indiana**.** Figure 1 shows the general location of Posey County relative to other municipal entities in the area. The City of Mt. Vernon is the largest city in Posey County. Other notable cities are Evansville to the east and Princeton to the northeast. Stewartsville is located on the northern portion of the county west of Poseyville and south of I-64.

Figure 1

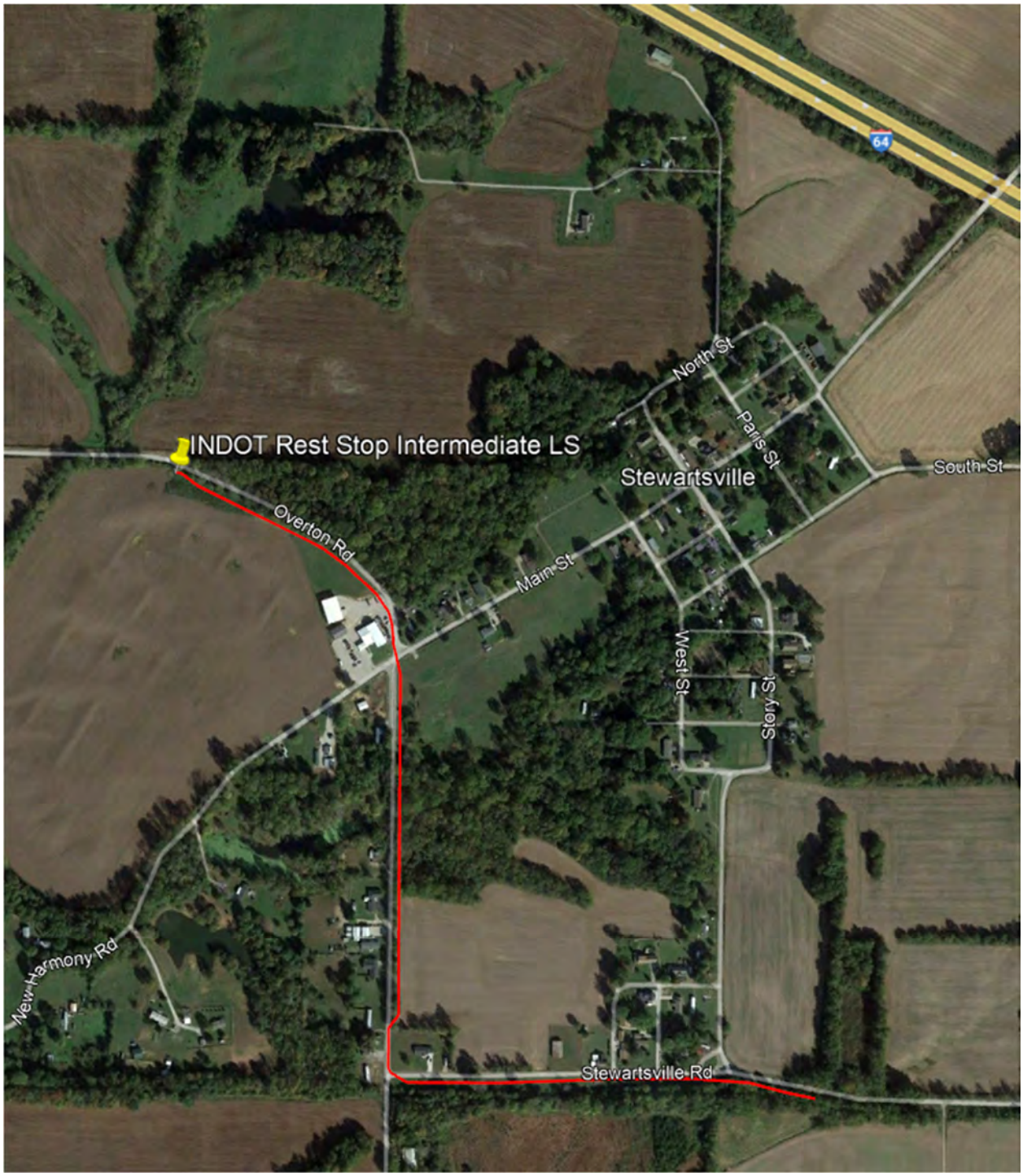
Source: https://www.in.gov/indot/files/2023_Roadway_Map-NEW-SIZExtended-e-version.pdf

Figure 2



Source: Black River Welcome Center Plans prepared by Beam, Longest and Neff for the Indiana Department of Transportation

Figure 3



Source: Google Earth

2. Area Map

The Stewartsville area is shown in Figure 2, which depicts a current Indiana Department of Transportation (INDOT) project that includes improvements to the Black River Welcome Center located along I-64 to the northwest of Stewartsville. INDOT will convey wastewater to the Town of Poseyville via. a new force main sewer.

3. Aerial Map

Figure 3 depicts the aerial map of the Stewartsville area along with the proposed force main sewer from the Black River Welcome Center project that will pump wastewater to the Town of Poseyville. The Posey County Regional Sewer District currently has no wastewater infrastructure in this portion of the county.

Chapter 2
Current Situation

A. History & Need

Posey County Regional Sewer District (RSD) was officially formed in January 2018 to provide wastewater service to unincorporated communities within the county. The Regional Sewer District worked with the Indiana Finance Authority (IFA) to address a severe sewage treatment need in the Harbortown Subdivision, which is located 2.25 miles east of Mt. Vernon on the south side of S.R. 62. A small treatment plant (Harbortown located 1.25 miles east of Mt. Vernon on the south side of S.R. 62) and grinder pumps were designed and construction was initiated January 2019 with completion in April 2020. An application was submitted by the district in August 2018 for a subsequent plan for the communities of Wadesville and Blairsville. The Wadesville-Blairsville treatment plant was completed in late 2023 and the collection system is ending construction at the time of this report in early 2024.

There are many small communities within Posey County which do not currently have public wastewater collection and treatment systems. Instead, the homes and businesses use on-site septic systems. Septic disposal fields (also called leach fields) are used to distribute the effluent from the septic tank into the ground. Disposal fields commonly consist of shallow, gravel filled trenches with distribution pipes running from the septic tank. Old septic systems tend to fail and the most frequent example of failure is ponding from overloaded leach fields. Ponded wastewater then overflow into nearby ditches, streams, and lakes. Appendix E contains excerpts from a 2009 Big Creek Watershed Management Plan report and subsequent E. Coli testing data and graphs from 2023 by the Posey County Soil & Water Conservation District. Although Big Creek watershed is outside Stewartsville, the results are indicative of other rural areas of Posey County and surrounding areas of unsewered communities.

Recently, the Indiana Department of Transportation (INDOT) has implemented a construction project that includes improvements to the Black River Welcome Center located along I-64 to the northwest of Stewartsville. INDOT will convey wastewater to the Town of Poseyville via. a new force main sewer with a lift station at the rest stop site and an intermediate lift station just west of Stewartsville. INDOT is working with the Posey County RSD to take over the collection system and become a customer of the RSD. Also, the Indiana Finance Authority (IFA) is planning to fund individual customer

connections along the new force main route to become RSD customers as well (properties within 300 feet of the new sewer line). Due to Stewartsville's close proximity to this new force main sewer, the RSD is pursuing funding for customer connections in this area of the RSD service area and is the principal factor for the development of this preliminary engineering report.

B. Existing Wastewater Treatment Plants

Posey County RSD currently owns two (2) wastewater treatment plants in the district. Harbortown is a 15,500 gallons per day average design flow, 67,000 gallons per day peak design flow Sequencing Batch Reactor wastewater treatment plant including a mechanical fine screen, two (2) process tanks, ultraviolet light disinfection, sludge dewatering facilities, and effluent plant lift station. The second wastewater treatment plant in the district is the recently constructed Wadesville-Blairsville plant which was designed for 200,000 gallons per day average flow, 730,000 gallons per day peak design flow and includes a mechanical fine screen, Biolac system containing an aeration basin with retrievable fine bubble diffusers and an integrated secondary clarifier, ultraviolet light disinfection, cascade post-aeration structure, and sludge dewatering facilities.

C. Existing Wastewater Collection System

The existing Harbortown collection system consists of approximately 10,100 feet of 1.25" thru 3" high density polyethylene (HDPE) low pressure sewer main and 27 individual grinder pumps. The existing Wadesville-Blairsville collection system consists of approximately 76,300 feet of 2" thru 6" HDPE low pressure sewer main and 420 individual grinder pumps. Although the Wadesville-Blairsville system is still under construction and final installed footage of pipe and number of grinders is to be determined.

D. Stewartsville Wastewater Flows

When planning for wastewater facilities, it is essential to estimate design wastewater flows and organic loadings. To estimate these, the number of residential, commercial and industrial users within the planning area should be evaluated. This analysis revealed a total of approximately 70 possible customers of which are predominantly residential connections. No industrial or commercial users were identified. Based upon state code and IDEM permitting requirements, the average daily flow is estimated at 310 gallons per day (gpd) per customer, and the peak daily flow is estimated at 4.2 times the average daily demand based upon a peaking factor related to the service population. Based upon past

experience and water usage records the actual average and peak flows will likely be 30 to 40% less (approx. 200 gpd) than the design flows for purposes of treatment volume and associated costs by the Town of Poseyville.

The projected initial average daily wastewater flow is estimated to be 21,700 gallons per day (gpd) and a peak hourly flow is estimated to be 91,140 gpd.

Again, based upon experience the initial average daily flow is only expected to be on the order of 14,000 gpd for 70 customers.

E. Condition of Existing Facilities

As previously noted, the existing wastewater treatment plants and collection systems were put into operation in 2020 and currently. Some of the original mechanical components for Harbortown will start to show wear and some equipment repair and replacements are expected to occur over time as the system ages.

Chapter 3
Future Situation

A. Population and Wastewater Flow Projections

A key to any wastewater utility planning report is an understanding of current and future demands of the facilities. For wastewater planning, this requires a sound, reliable projection of future population and an understanding of land use and development trends occurring within the service area or future service areas.

Population projections for Posey County are estimated to decrease over the next twenty (20) years, thus unfortunately there is expectation of a declining population during the study period. Population projections are not available for townships or Cities, however for Posey County the population in 2020 was 25,053 and is projected to decrease to 21,979 by 2040, which is a 12.3% decrease.

Posey County Regional Sewer District is hopeful that providing wastewater collection and treatment service to rural customers will help slow the projected decline in population of the county and reverse the projected trend. The service area and likely growth for the Stewartsville is considered to be somewhat limited.

Thus, for purposes of this report potential future added customers were also evaluated in the collection system design including vacant or undeveloped lots in the area. It was estimated that 10 future customers could be added to the system in the future, which results in 24,800 gpd average flow and 104,160 gpd peak hourly flow.

Chapter 4

Evaluation of Alternatives

A. Wastewater Collection and Treatment Alternatives

1. No Action

An alternative, which can be applied to the wastewater needs, is to take no action. The alternative of no action to develop pollution control facilities offers neither short nor long term benefits. Currently, conditions within the unincorporated community of Stewartsville are not conducive to on-site treatment systems. As time progresses, the condition of the on-site septic systems would only further deteriorate. The no action alternative would thus increase the potential for serious health hazards.

Thus, the "No Action" Alternative is therefore not considered a viable long-term solution and is not considered further.

2. Conventional Gravity Sewer System

This type of sewer system is the most common, most widely used wastewater collection system. Conventional gravity sewers consist of 8-inch or larger diameter pipes constructed with specific slopes. Manholes are provided at least every 400 feet and at every change in direction, pipe slope or pipe size. The manhole is usually 4 feet in diameter, which allows sufficient room for a worker to reach the pipe for cleaning and inspection. The network of sewers allows sewage to drain by gravity to low-lying areas. Pump stations are required where sewers get excessively deep or gravity flow is no longer available. From the pump stations, the sewage is pumped through pipelines called force mains. Force mains, being under pressure, are constructed like water mains, buried approximately 4 feet deep and follow the terrain. A simplified sketch of a conventional gravity system is presented as Figure 4. Conventional gravity sewers have been and continue to be very economical in high population density urban areas. The deep construction and large pipe sizes make a conventional system very expensive in low population density rural areas, and in areas with shallow rock or high groundwater. An overall map that presents a possible gravity sewer collection system layout is located in Appendix A.

Probable construction costs are presented in Table 1 for the Conventional Gravity Sewer System Alternative. Probable O&M costs are also presented.

Figure 4
Conventional Gravity Sewer System

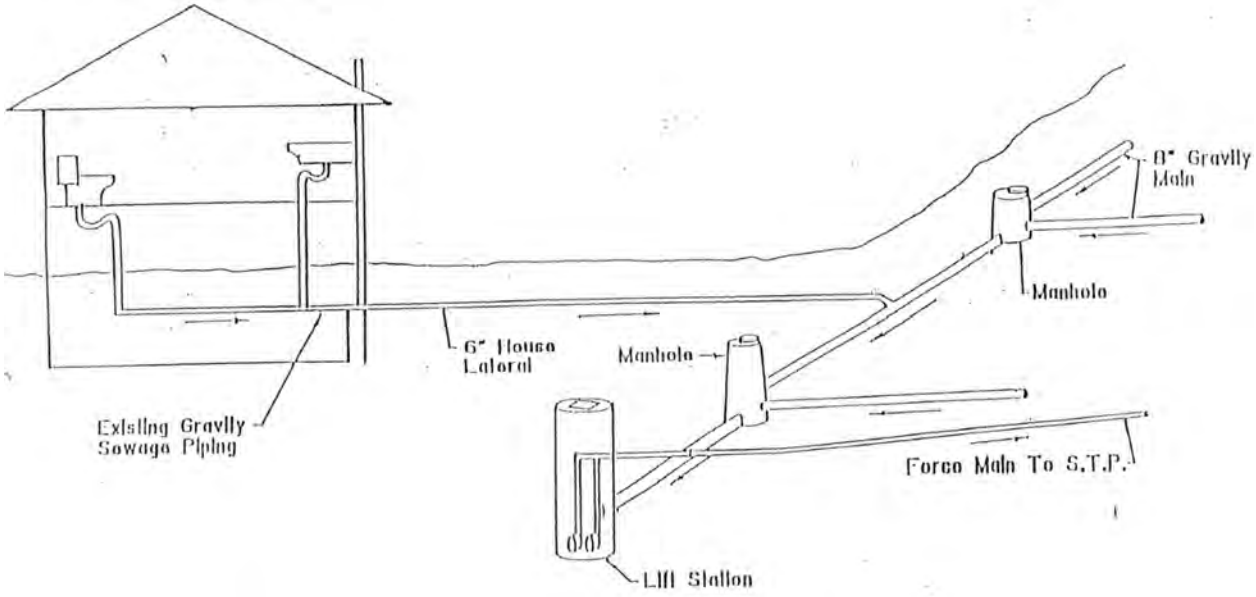


TABLE 1
PROBABLE CONSTRUCTION COSTS
FOR
GRAVITY SEWER ALTERNATE
FOR THE
POSEY COUNTY REGIONAL SEWER DISTRICT
POSEY COUNTY, INDIANA
FEBRUARY, 2024

I. PROBABLE CONSTRUCTION COST

ITEM NO.	DESCRIPTION	ESTIMATED QUANTITY		ESTIMATED UNIT COST	ESTIMATED TOTAL COST
1.	8" SEWER, 0 - 6' DEPTH	5,580	L.F.	$ 90.00	$ 502,200.00
2.	8" SEWER, 6' - 8' DEPTH	3,360	L.F.	$ 95.00	$ 319,200.00
3.	8" SEWER, 8 '- 10' DEPTH	540	L.F.	$ 110.00	$ 59,400.00
4.	8" SEWER, 10' - 12' DEPTH	90	L.F.	$ 130.00	$ 11,700.00
5.	8" SEWER, 12' - 14' DEPTH	620	L.F.	$ 160.00	$ 99,200.00
6.	4" PVC, FORCE MAIN	6,440	L.F.	$ 35.00	$ 225,400.00
7.	6" SERVICE SEWER ALL DEPTHS	14,000	L.F.	$ 75.00	$ 1,050,000.00
8.	4" PVC FORCE MAIN, BY UNCASED BORING	240	L.F.	$ 81.00	$ 19,440.00
9.	MANHOLE 0 - 6' DEPTH	40	EA.	$ 6,000.00	$ 240,000.00
10.	ADDITIONAL DEPTH OF MANHOLE	63	VF.	$ 500.00	$ 31,500.00
11.	MANHOLE DROP	6	EA.	$ 2,500.00	$ 15,000.00
12.	CLEANOUT AND FLUSHING STATION	5	EA.	$ 6,000.00	$ 30,000.00
13.	AIR RELIEF VALVE	5	EA.	$ 9,000.00	$ 45,000.00
14.	8" X 6" WYE	70	EA.	$ 350.00	$ 24,500.00
15.	CONNECTION TO EX. MAIN	2	EA.	$ 5,000.00	$ 10,000.00
16.	STONE BACKFILL AND RESURFACING	6,000	TON	$ 30.00	$ 180,000.00
17.	BITUMINOUS RESURFACING	1,800	L.F.	$ 58.00	$ 104,400.00
18.	CONCRETE RESURFACING	200	L.F.	$ 90.00	$ 18,000.00
19.	LIFT STATIONS	5	EA.	$ 180,000.00	$ 900,000.00
20.	TRAFFIC CONTROL DURING CONSTRUCTION	1	L.S.	$ 35,000.00	$ 35,000.00
21.	MOBILIZATION, BONDS, AND INSURANCE	1	L.S.	$ 179,000.00	$ 179,000.00
22.	EROSION CONTROL PLAN AND IMPLEMENTATION	1	L.S.	$ 45,000.00	$ 45,000.00
				SUB-TOTAL	**$ 4,143,940.00**
				CONSTRUCTION CONTINGENCIES	$ 414,060.00
				TOTAL PROBABLE CONSTRUTION COSTS	**$ 4,558,000.00**

II. PROBABLE COLLECTION SYSTEM O&M COSTS

1.	LINE MAINTENANCE ($0.25 per inch diameter per linear foot per year)	$48,100.00
2.	TREATMENT COST (estimated at $5.40/1,000 gal + $723.30/mo. base charge)	$36,300.00
3.	INSURANCE	$9,100.00
4.	MISC. AND CONTINGENCIES	$9,500.00
	TOTAL ANNUAL O&M COSTS	**$103,000.00**

3. Low Pressure Grinder Pump Sewer System

Low pressure grinder pump collection systems use one or more small pumps located in pump pits connected to one or more building gravity laterals. The pumps grind the sewage solids into small particles. The pumps discharge into a collection system consisting of small pressure force mains, generally 1.5 to 4-inches in diameter. The force mains follow the existing ground elevations and need to be only deep enough to prevent freezing. Power for the pumps must come from one of the buildings connected to the pump pit or from a meter drop line. Figure 5 presents a simplified sketch of a grinder pump system. This type of wastewater collection system is economical in low density rural areas and in areas where the topography makes construction of gravity sewers unfeasible, such as areas around lakes. The existing collection system for Posey Regional Sewer District is a low pressure grinder system as well. An overall map that presents a possible low pressure sewer collection system layout is located in Appendix A.

Probable construction costs are presented in Table 2 for the Low Pressure Grinder Pump Sewer System Alternative. Probable O&M costs are also presented.

Figure 5
Low Pressure Grinder Pump Sewer System

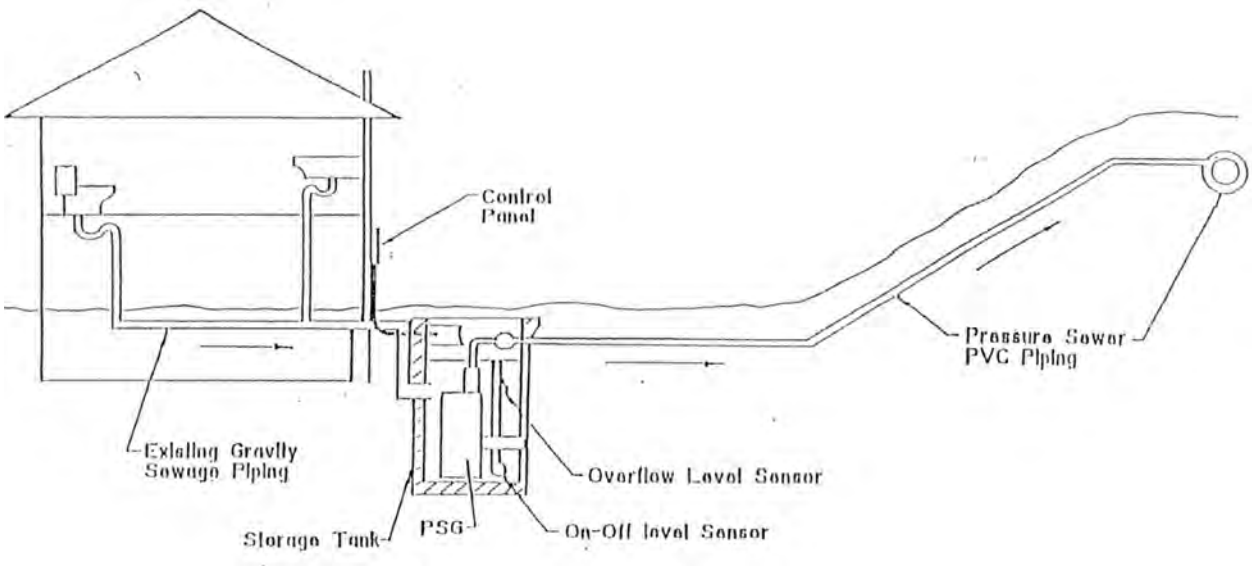


TABLE 2
PROBABLE CONSTRUCTION COSTS
FOR
LOW PRESSURE SEWER ALTERNATE
FOR THE
POSEY COUNTY REGIONAL SEWER DISTRICT
POSEY COUNTY, INDIANA
FEBRUARY, 2024

I. PROBABLE CONSTRUCTION COST

ITEM NO.	DESCRIPTION	ESTIMATED QUANTITY		ESTIMATED UNIT COST	ESTIMATED TOTAL COST
1.	1-1/2" FORCE MAIN, BY OPEN CUT PLACEMENT	220	L.F.	$ 23.00	$ 5,060.00
2.	2" FORCE MAIN, BY OPEN CUT PLACEMENT	4,280	L.F.	$ 25.00	$ 107,000.00
3.	3" FORCE MAIN, BY OPEN CUT PLACEMENT	4,320	L.F.	$ 28.00	$ 120,960.00
4.	4" PVC FORCE MAIN, BY OPEN CUT PLACEMENT	1,200	L.F.	$ 35.00	$ 42,000.00
5.	1-1/2" FORCE MAIN, BY UNCASED BORING	40	L.F.	$ 53.00	$ 2,120.00
6.	2" FORCE MAIN, BY UNCASED BORING	200	L.F.	$ 58.00	$ 11,600.00
7.	3" FORCE MAIN, BY UNCASED BORING	240	L.F.	$ 65.00	$ 15,600.00
8.	4" PVC FORCE MAIN, BY UNCASED BORING	120	L.F.	$ 81.00	$ 9,720.00
9.	1-1/4" SERVICE LINE, BY OPEN CUT PLACEMENT	8,400	L.F.	$ 22.00	$ 184,800.00
10.	1-1/4" SERVICE LINE, BY UNCASED BORING	2,100	L.F.	$ 51.00	$ 107,100.00
11.	SIMPLEX GRINDER PUMP STATION	70	EA.	$ 7,750.00	$ 542,500.00
12.	SERVICE CONNECTION AND CHECK VALVE ASSEMBLY	70	EA.	$ 1,900.00	$ 133,000.00
13.	CLEANOUT AND FLUSHING STATION	15	EA.	$ 6,000.00	$ 90,000.00
14.	AIR RELIEF VALVE	6	EA.	$ 9,000.00	$ 54,000.00
15.	4" OR 6" GRAVITY SERVICE LATERAL TO GRINDER PUMP STA.	3,500	L.F.	$ 45.00	$ 157,500.00
16.	CONNECTION TO EX. MAIN	3	EA.	$ 5,000.00	$ 15,000.00
17.	STONE BACKFILL AND RESURFACING	420	TON	$ 30.00	$ 12,600.00
18.	TRAFFIC CONTROL DURING CONSTRUCTION	1	L.S.	$ 25,000.00	$ 25,000.00
19.	MOBILIZATION, BONDS, AND INSURANCE	1	L.S.	$ 115,000.00	$ 115,000.00
20.	EROSION CONTROL PLAN AND IMPLEMENTATION	1	L.S.	$ 35,000.00	$ 35,000.00
				SUB-TOTAL	**$ 1,785,560.00**
				CONSTRUCTION CONTINGENCIES	$ 178,440.00
				TOTAL PROBABLE CONSTRUTION COSTS	**$ 1,964,000.00**

II. PROBABLE COLLECTION SYSTEM O&M COSTS

1.	LINE MAINTENANCE ($0.25 per inch diameter per linear foot per year)	$7,100.00
2.	TREATMENT COST (estimated at $5.40/1,000 gal + $723.30/mo. base charge)	$36,300.00
3.	INSURANCE	$3,900.00
4.	MISC. AND CONTINGENCIES	$4,700.00
	TOTAL ANNUAL O&M COSTS	**$52,000.00**

B. Present Worth Cost Analysis

The present value factor (P) is determined by:

$P = (1 / (1 + r)^n)$

Where: r = the discount rate, in this case 2.0%
(OMB Circular A-94 with a real interest rate of 2.0%)
n = the project term, 20 years

Employing the above figures, the present worth (P) of the salvage value at the end of a 50-year life expectancy is calculated by multiplying its value by 0.369

Present worth of annual operation, maintenance, and equipment replacement costs is calculated by multiplying its value by:

$[(1 + r)^n - 1] \div [r(1 + r)^n] = 19.184$

The following table presents the present worth analysis.

TABLE 3

Item	Alternate 2. Gravity Sewer Alternate	Alternate 3. Low Pressure Sewer Alternate
Cost Summary:		
a. Total Capital Cost	$4,558,000	$1,964,000
b. Annual O&M Increase	$103,000.00	$52,000.00
Present Worth Analysis:		
c. Total Capital Cost	$4,558,000	$1,964,000
d. Present Worth, Annual O&M (Present worth factor = 19.184)	$1,975,961	$997,572
e. Present Worth Salvage	$1,683,294	$725,316
(Present worth factor = 0.369)		
Total Present Worth, (c + d) minus e	**$4,850,666**	**$2,236,256**

Based upon the present worth analysis the Low Pressure Grinder Pump Sewer System Alternate is the lowest present worth cost and preferred alternative.

Chapter 5
Evaluation of Environmental Impacts

This chapter examines the potential environmental impacts of proposed improvements and the necessary mitigation measures. Due to the nature of the project, the wastewater system improvements will have a minor short-term impact. With proper mitigation efforts, those impacts can be minimized even further.

The evaluation of environmental impacts involves site inspection and a consideration of local land use, soils types and conditions, floodway issues, and coordination with state and local agencies. During the evaluation process, it is also appropriate to identify the various permits that may be required to implement a proposed alternative.

All improvements recommended as part of this project will be completed within existing right-of-ways or utility easements and along existing utility corridors where possible. Construction related impacts will be confined to areas of surface disturbance. Most land disturbance will be limited to superficial, localized disruption created by the entrance of construction traffic for delivery and installation of wastewater improvements. Mitigative measures should adequately address any temporary impacts associated with construction activities.

A. Wetlands

National Wetland Inventory Maps are presented in Appendix A. There will be no direct or indirect impacts to wetlands caused by the proposed project. All mitigation measures to lessen and compensate for wetland impacts cited in comment letters about the project from the Indiana Department of Natural Resources and the U.S. Fish and Wildlife Service will be implemented. Directional drilling will be utilized for stream crossings to avoid disturbance and impact.

B. Disturbed and Undisturbed Land

The project area is located along existing right-of-ways or utility easements and along existing utility corridors. There will be no significant impact to undisturbed land in the project area.

C. Surface Waters

None of the proposed improvements will adversely affect exceptional use streams, State resource waters, or Natural and Scenic Rivers. Normal sediment and erosion control management practices and good project site housekeeping will be utilized to minimize any impacts to surface water features. Directional drilling will be utilized for stream crossings to avoid disturbance and impact.

D. Groundwater

The proposed project will not have an adverse impact on the groundwater or other wells in this area. There should be minimal dewatering associated with typical construction practices as part of the project. There are no sole source aquifers in the project area.

E. 100 Year Floodplain

The project area was reviewed with respect to facilities located within a designated floodplain or floodway. No construction areas are located in or will negatively impact any 100-year floodplain areas. A Floodplain Map of the project area is included in Appendix B.

No adverse impacts to floodplains or floodways are anticipated, and no structures will be modified in a manner which could affect flood elevations or upstream drainage areas.

F. Plants and Animals

There are no known endangered or threatened species of plants or animals that would be negatively impacted within the planning area. All construction activity will be confined to existing utility easements or public right-of-way or utility corridors. There are no known occurrences of rare or State-listed animals or plants, significant natural communities, or other significant habitats, on or in the immediate vicinity of the project site.

G. Prime Farmland

No conversion of prime farmland and no construction will take place beyond existing utility corridors or adjacent to public right-of-ways. Any areas which are disturbed by construction activity will be immediately restored to original condition to re-establish ground cover.

If required by State or Local regulations, an erosion control plan will be implemented to prevent soil erosion. Any storm-water runoff from the site will be consistent conditions which existed prior to construction activity.

H. Air Quality

No permanent air emissions will be produced by the proposed project or its alternatives. There should be no odors produced by construction or minimal odors produced by the operation of the proposed facilities. Therefore, no indirect or cumulative impacts on air quality within the planning area should occur. Dust produced by construction may temporarily affect air quality. Project connected emission sources are machinery exhausts, workers' automobile exhausts and power supplier emissions due to electricity used by the project. All these sources will be direct and temporary impacts and will be controlled by requiring equipment to be in good working order.

To minimize the affects on air quality, construction activities shall be limited to normal working periods and proper clean up practices shall be implemented. Surface wetting practices will be utilized to control dust emissions. Any other mitigation measures cited in comment letters will be implemented. Air quality will not be adversely affected by this proposed project or any of its alternatives.

I. Open Space and Recreational Opportunities

Proposed construction activities will neither create nor eliminate open space or recreational opportunities within the project area.

J. Lake Michigan Coastal Program

The proposed project will have no impact on the Lake Michigan Coastal Zone.

K. Soils

Soils maps are provided from the Posey County soil survey mapping publications within the project area. The soils in the project area are conducive to construction activities and will not impact construction.

A soils map of the project area is provided in Appendix B.

L. National Natural Landmarks

There are no known National landmarks in the project area. Construction and operation of the proposed facilities will have no impact on National landmarks.

M. Mitigation Measures

The following mitigation measures will be required to be followed during construction along with any other mitigation measures cited in comment letters from the environmental agencies.

1. Upon completion of construction, the contractor shall remove all equipment, temporary structures, waste materials and rubbish from the vicinity of which the work was being done. The premises should be left in a neat and presentable condition. Therefore, there should be no negative indirect or cumulative impacts to the visual aesthetics of the project area.

2. No fill material will be placed in flood areas that would impact the floodplain and the original grade will be restored when work is completed. Directional drilling will be utilized for stream crossings to avoid wetland or stream disturbance and impact.

3. The area around all construction will be restored to its original condition after construction is complete to minimize the direct impacts. All disturbed areas of this project located outside of the pavement will be refurbished and seeded to establish cover and prevent erosion. Siltation devices or temporary seeding will be utilized, where applicable. The contractor in charge of construction must abide by local and state requirements to minimize soil erosion. This will include complying with IDEM's rule for construction storm water runoff, if applicable.

4. To minimize the affects on air quality, construction activities shall be limited o normal working periods and proper clean up practices shall be implemented. Surface wetting practices will be utilized to control dust emissions.

Chapter 6
Selected Plan

A. Scope of Recommended Improvements

Alternative 3 – 3. Low Pressure Grinder Pump Sewer System is the recommended alternative.

a. Description – This alternative includes installation of a low pressure grinder pump wastewater collection system in the Stewartsville area and connections to the 6" INDOT force main project. Flows from the current INDOT project are being conveyed to the Town of Poseyville and this project will connect and convey wastewater via. the planned INDOT force main sewer.

b. Design Criteria – Design, construct, and install all improvements in accordance with IDEM minimum standards of design for public wastewater collection systems. The collection system includes individual grinder pumps, 1-1/2" thru 4" force main sewers, and associated appurtenances.

c. Environmental Impacts – The construction would have minimal or short-term environmental impacts as described in the environmental chapter. Construction would not impact the 100-year flood plain or wetlands. After construction, all disturbed areas must be resurfaced to original conditions. Directional drilling will be utilized for stream crossings to avoid wetland disturbance and impact.

d. Land Requirements – The project area is located along existing right-of-ways or utility easements and along existing utility corridors where possible.

e. Potential Construction Problems – The problems associated with construction are believed to be minimal, but may include weather conditions, conflicts with other utilities, and coordination of the operation of the existing wastewater system during construction.

f. Conclusions – The proposed improvements will address short and long term needs of the Stewartsville community by eliminating septic systems.

As this is a preliminary engineering report, it is anticipated that the use of this document is largely for planning purposes and in support of funding activities. As the project moves forward, the project detailed scope and design will be developed and the costs will be refined as needed.

B. Project Cost

The cost estimates presented herein are considered appropriate for the level of detail associated with the preliminary planning phase of a project. The costs are based on budgetary information obtained from various equipment vendors and suppliers, available information and historical data from bid tabulations for public works projects of comparable work and similar scope. A contingency has been added to account for unanticipated costs which may be incurred during the course of construction. This approach reduces the likelihood of budget surprises when the detailed design and bidding process is complete.

The estimated capital costs for the recommended improvements were presented in Table 2. Table 4 summarizes the capital improvement costs and includes estimated non-construction costs associated with design, bidding, construction engineering, legal, bond counsel, rate consulting fees, etc.

TABLE 4
PROBABLE PROJECT COST
FOR
WASTEWATER SYSTEM IMPROVEMENTS - LOW PRESSURE GRINDER PUMP SEWER SYSTEM
FOR THE
POSEY COUNTY REGIONAL SEWER DISTRICT
POSEY COUNTY, INDIANA
FEBRUARY, 2024

I. PROBABLE CONSTRUCTION COST		**$1,964,000.00**
II. PROBABLE NON-CONSTRUCTION COSTS		
1.	Administration and Labor Standards	$25,000.00
2.	Permits & Advertising	$2,000.00
3.	Legal	$30,000.00
4.	Rate Accountant	$35,000.00
5.	Bond Counsel	$40,000.00
6.	Preliminary Engineering	$26,000.00
7.	Engineering	$187,000.00
8.	Bidding, General Observation / Contract Administration	$49,000.00
9.	Inspection	$168,000.00
10.	Archaeological for Environmental Review (if required)	$15,000.00
11.	SRF Closing Fees	$35,000.00
12.	County Highway Bond (if required)	$50,000.00
13.	Non-Construction Contingencies	$33,000.00
	TOTAL PROBABLE NON-CONSTRUCTION COST	**$695,000.00**
III. PROBABLE PROJECT COST		**$2,659,000.00**
IV. PROBABLE ANNUAL ADDED O&M COSTS		**$52,000.00**

C. Project Implementation Schedule

Implementing the recommended improvements would follow a normal progression of design, public bidding and construction, with time allowed for regulatory review and plan approvals.

The following time-table presents the anticipated schedule of activities and milestones assuming SRF funding of the project along with a possible bond anticipation note to cover preliminary costs. A bond anticipation note (BAN) is a short-term interest-bearing security issued in advance of a larger, future funding instrument. The issuing entity uses the note as short-term financing, with the expectation that the proceeds from a larger, future bond issue will cover the costs of the short-term security.

TABLE 5
Project Implementation Schedule

Task Description	Est. Completion Date
SRF Loan/Grant Application and PER Submittal	March, 2024
Public Hearing for PER	April, 2024
Anticipated SRF Project Approval / Grant and Loan Offer	July/August, 2024
Proceed with Design, Plans, and Specifications	August, 2024
Submit Final Plans and Specifications to IDEM	December, 2024
Advertise for Bids	December, 2024
Receive Bids for Construction Project	January, 2025
Receive IDEM Construction Permit	February, 2025
Award Construction Contract	March, 2025
SRF Loan Closing	March, 2025
Initiate Construction	April, 2025
Substantial Completion of Construction	December, 2025
Final Completion, Initiate Operation	January, 2026

Chapter 7 – Legal, Financial, Managerial Capabilities

1. Management Resolutions

a. Designated Signatory Authorization refer to **Appendix C**

b. Preliminary Engineering Report Acceptance Resolution refer to **Appendix** C

2. Financial

a. SRF Financial Information Form refer to **Appendix C**

3. Land Acquisition

No land acquisition is anticipated as part of this project.

4. Asset Management Program (AMP)

The Posey County Regional Sewer District has developed an Asset Management Program that meets the requirements defined by the State Revolving Fund's Asset Management Program Guidelines pursuant to Indiana Code 5-1.2-10-16.

Chapter 8 – Public Participation

1. Public Participation Information

As required, the Posey County Regional Sewer District will hold a public hearing on _____ to discuss the project. The public hearing will be advertised in the local newspaper ten (10) days prior to the hearing. The PER will be available for public review 10 days prior to the hearing. Comments will be accepted at the hearing and for five (5) days afterwards.

The following items will be included in **Appendix D** of this report once available:

• Notice of Public Hearing

• Publisher's Affidavit

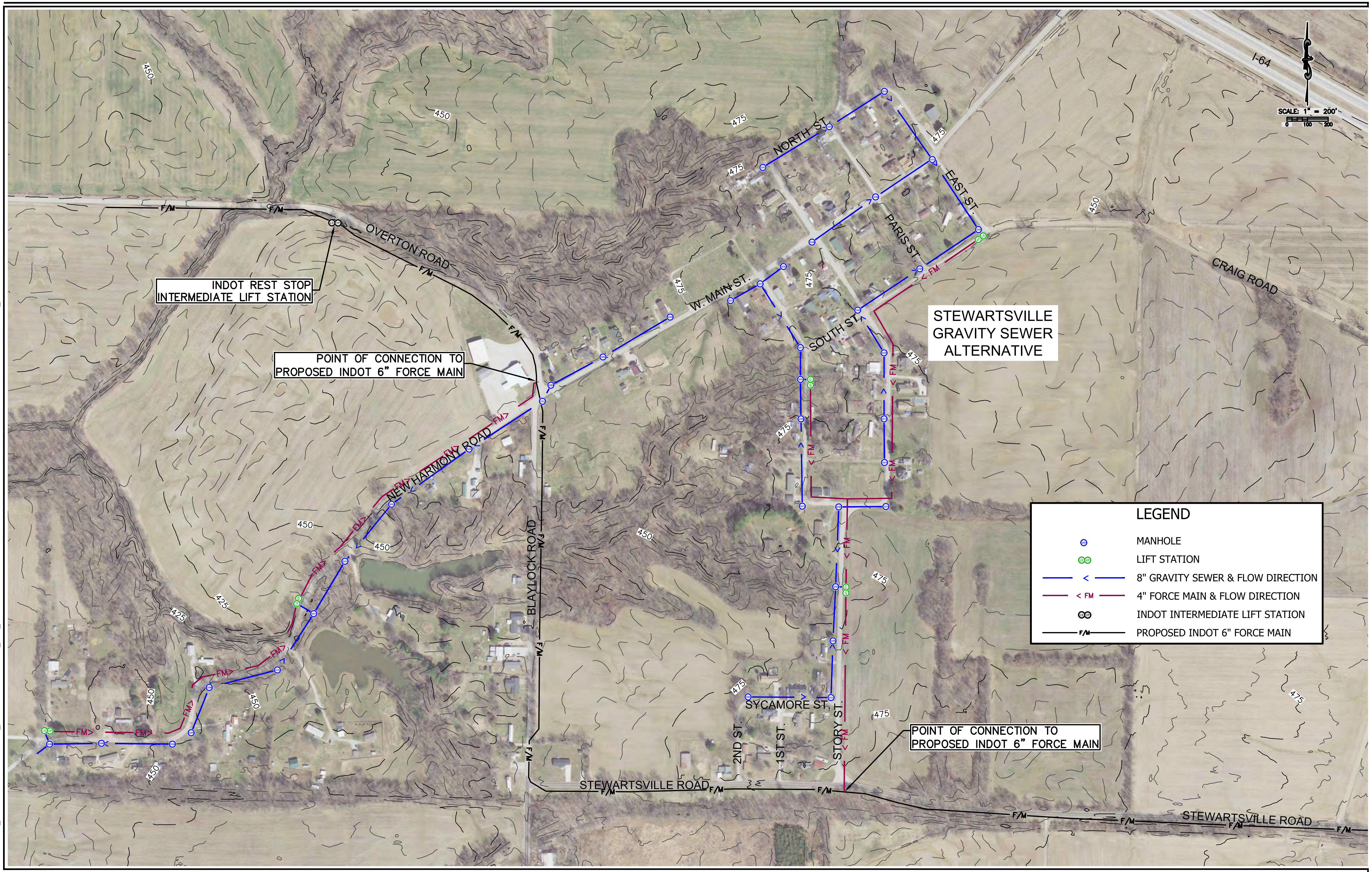
• Sign-in sheet from hearing

• Public Hearing meeting minutes

• Written comments (if applicable)

• Mailing labels

APPENDIX A
IMPROVEMENTS ALTERNATIVES CONSIDERED MAPS



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Quality Engineering Services Since 1959
6809 Corporate Drive, Indianapolis, IN 46278 Ph: 317-334-0262
802 West Broadway St., P.O. Box 295 Loogootee, IN 47553 Ph: 812-295-2800
Website: midwesterneng.com Fax: 812-295-2801 Email: meinc@midwesterneng.com
Consultants · Civil · Mechanical · Electrical

WASTEWATER SYSTEM IMPROVEMENTS
STEWARTSVILLE AREA
FOR THE
POSEY COUNTY REGIONAL SEWER DISTRICT
POSEY COUNTY, INDIANA

REVISIONS

PRELIMINARY
NOT FOR CONSTRUCTION

DATE	JAN. 2024
DESIGN	R.D.W.
DRAWN	M.K.W.
Q.C. CHECK	.
PROJECT NUMBER	2023019-00

1

1 OF 1

FILE NUMBER

Author: MWILKINS; Plot Date: 1/15/2024 3:44 PM
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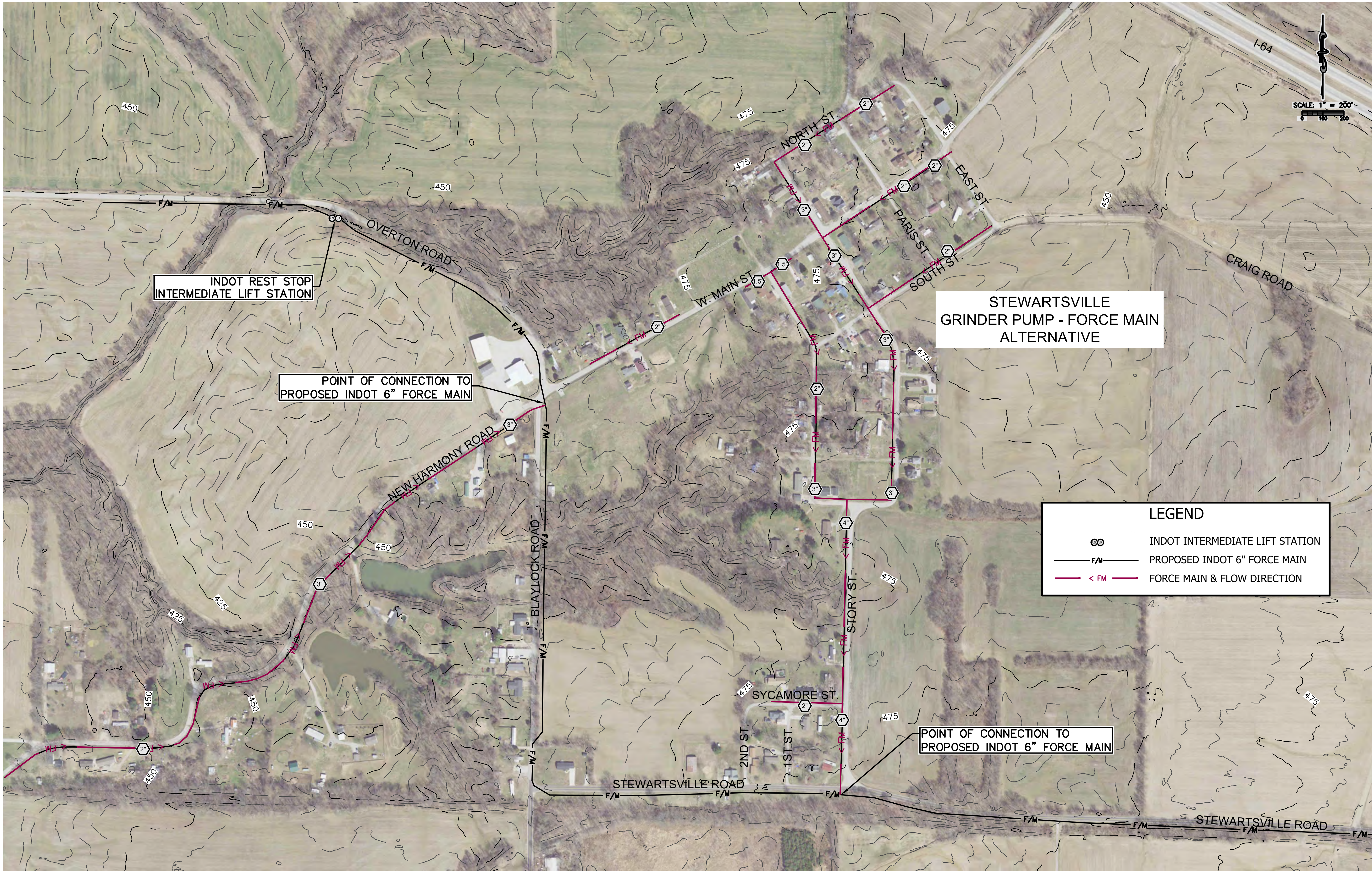

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WASTEWATER SYSTEM IMPROVEMENTS
STEWARTSVILLE AREA
FOR THE
POSEY COUNTY REGIONAL SEWER DISTRICT
POSEY COUNTY, INDIANA

REVISIONS

PRELIMINARY
NOT FOR CONSTRUCTION

DATE: JAN. 2024
DESIGN: R.D.W.
DRAWN: M.K.W.
Q.C. CHECK:
PROJECT NUMBER: 2023019-00

1

1 OF 1

FILE NUMBER

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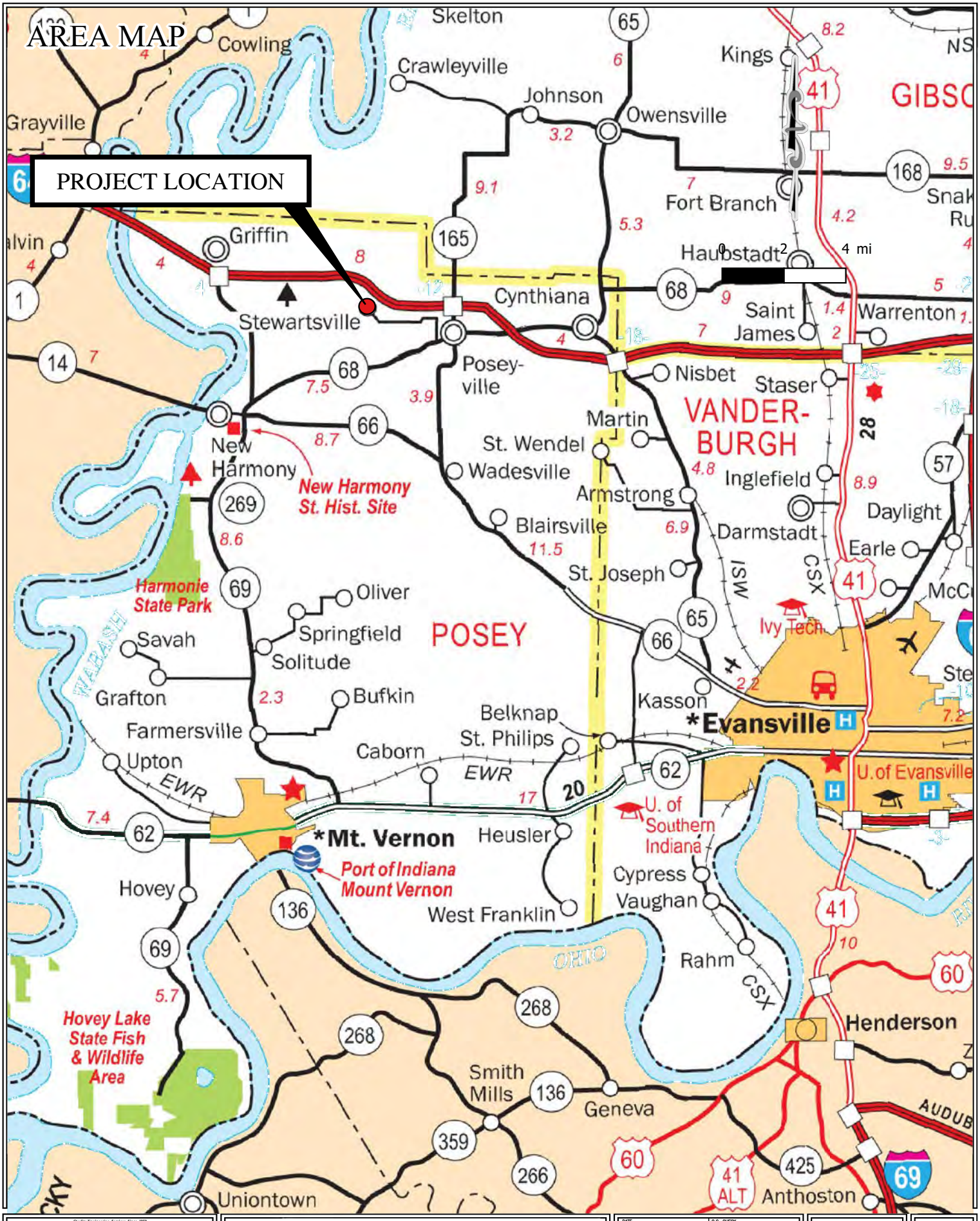
APPENDIX B
ENVIRONMENTAL MAPS



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WASTE WATER SYSTEM PER STEWARTSVILLE
FOR THE
POSEY COUNTY RURAL SEWER DISTRICT
POSEY COUNTY, INDIANA

DATE	Q.C. CHECK
JAN. 2024	
DESIGN	PROJECT NUMBER
RDW	2023019-00
DRAWN	FILE NUMBER
MKW	

FIGURE 1 of

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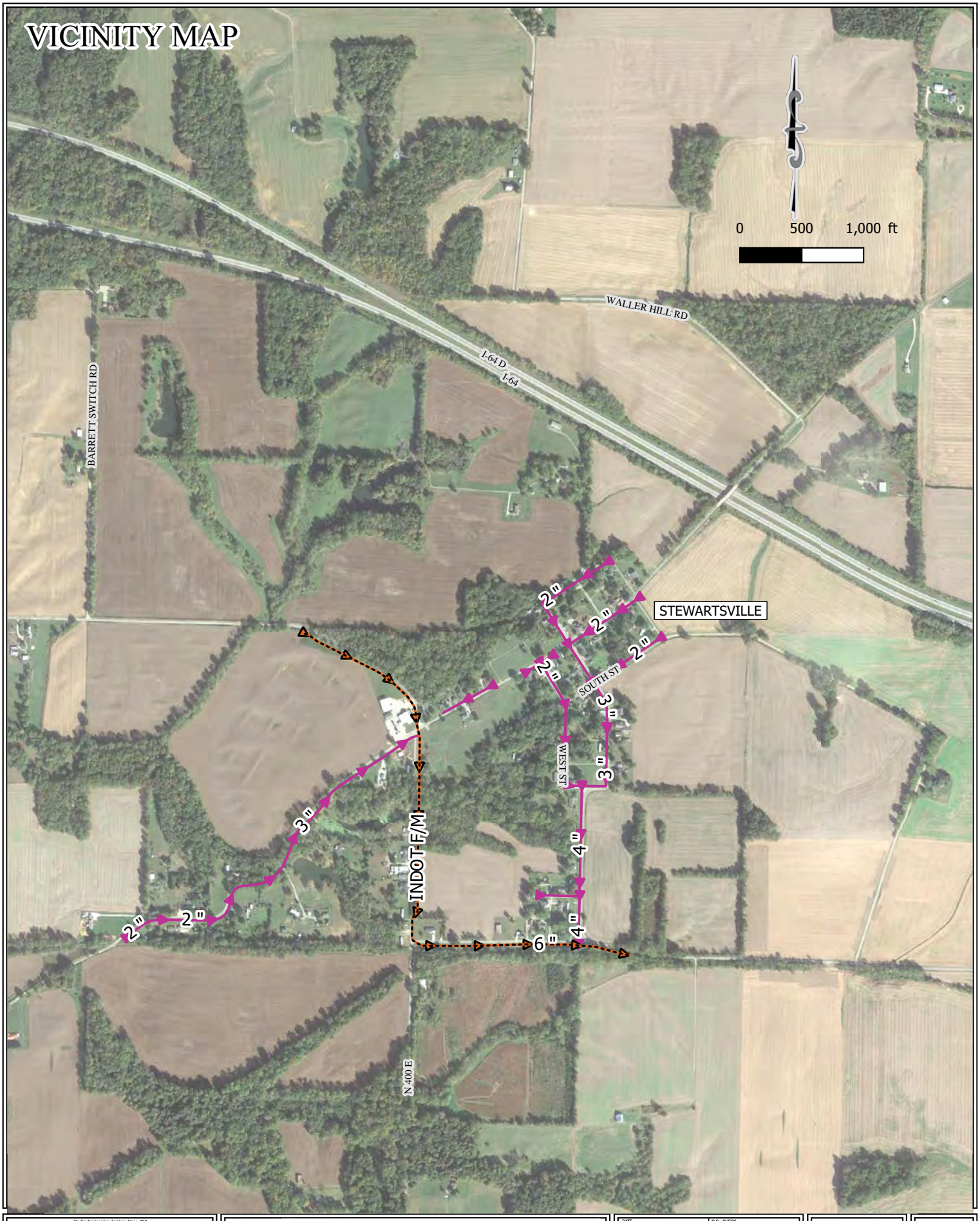
VICINITY MAP



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802 West Broadway St., P.O. Box 295 Logootee, IN 47553 Ph: 812-295-2680
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WASTE WATER SYSTEM PER STEWARTSVILLE
FOR THE
POSEY COUNTY RURAL SEWER DISTRICT
POSEY COUNTY, INDIANA

DATE: JAN. 2024	Q.C. CHECK:
DESIGN: RDW	PROJECT NUMBER: 2023019-00
DRAWN: MKW	FILE NUMBER:

FIGURE 2 OF ____

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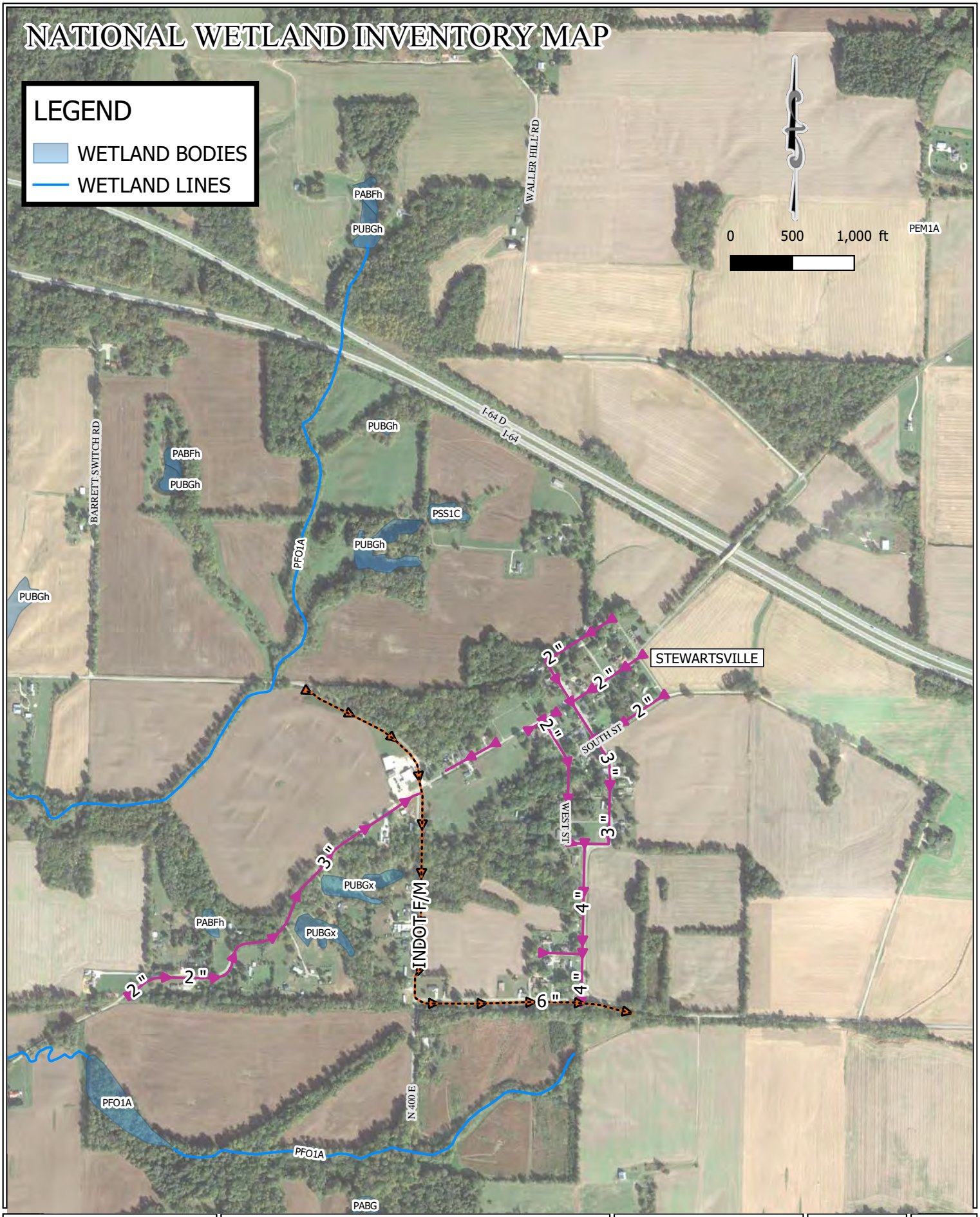
NATIONAL WETLAND INVENTORY MAP



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WASTE WATER SYSTEM PER STEWARTSVILLE
FOR THE
POSEY COUNTY RURAL SEWER DISTRICT
POSEY COUNTY, INDIANA

DATE	Q.C. CHECK
JAN. 2024	
DESIGN	PROJECT NUMBER
RDW	2023019-00
DRAWN	FILE NUMBER
MKW	

Midwestern Engineers, Inc.
802 West Broadway Street
P.O. Box 295
Loogootee, Indiana 47553

FIGURE
3
___OF___

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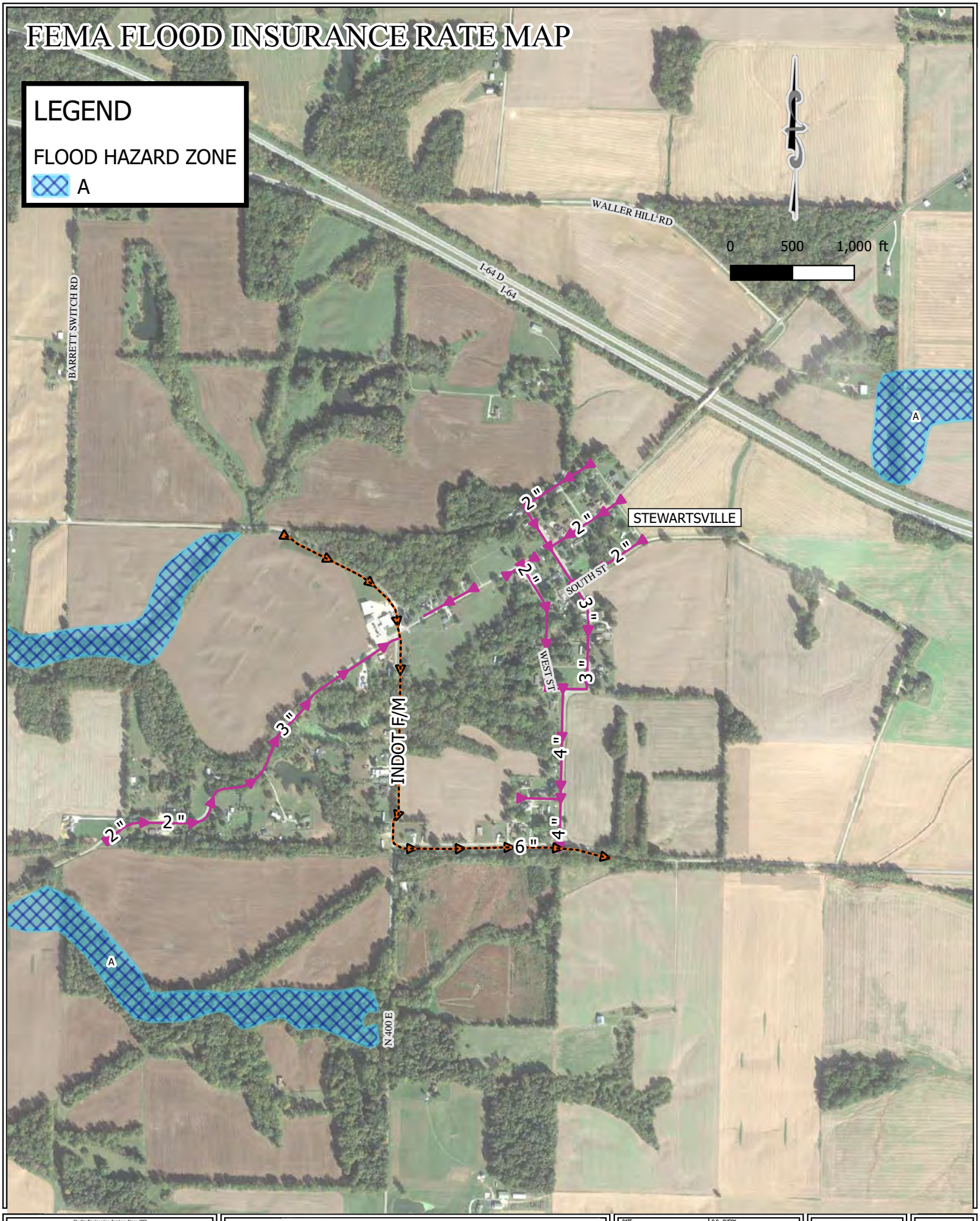
FEMA FLOOD INSURANCE RATE MAP



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WASTE WATER SYSTEM PER STEWARTSVILLE
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POSEY COUNTY RURAL SEWER DISTRICT
POSEY COUNTY, INDIANA

DATE: JAN. 2024	Q.C. CHECK:
DESIGN: RDW	PROJECT NUMBER: 2023019-00
DRAWN: MKW	FILE NUMBER:

FIGURE 4 of ___

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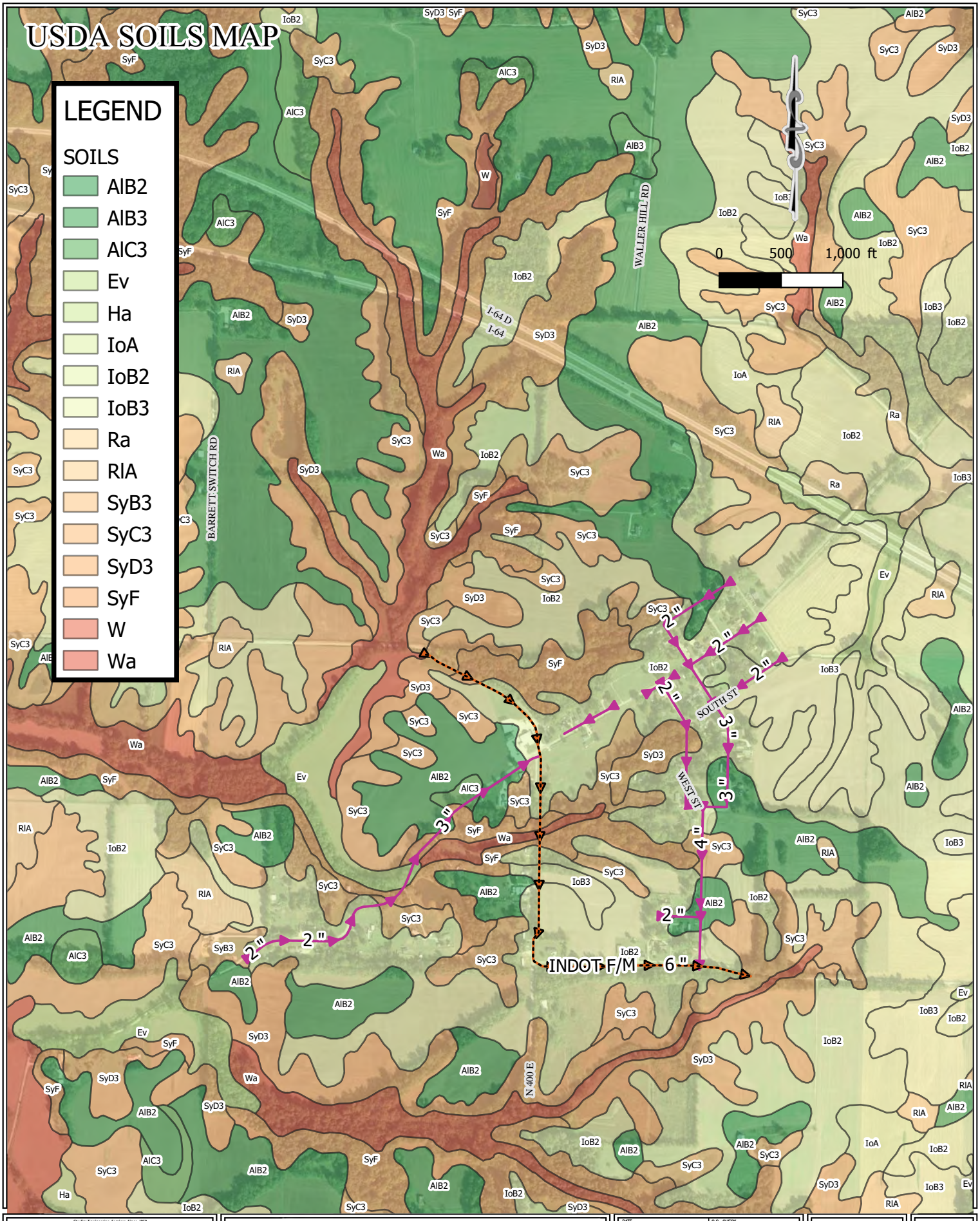

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FOR THE
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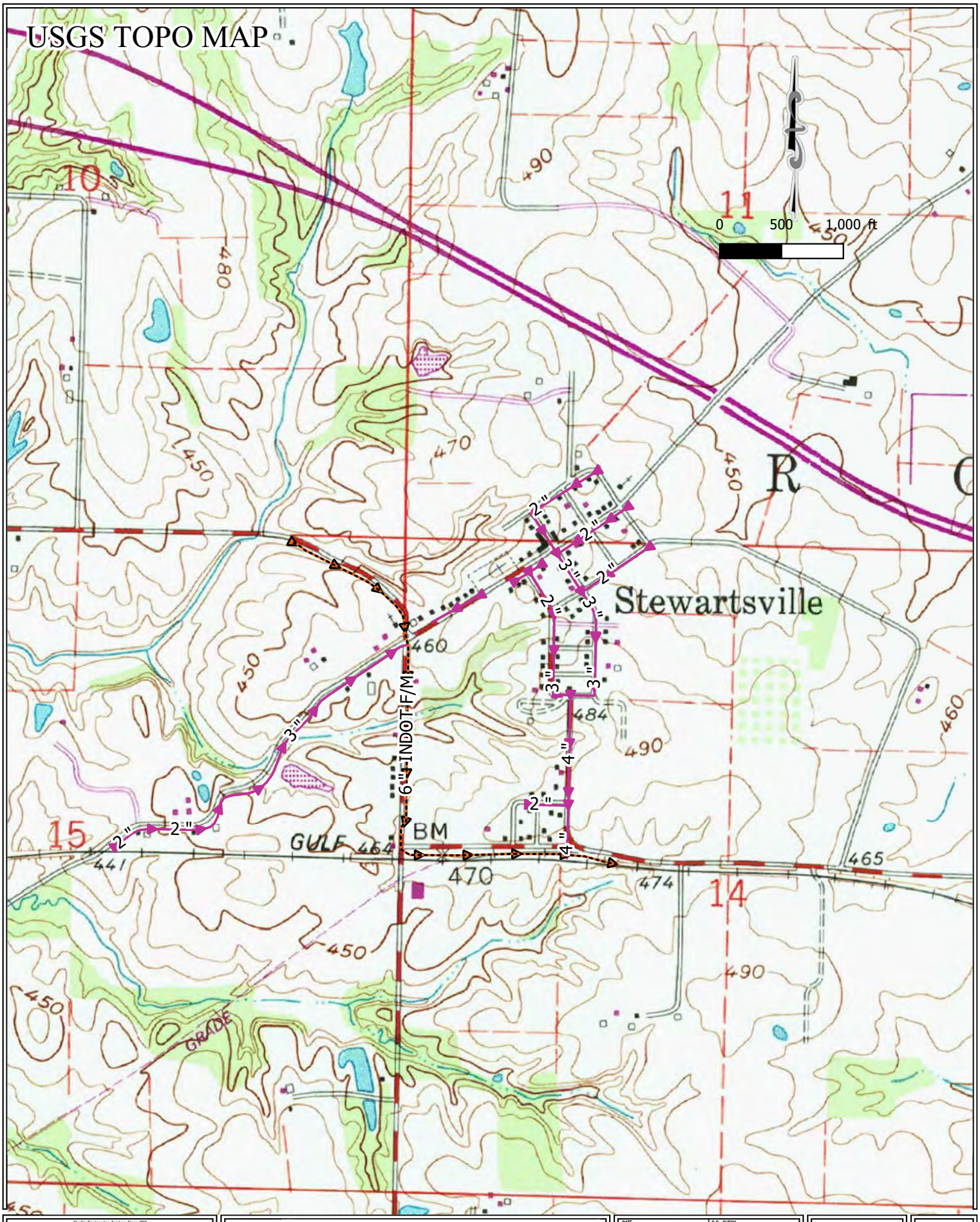
POSEY COUNTY, INDIANA

DATE: JAN. 2024	Q.C. CHECK
DESIGN: RDW	PROJECT NUMBER: 2023019-00
DRAWN: MKW	FILE NUMBER

FIGURE 5 OF ___

Path: Z:\CAD_Incomplete\2023019-00_Posey County RSD_WW_Marrs Center & Stewartsville\020_Preliminary\POSEY COUNTY RSD - Waste Water System PER\Posey

USGS TOPO MAP



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POSEY COUNTY, INDIANA

DATE: JAN. 2024	Q.C. CHECK:
DESIGN: RDW	PROJECT NUMBER: 2023019-00
DRAWN: MKW	FILE NUMBER:

Midwestern Engineers, Inc.
802 West Broadway Street
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Loogootee, Indiana 47553

FIGURE 6 OF ____

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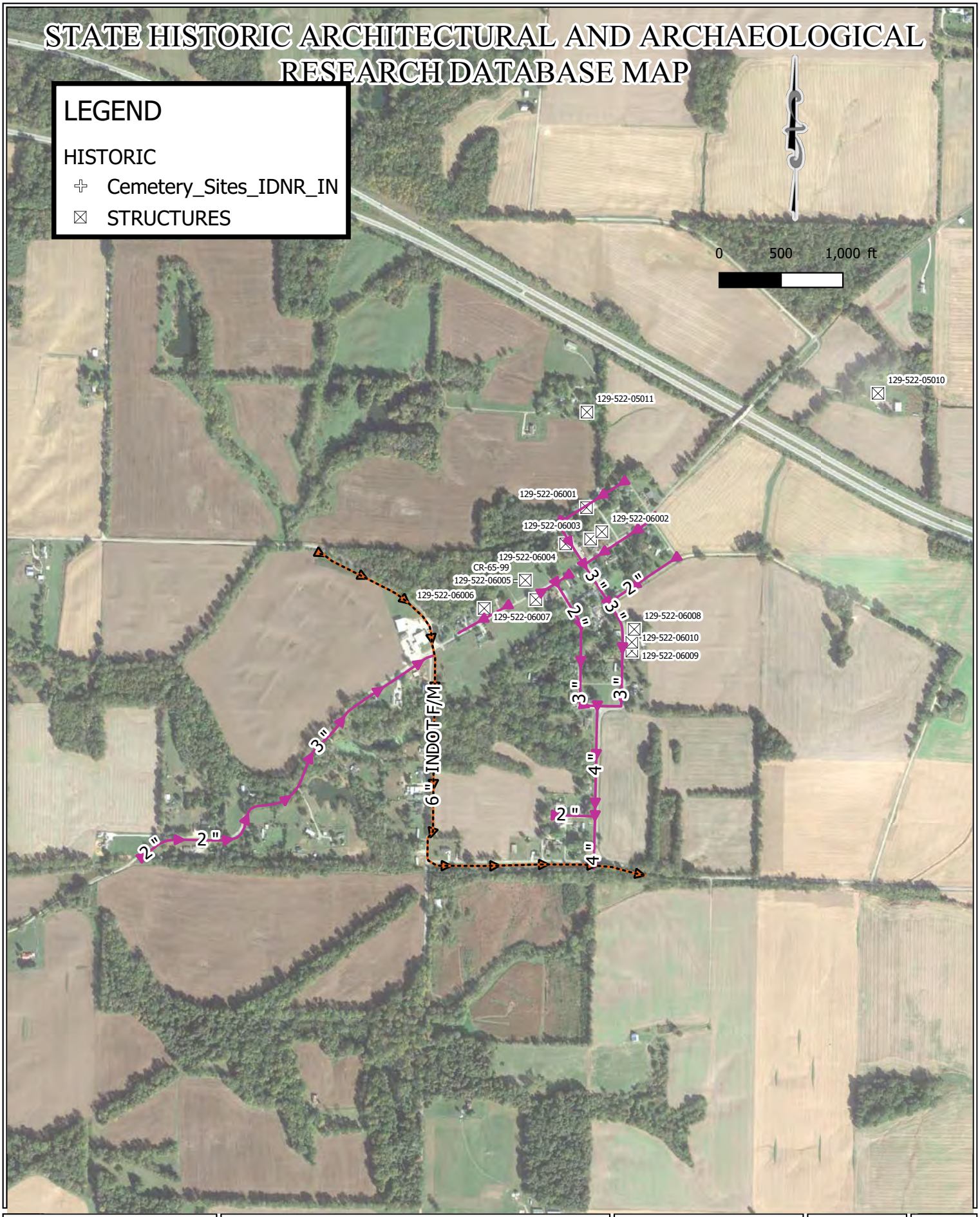
STATE HISTORIC ARCHITECTURAL AND ARCHAEOLOGICAL RESEARCH DATABASE MAP



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DATE: JAN. 2024	Q.C. CHECK:
DESIGN: RDW	PROJECT NUMBER: 2023019-00
DRAWN: MKW	FILE NUMBER:

FIGURE 7 of ___

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STATE HISTORIC ARCHITECTURAL AND ARCHAEOLOGICAL RESEARCH DATABASE MAP

SHAARD_ID	NAME	RATING	GLOBALID
CR-65-99	Stewartsville		
129-522-06005	Stewartsville Cemetery	Contributing	{D84A9BC8-61D3-4947-8EFA-144801D54570}
129-522-06007	House	Contributing	{E1F1577A-6EB9-4A43-9A11-FA56729DE3C5}
129-522-05010	Huebler House	Contributing	{EEFB57F0-9162-44BA-A1FD-5F2AFE0306DE}
129-522-06010	House	Contributing	{4CB1FB13-B54D-44E6-A333-CC2448D7C2E2}
129-522-06009	House	Contributing	{17E5887A-AC91-40B0-BFC6-F1D970084C0D}
129-522-06001	House	Contributing	{DCE98586-09B1-4685-9363-A20A13085199}
129-522-06003	Hines House	Contributing	{449BA9E7-33A0-45C7-9DC5-167C0EDBFD70}
129-522-06004	Commercial Building	Contributing	{52BADFF1-707F-40A9-82C7-118DF3864101}
129-522-06006	House	Contributing	{0F49547D-4839-4F59-962B-B3B1CAF25B82}
129-522-06008	House	Notable	{16538839-E327-49BC-8C2F-813597E807B0}
129-522-06002	Falls House	Notable	{6B8CBD65-AA44-4C6B-B8FD-B84632A20540}
129-522-05011	Farm	Notable	{5A5833B5-47BD-4673-A3AB-3799485DB9FC}

APPENDIX C
SRF RESOLUTIONS AND FORMS

AUTHORIZED REPRESENTATIVE RESOLUTION

WHEREAS, the Posey County Regional Sewer District of Posey County, Indiana, herein called (the "Participant"), has plans for a wastewater infrastructure improvement project to meet State and Federal regulations, such as the NPDES discharge limitations, and the district intends to proceed with the construction of such works if adequate funding is available:

WHEREAS, the Posey County Regional Sewer District has adopted this Resolution dated March 22, 2024.

NOW, THEREFORE, BE IT RESOLVED by the Posey County Regional Sewer District, the governing body of said Posey County Regional Sewer District, that:

1. Pat Beamon be authorized to make application for an SRF Loan and provide the State Revolving Fund Loan Program such information, data and documents pertaining to the loan process, including but not limited to all loan closing documents such as the financial assistance agreement, bond specimen, etc. as may be required, and otherwise act as the authorized signatory of the district.

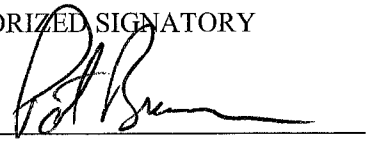
2. The district agrees to comply with all requirements of the Indiana Finance Authority, the State of Indiana and all Federal requirements as they pertain to the SRF Loan Program.

3. That two copies of the resolution be prepared and submitted as part of the district's Preliminary Engineering Report.

ADOPTED this 22nd day of March, 2024.

The Posey County Regional Sewer District of Posey County, Indiana By and Through Its Board

AUTHORIZED SIGNATORY

BY: 

Pat Beamon, President

ATTEST: 

Scot Keller, Secretary

PER ACCEPTANCE RESOLUTION

WHEREAS, the Posey County Regional Sewer District of Posey County, Indiana, has caused a Preliminary Engineering Report, PER, dated March, 2024, to be prepared by the consulting firm of Midwestern Engineers, Inc.; and

WHEREAS, said PER has been presented to the public at a public hearing held April , 2024, for their comments; and

WHEREAS, the Posey County Regional Sewer District Board finds that there was not sufficient evidence presented in objection to the recommended project in the Preliminary Engineering Report.

NOW, THEREFORE, BE IT RESOLVED by the Posey County Regional Sewer District, the governing body of said Posey County Regional Sewer District, that:

The Wastewater System Improvements Preliminary Engineering Report dated March, 2024 be approved and adopted by the Posey County Regional Sewer District Board; and That said PER be submitted to the State Revolving Fund Loan Program for review and approval.

Passed and adopted by the Posey County Regional Sewer District Board this ____ day of April, 2024.

AUTHORIZED SIGNATORY

BY: _____

Pat Beamon, President

ATTEST: _____

Scot Keller, Secretary

APPENDIX D
PUBLIC PARTICIPATION

NOTICE OF PUBLIC HEARING
POSEY COUNTY REGIONAL SEWER DISTRICT
WASTEWATER PRELIMINARY ENGINEERING REPORT (PER)

The Posey County Regional Sewer District will hold a public hearing at _____ __.M. CT on_____, April __, 2024, at the Hovey House, 330 Walnut Street, Mt. Vernon, Indiana 47620. The District's engineering consultant, Midwestern Engineers Inc., will present the recommended project, which will include construction of a wastewater collection system in and near the Community of Stewartsville. The project will be funded, in part, through a Wastewater State Revolving Fund (WWSRF) loan.

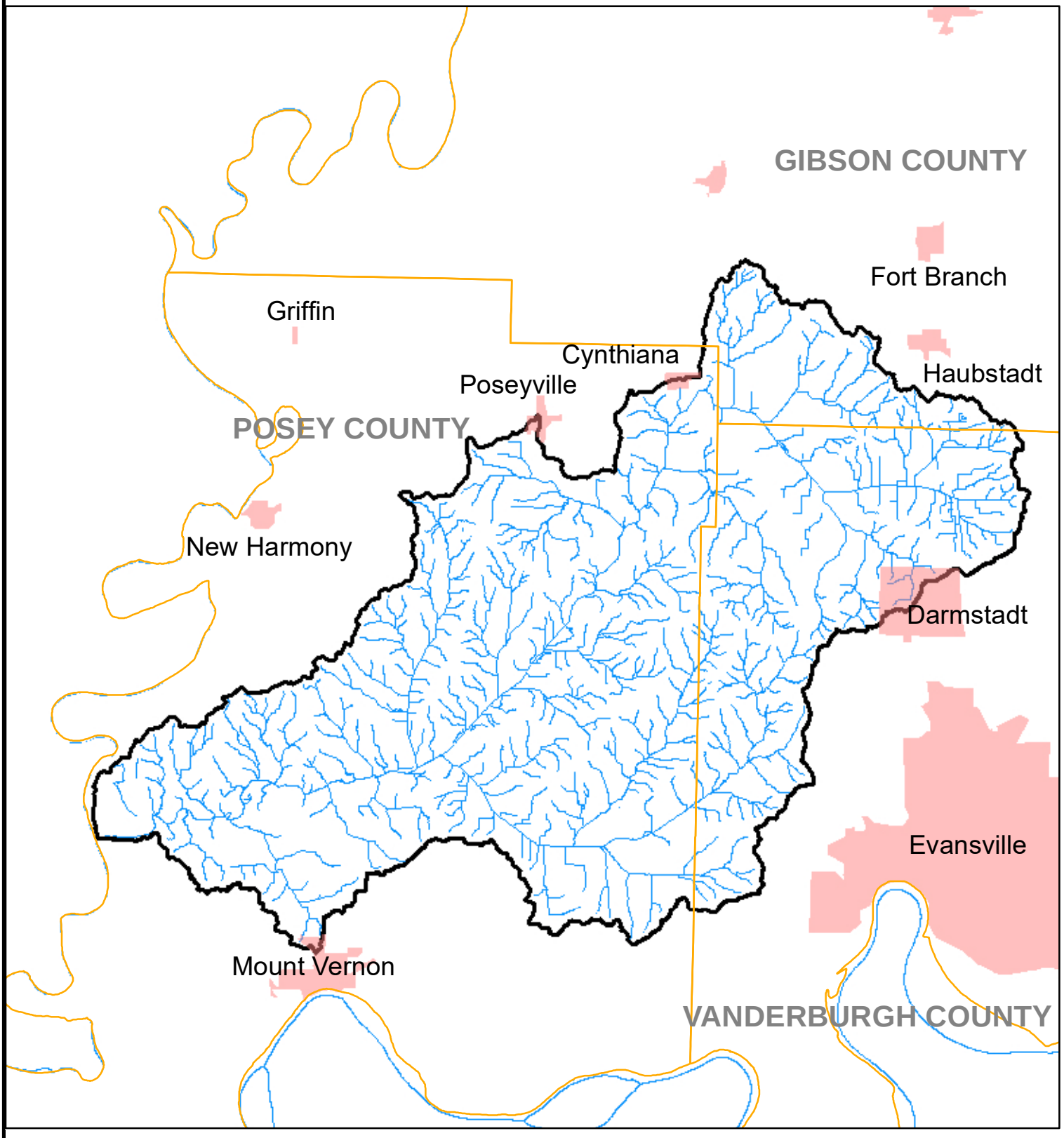
Copies of the required Preliminary Engineer Report (PER) are available for public viewing starting _____, 2024 through _____, 2024 at _____ _____.

There will be the opportunity for questions and comments from the public at this meeting. Written comments from the public will be accepted five (5) days following the public hearing. Your participation is welcomed and encouraged. If you will require special assistance at the meeting, please contact _____, (812)_____. Written comments regarding this project should be sent to _____ _____ prior to _____,2024.

Publish One time – (10 DAYS PRIOR TO PUBLIC HEARING)
Posey County News

APPENDIX E
E. COLI WATER QUALITY DATA

Big Creek WMP 7-6



This map is intended to serve as an aid in graphic representation only. This information is not warranted for accuracy or other purposes.

Mapped By:
Tom Reeve, Office of Water Quality
Date:06/06/2012

0 1 2 4 Kilometers
0 1 2 4 Miles

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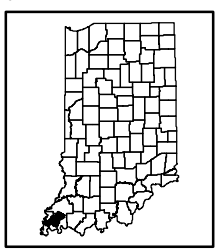


IDEM

Legend
- Watershed
- Streams
- Incorporated Area
- County

Sources:
Data - Obtained from the State of Indiana Geographical Information Office Library
Map Projection: UTM Zone 16 N **Map Datum:** NAD83

Big Creek Watershed Management Plan

Prepared with funding from the EPA 205(j) Grant

By Blair Borries & the Posey County Soil & Water Conservation District

Final Draft: 2/21/2009

Problem Statement #2 – Pathogens

Concern	Section(s) Validated
contaminants in the water	3.1.1, 3.1.2, 3.1.3
confined feeding	
groundwater quality	NOT VALIDATED, no data
packaged sewer treatment facilities	NOT VALIDATED, see section 3.5
contaminants in the water	3.1.3
lack of centralized wastewater treatment opportunities	3.6
lack of filter/buffer strips	3.3.1, 3.4
surface water quality	3.1.3
Pastures*	3.3.6

E. coli levels above the state standard for full body contact have been found in 100% of Big Creek waterways and levels above the recommended threshold for partial body contact have been found in about 10% of the waterways. *E coli* is an indicator that pathogens harmful to human and animal health are likely present. Pathogens make the streams and creeks unsafe, limiting recreation and fishing.

Stressors:
E. coli
Fecal Coliforms
other blood-borne pathogens

Sources:

1. *Households with Septic Systems and field bed areas or direct discharges*

   Septic systems are the only available wastewater treatment options in areas within the watershed not served by municipal sewer lines. Septic systems generally consist of a septic tank that allows for solids to settle and a field bed that spreads the liquid effluent out over the subsurface so that biological treatment can occur as it percolates through the sub-soil. Some households may still have a system that only has a septic tank and a direct discharge pipe rather than a field bed, but the number is not known. Septic systems are not a perfect system for treating contaminants associated with wastewater. When a confining layer restricts groundwater from traveling downward, or when excessive soil moisture occurs, groundwater will move up rather than down carrying untreated wastewater containing pathogens and other pollutants to the surface. High

water use or a system not sized for the amount of people using it can also increase this occurrence of this phenomenon.

The number of households with septic systems was estimated by identifying the areas with municipal services available and using 2000 census data to determine the households within these areas. Acreage for the field beds was estimated by multiplying the household value by a normal field bed area of 4000 square meters. This is useful in comparing the magnitude of the source to other area based magnitudes.

2. *Manure Use and Storage at Confined Feeding Operation*

Runoff containing recently applied manure or improperly stored manure often contains *E. coli* and other pathogens. Manure is applied to crop fields to increase fertility and to deal with the waste associated with confined animal production. Solid manure produced at poultry operations and on feedlots is surface applied with a spreader. Semi-solid and liquid manure produced at dairy operations and hog operations respectively is surface applied with a pump or injected into the soil. It is a common practice to maintain aerobic activity in lagoons by pumping the liquid onto crop land. Manure stored at these sites can contaminate runoff when it is stored without a roof or a densely vegetated filter area.

Sources were identified as farms with Confined Feeding Operation permits from IDEM. The amount of manure produced at a farm depends on the amount of animals and their weight. Any farm meeting or exceeding a threshold number of animals based on the weight of the animal must apply for a confined feeding operation permit under Indiana law. Assuming these farms do in fact have this threshold of animals at any given time, they are the most likely to produce the most manure. And since transportation costs are usually the most prohibitive in the reasons for not using manure as fertilizer, areas surrounding these CFOs can be considered likely areas where the manure is applied.

3. *Livestock with Stream Access*

Pastures along streams that do not have fencing or an appropriate stream crossing for livestock expose especially sensitive stream side areas to trampling and compaction from the increased livestock traffic. This results in the destruction of the stream-side buffer that would normally filter upslope runoff and for the animals in the stream, bypasses the filtering altogether. In addition when animals walk in the stream bottoms, they may dislodge particles containing *E. coli* and other pathogens engaging them in stream flow during sensitive dry periods.

4. *Bare Pasture Areas*

Bare areas in pastures occur primarily where livestock congregate (typically feeding areas, watering areas, and shaded areas), regularly travel (i.e. cow paths), or in pastures that are not large enough to support the number of animals that graze there. These areas are subject to high levels of sheet and rill erosion especially on steep slopes due to the lack of vegetative cover. The sediment transported through runoff may carry *E. coli* and other pathogens and un-vegetated areas cannot filter polluted runoff as effectively as a densely vegetated pasture area.

5. *Ponds and Lagoons in Need of Repair*

Ponds are often used in association with livestock as a watering source and a way to break up steep slopes that are common in pastures. Lagoons are commonly associated with storage of semi-solid manure and feedlots and are located immediately downstream of these areas to collect liquids and runoff. Ponds and lagoons are in need of repair and become a source when dams begin to break or when spillways are no longer covered in dense vegetation that can filter contaminated runoff when the ponds and lagoons overflow.

6. *Dead Wildlife in Streams*

   Although inconsequential during wet periods, dead wildlife left in streams by natural causes or by people can cause *E. coli* and other pathogen problems during low flow dry periods. Sources were identified during water monitoring rounds, but it is not clear if these areas experience sources each year.

4.2.1: Source Locations & Magnitudes

Table 4.2.1-A: Pathogen Sources: Locations and Magnitude shows the magnitude and location of the pathogen sources. This table provides both an assessment of the magnitude and location of the source and evidence that the source is significant according to the impairments associated with pathogens. The amount of each sources as determined from the results of the windshield and GIS inventory is compared to the level of impairment determined from the water monitoring that occurred through the project. The amount or magnitude of the source is shown as area or length of each source occurring in each sub-watershed. The impairment based on E. coli shown in the right hand columns of the table. "Partially impaired" indicates that a sub-watershed exceeded standards between 1 and 10% of the samples collected. "Impaired" indicates that the sub-watershed exceeded standards between 11 and 50% of the samples collected. "Severely impaired" indicates that the sub-watersheds exceeded standards greater than 50% of the samples collected. Two hundred thirty five colonies/100 mL is used as the E. coli standard. This relationship is described below for each of the sources.

In general, the impact of the sources is best understood when they are considered all together since each source can affect the level of *E. coli* during different hydrologic conditions. The combination of several sources results in a higher incidence of impairment than a greater magnitude of a single source, though a higher load of *E. coli* will commonly be associated with larger magnitudes.

1. *Households with Septic Systems and field bed areas or direct discharges*

   The relationship between the number of households on septic systems or in other terms the estimated acreage of field beds is difficult to confirm since the amount of households with septic systems is consistent throughout much of the watershed and every sub-watershed was found to be impaired for *E. coli.* The number of households with septic systems varies from 156 in the Big Creek – Alexander Creek Sub-watershed to 2129 households in the Little Creek – Headwaters Sub-watershed. Areas with the lesser number of households did not necessarily fall into the impaired rather than severely impaired category. This may be due in large part to the care and condition of individual septic systems which is independent of their geographic location. There is, however, a stronger relationship between the number of households and the percent reduction needed to achieve the standard. This phenomenon is explained above and relates to

the variations in impact for each source depending on hydrologic condition. A combination of several sources will result in a higher incidence of water standards being exceeded even though the magnitude of a single source in another sub-watershed is greater.

2. *Manure Use and Storage at Confined Feeding Operation*

   The number of confined feeding operations did have a correlation to the incidence of water samples exceeding the *E. coli* standard. This is most clear in the Pond Flat – Headwaters (010), Pond Flat – Jordan Creek (030), and Buente Creek – Maidlow Ditch (020) Sub-watersheds. These sub-watersheds had 1, 2, and 3 CFOs respectively and all three were found to be severely impaired based on the *E. coli* standard. As discussed seperately, however, each of these sub-watersheds also possessed additional sources including households with septic systems, bare pasture areas, and livestock with stream access. Other sub-watersheds that included CFOs and were found to be severely impaired based on *E. coli* included the Little Creek – Lower and Barr Creek Sub-watersheds with one in each. This accounts for 5 of the 6 sub-watersheds found to be severely impaired.

3. *Livestock with Stream Access*

   Livestock with stream access in 9 of the 16 sub-watersheds and in combination with other sources is associated with an increased number of water samples exceeding the standard. In the Big Creek – Alexander Creek it is the only source that is not the lowest in magnitude among the sub-watersheds. This indicates that livestock having stream access is at least partly responsible for impairments based on *E. coli.*

4. *Bare Pasture Areas*

   Bare pasture areas occupied, at most, 14 acres in any sub-watershed for a total of only 85 acres in the entire watershed. Due to its slight impact compared to other sources it is difficult to correlate with impairments since all sub-watersheds with bare pasture areas had a significant amount of some other source. It is best confirmed through the nature of the source which indicates that not only is the area devoid of vegetation and thus unable to filter pollutants, but is also likely where most livestock congregate and where the most manure will accumulate.

5. *Ponds and Lagoons in Need of Repair*

   Ponds and lagoons in need of repair were only identified in 2 sub-watersheds, but is still a significant source by itself in those areas where it occurs. This is due to the high amount of contamination that can occur from such a small area since it is where manure is stored

6. *Dead Wildlife in Streams*

   Similar to ponds and lagoons, the incidence of dead wildlife in streams is small, but it occurs in hot dry months when streams are most susceptible to contamination and can be caused to exceed standards with a very limited input of contaminant.

	Sources							Impairments
Sub-watershed	1. Households with septics (est. #)	Estimated Field Bed Acreage	2. # CFO permits (active)	3. Feet of Cattle Access	4. Bare Pasture Acres (%)	5.Ponds, Lagoons in Need of Repair	6. Sightings of dead wildlife in creek bottoms	*E. coli*
010	542	50 (0.4%)	1		13 (0.1%)			**SEVERELY IMPAIRED**
020	472	43 (0.5%)	3		4 (0.1%)	1		**SEVERELY IMPAIRED**
030	489	45 (0.4%)	2	1416	5 (0.1%)			**SEVERELY IMPAIRED**
040	467	43 (0.4%)			0 (0.0%)			**IMPAIRED**
050	363	33 (0.4%)	1	1229	5 (0.1%)			**SEVERELY IMPAIRED**
060	331	30 (0.4%)	2	1417	3 (<0.1%)			**IMPAIRED**
070	344	32 (0.4%)		555	14 (0.2%)	1		**IMPAIRED**
080	633	58 (0.4%)			14 (0.1%)			**IMPAIRED**
090	2129	195 (1.5%)		659	5 (<0.1%)		1	**IMPAIRED**
100	935	86 (1.3%)		1278	1 (<0.1%)		1	**IMPAIRED**
110	620	57 (0.6%)			1 (<0.1%)			**IMPAIRED**
120	516	47 (0.4%)	1	2536	7 (0.1%)			**SEVERELY IMPAIRED**
130	359	33 (0.3%)			2 (<0.1%)		2	**IMPAIRED**
140	183	17 (0.2%)			4 (<0.1%)			**SEVERELY IMPAIRED**
150	396	36 (0.3%)	1	1930	5 (<0.1%)			**IMPAIRED**
160	156	14 (0.2%)		1794	2 (<0.1%)			**IMPAIRED**
Total	8963	823 (0.5%)	11	12814	85 (0.1%)		4	

Table 4.2.1-A: Pathogen Sources: Locations and Magnitude

In addition to the load duration curves developed for the sediment problem, *E. coli* load duration curves were developed for each sample point. The same methodology was used and the results are detailed below. *Figure 4.2.2-A: E. coli Load Duration Curve for Big Creek at Highway 66* shows an example of the curve using data from sample point 23 on Big Creek at Highway 66. Reductions for each hydrologic condition are also shown as an illustration of how the reductions are determined.

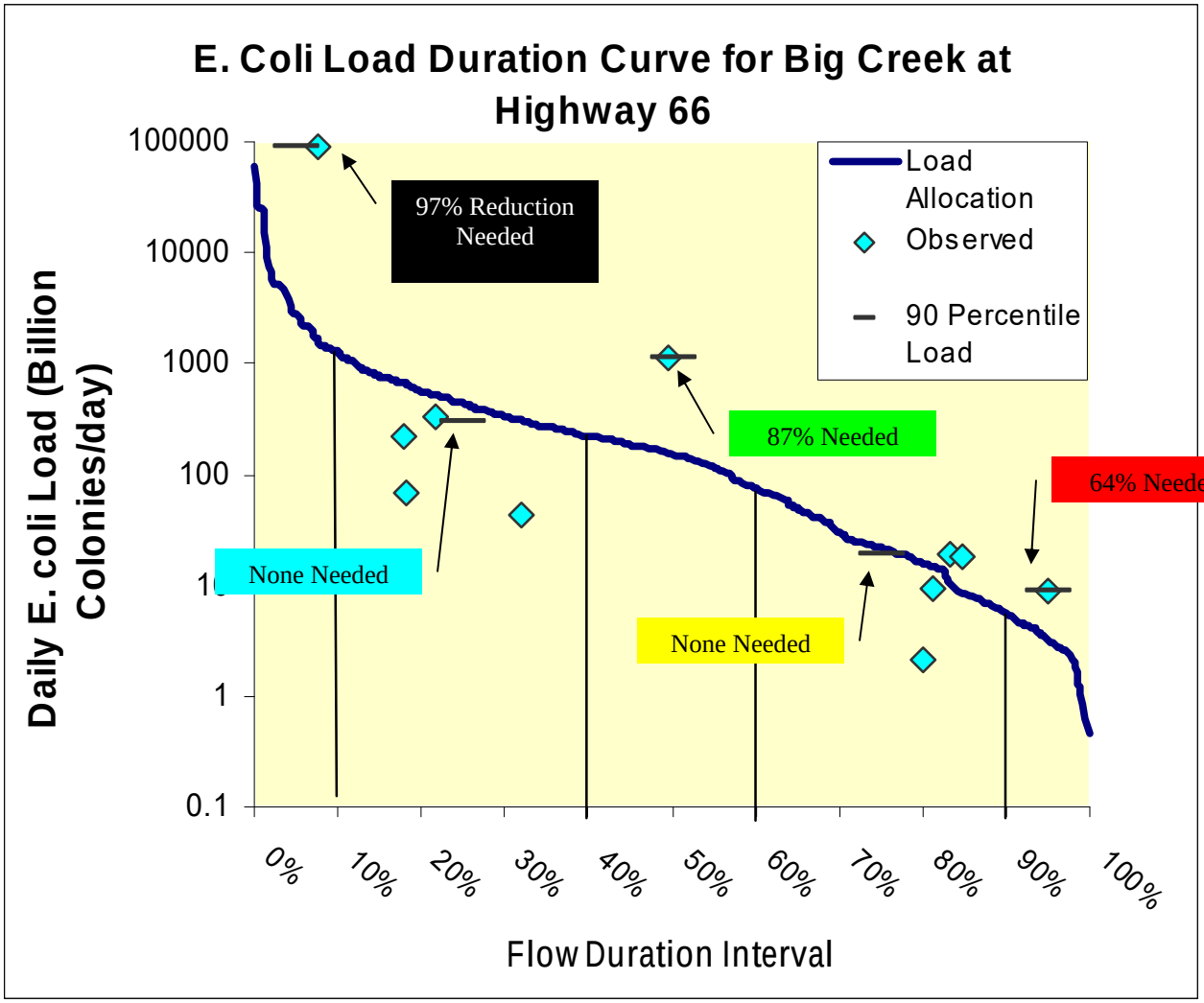


Figure 4.2.2-A: E. coli Load Duration Curve

As is shown in the figure, reductions are needed during three of the hydrologic conditions. The greatest reduction are needed during High flow conditions (97% as shown in the black box), but since this represents extreme events, the next highest needed reduction is used, which is 87% for the mid-range flow conditions. Using this method, it's assumed that in order to achieve the desired concentration for total suspended solids, an 87% reduction in *E. coli* loading within the area draining to sample point 23 must occur.

The results of the load duration curve method for determining reductions are summarized in *Table 4.1.2-A: Reductions Needed to Achieve E. coli Standard.* The table reports the calculated reductions for each sample point during each hydrologic condition and a final overall reduction in the farthest column to the right. The largest reductions are shown in bold and the low and high conditions are shown in italics as they are disregarded in deciding the overall reduction. Sample points that have other sample points nested within their drainage area were subject to additional adjustments to reflect the load reduction that is expected from the upstream areas. For example, even though sample point 23 (Big Creek at Highway 66) was determined to require a 87% reduction, the reductions required of the areas draining to sample points 25, 26, and 27 which are nested within the drainage area of sample point 23, exceed that of the necessary reduction for 23. For this reason the area draining to sample point 23, but not to sample points 25, 26, and 27 is deemed to need only 8% reduction in *E. coli* rather than 87%. In the table, reductions noted with an asterisk are those that were adjusted as described.

	Reduction Needed to Achieve *E. coli* Standard					
Sample Point	*Low*	**Dry**	**Mid-Range**	**Moist**	*High*	**Reduction**
1	*0.0%*	0.0%	n/a	0.0%	*n/a*	NONE*
2	*n/a*	n/a	n/a	0.0%	*n/a*	NONE
3	*n/a*	n/a	n/a	0.0%	*n/a*	NONE
4	*23.7%*	0.0%	n/a	0.0%	*n/a*	NONE*
5	*n/a*	**74.8%**	n/a	0.0%	*n/a*	74.8%
6	*n/a*	0.0%	n/a	0.0%	*n/a*	NONE
7	*n/a*	**88.5%**	n/a	0.0%	*n/a*	88.5%
8	*n/a*	**24.5%**	n/a	0.0%	*n/a*	20.3%*
9	*n/a*	**41.5%**	n/a	0.0%	*n/a*	41.5%
10	*n/a*	**87.6%**	n/a	0.0%	*n/a*	87.6%
11	*n/a*	0.0%	n/a	0.0%	*n/a*	NONE*
12	*0.0%*	0.0%	n/a	0.0%	*n/a*	NONE*
131	*n/a*	0.0%	n/a	0.0%	*n/a*	NONE
132	*n/a*	0.0%	n/a	**16.5%**	*n/a*	16.5%
14	*n/a*	0.0%	n/a	**16.6%**	*n/a*	1.2%*
15	*n/a*	0.0%	n/a	**17.7%**	*99.2%*	17.7%
16	*n/a*	0.0%	43.4%	0.0%	*87.8%*	NONE*
17	*n/a*	n/a	**62.3%**	37.6%	*92.5%*	62.3%
18	*n/a*	n/a	**54.7%**	0.0%	*93.9%*	54.7%
19	*0.0%*	0.0%	90.6%	0.0%	*97.3%*	35.6%*
20	*n/a*	n/a	**81.1%**	0.0%	*89.1%*	81.1%*
21	*n/a*	n/a	**77.4%**	0.0%	*89.1%*	77.4%
22	*n/a*	n/a	**24.6%**	3.7%	*80.5%*	24.6%
23	*63.5%*	0.0%	**86.7%**	0.0%	*96.9%*	7.5%*
24	*n/a*	n/a	**71.7%**	42.6%	*91.1%*	71.7%
25	*n/a*	70.0%	**74.9%**	0.0%	*93.0%*	74.9%
26	*n/a*	0.0%	**94.3%**	0.0%	*87.8%*	94.3%
27	*0.0%*	0.0%	**82.6%**	0.0%	*96.7%*	NONE*
28	*n/a*	66.5%	**92.7%**	0.0%	*96.5%*	92.7%
29	*0.0%*	0.0%	**83.8%**	0.0%	*97.6%*	NONE*
30	*n/a*	0.0%	**82.6%**	17.4%	*86.0%*	82.6%
31	*32.2%*	74.4%	**92.5%**	13.9%	*99.2%*	NONE*
32	*70.4%*	63.9%	**97.2%**	31.8%	*99.2%*	52.5%*
33	*n/a*	59.1%	**91.6%**	18.5%	*99.2%*	91.6%
34	*5.1%*	42.1%	**96.2%**	37.0%	*99.2%*	96.2%

*Values adjusted to reflect reductions expected upstream

Table 4.2.2-A: Reductions Needed to Achieve E. coli Standard

4.2.3: Critical Areas

Critical areas were identified from the problem sources and water monitoring data was used to prioritize the critical areas. The water quality standard for *E. coli* was used to set a desired load. Reduction needed was calculated by subtracting the actual load from the desired load, assuming the concentration is within the standard and the flow remains the same. For each point whose drainage area includes one or more other sample points, the reduction needed in the nested drainage area is subtracted from the needed reduction from the next downstream sample point. This means that the reduction needed at each sample downstream of other points is calculated assuming necessary reductions were achieved in the upstream areas as calculated. The highest priority areas are those with the most reduction needed. The resultant map is shown in *Figure 4.2.3-A: Critical Areas for Achieving E. coli Standard*. Priority is shown as dark blue (1-20% - Lowest), light blue (21-40%), yellow (41-60%), orange (61-80%), or red outline (81-97.2% - Highest) depending on the level of reduction needed. Critical sources are shown as a variety of symbols and colors. A closer look at the critical area map can be found in Appendix E: Critical Areas – Sub-watershed Maps.

Within these critical areas as determined by water monitoring, the highest priority sources for restoration are those with the greatest estimated magnitude and the most important factors in each sub-watershed are those with the greatest acreage. They will have the most likelihood of being mitigated by having a greater pool of participants from which to choose especially when there is great variation in the magnitude per acre of the sources within the sub-watershed.

The most substantial *E. coli* loading reductions are needed in the Pond Flat - Headwaters (010), Pond Flat – Jordan Creek (020), Neu Creek (110), and Big Creek – McAdoo Creek sub-watersheds. In the Pond Flat – Headwaters and Pond Flat – Jordan Creek sub-watersheds, the most common sources by area are livestock. In the Neu Creek sub-watershed, the only source identified was Septic Systems and in the Big Creek – McAdoo Creek sub-watershed, livestock sources were the most common.

Critical Areas for Achieving *E. coli* Standard

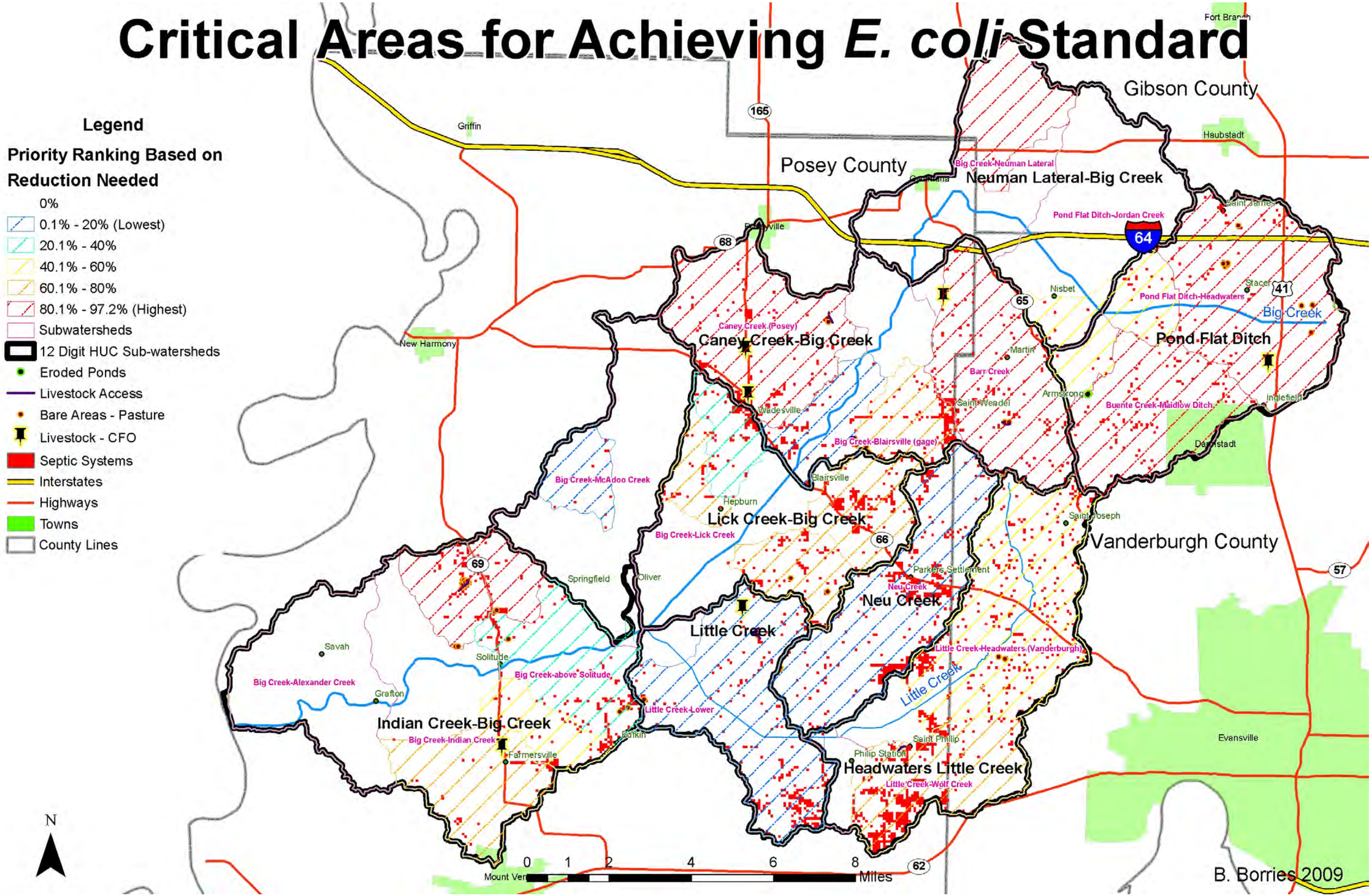
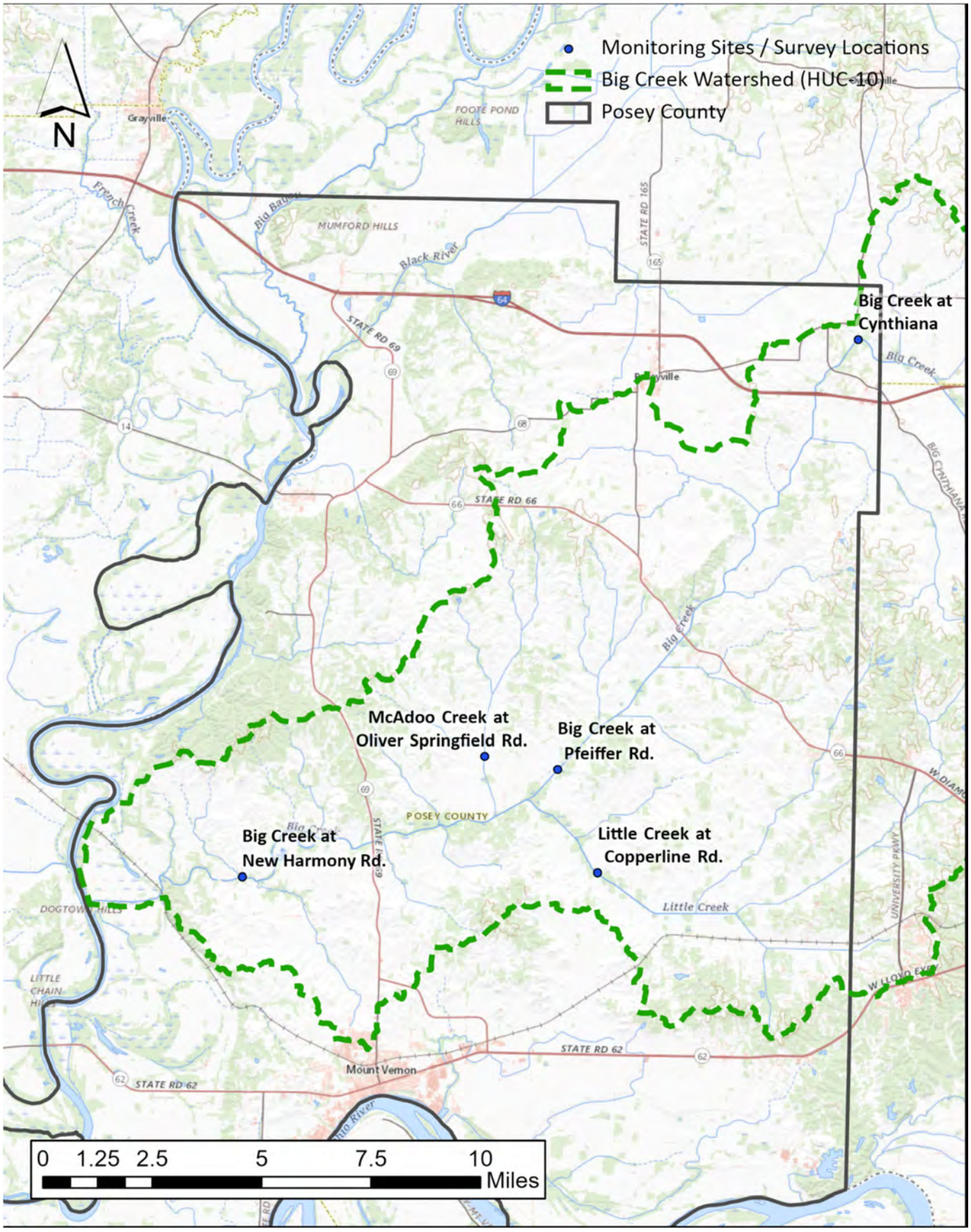
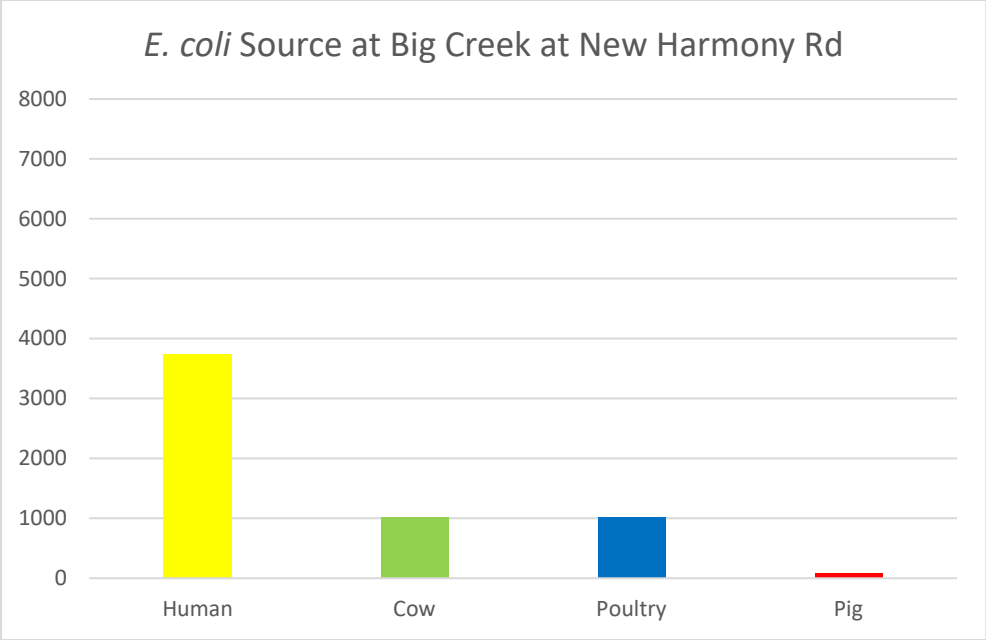


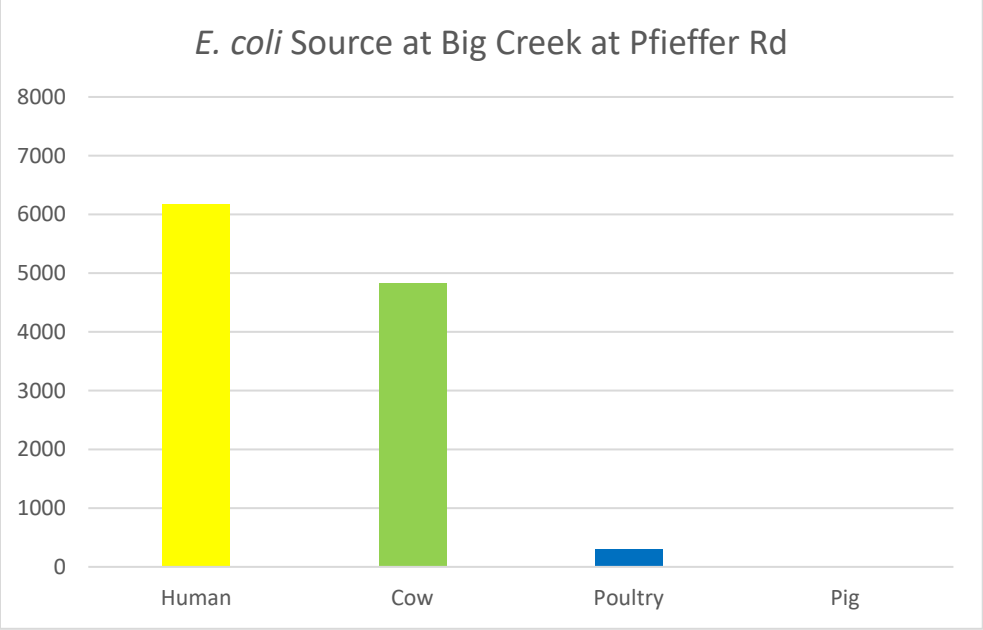
Figure 4.2.3: Critical Areas for Achieving E. coli Standard

2023 TESTING DATA

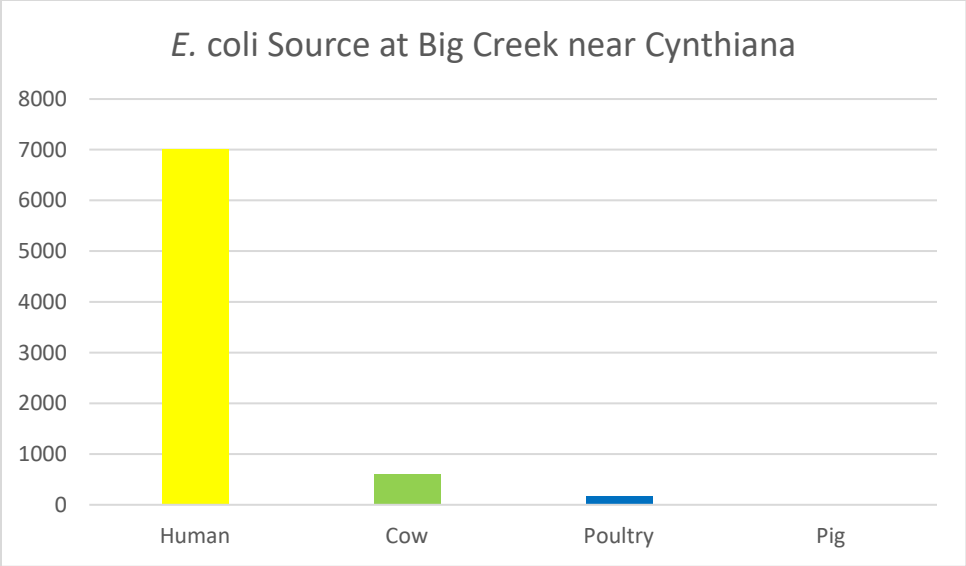

Monitoring Sites / Survey Locations
Big Creek Watershed (HUC-10)
Posey County
N
Big Creek at Cynthiana
McAdoo Creek at Oliver Springfield Rd.
Big Creek at Pfeiffer Rd.
Big Creek at New Harmony Rd.
Little Creek at Copperline Rd.
POSEY COUNTY
Mount Vernon
Grayville
STATE RD 69
STATE RD 66
STATE RD 62
STATE RD 165
Big Creek
Little Creek
French Creek
Black River
MUMFORD HILLS
FOOTE POND HILLS
DOGTOWN HILLS
0 1.25 2.5 5 7.5 10
Miles



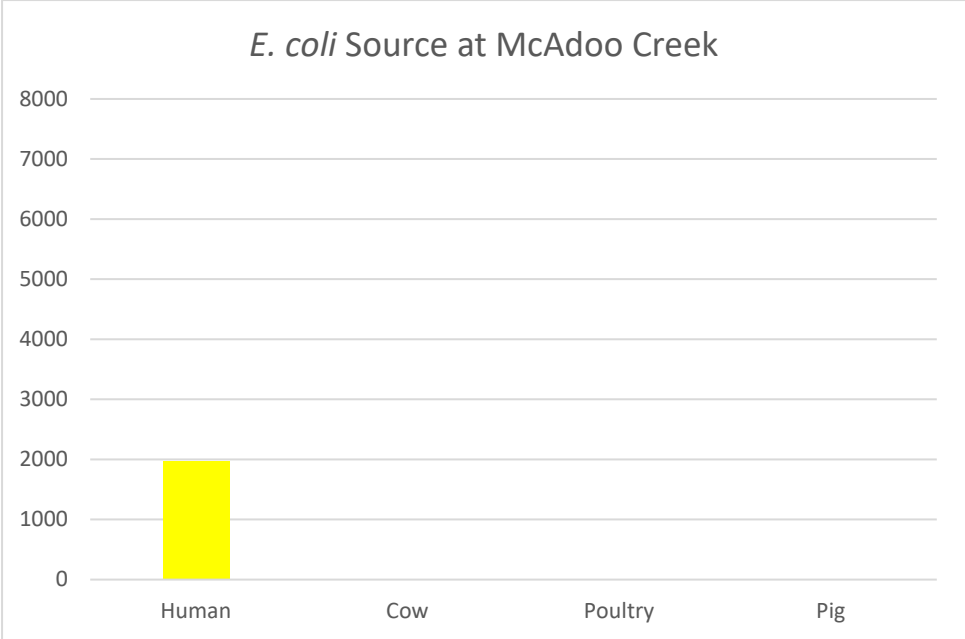
New Harmony	Human	3740
	Cow	1010
	Poultry	1010
	Pig	91



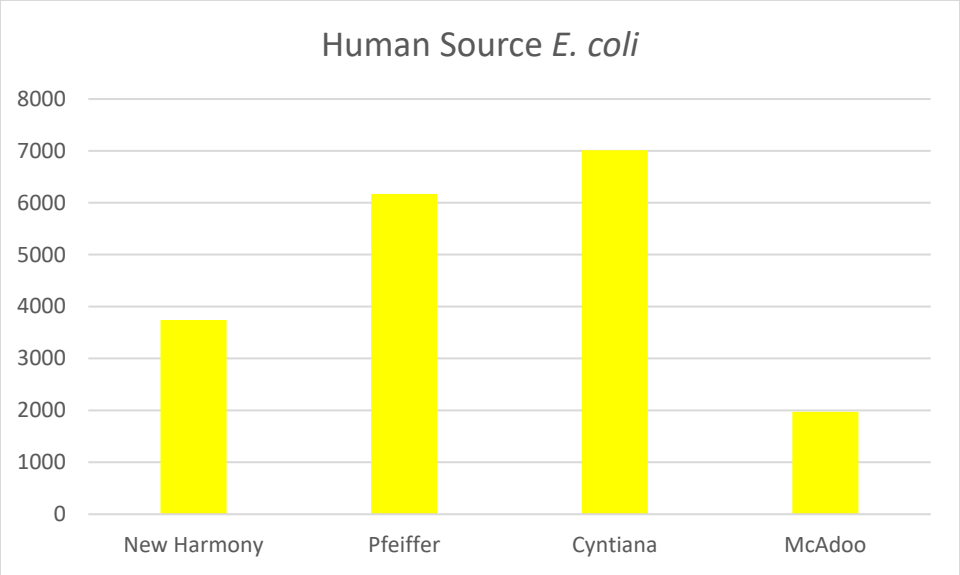
Pfieffer Rd	Human	6170
	Pig	4820
	Cow	305
	Poultry	0



Cynthiana	Human	7010
	Pig	610
	Cow	174
	Poultry	0



McAdoo Creek	Human	1970
	Cow	0
	Pig	0
	Poultry	0



New Harmony	Human	3740
Pfeiffer	Human	6170
Cyntiana	Human	7010
McAdoo	Human	1970

Sample ID	Analysis Requested	Marker Quantified	Result Unit
BC New Harmony	Human	3740	copies per 100ml
BC New Harmony	Cow	1010	copies per 100ml
BC New Harmony	Poultry	1010	copies per 100ml
BC New Harmony	Pig	91	copies per 100ml
BC Pfeiffer	Human	6170	copies per 100ml
BC Pfeiffer	Pig	4820	copies per 100ml
BC Pfeiffer	Cow	305	copies per 100ml
BC Pfeiffer	Poultry	0	copies per 100ml
Cynthiana	Human	7010	copies per 100ml
Cynthiana	Pig	610	copies per 100ml
Cynthiana	Cow	174	copies per 100ml
Cynthiana	Poultry	0	copies per 100ml
Little Creek	Human	0	copies per 100ml
Little Creek	Cow	0	copies per 100ml
Little Creek	Pig	0	copies per 100ml
Little Creek	Poultry	0	copies per 100ml
McAdoo	Human	1970	copies per 100ml
McAdoo	Cow	0	copies per 100ml
McAdoo	Pig	0	copies per 100ml
McAdoo	Poultry	0	copies per 100ml

LUMINULTRA

15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016

ACCREDITED CERT# 4126.01

Fecal Host Quantification ID Test Results Report

Detection and quantification of the fecal host associated gene biomarker by quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

ND: Not Dete DNQ: Detected, Not Quantifiable ROQ: Detected, Quantifiable

Submitter: Posey County Soil and Water Conservation District
Report Generated: 4/20/2023

SM #	Sample ID	Date Collected	Time Collected	Analysis Requested	Ct, Rep1	Ct, Rep2	Marker Quantified	Results Qualifier	LOD (Limit of detection)	LOQ (Limit of Quantification)	Result Unit
SM23D07001	McAdoo	4/6/2023		Human_HF183	31.88	31.35	1.97E+03	ROQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07002	Little Creek	4/6/2023		Human_HF183	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM23D07003	BC New Harmony	4/6/2023		Human_HF183	30.69	30.57	3.74E+03	ROQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07004	BC Pfeiffer	4/6/2023		Human_HF183	29.95	29.81	6.17E+03	ROQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07005	Cynthiana	4/6/2023		Human_HF183	29.85	29.54	7.01E+03	ROQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07006	McAdoo	4/6/2023		Cow_CowM2(EPA1)	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM23D07007	Little Creek	4/6/2023		Cow_CowM2(EPA1)	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM23D07008	BC New Harmony	4/6/2023		Cow_CowM2(EPA1)	35.45	33.52	1.01E+03	DNQ	1.50E+03	5.00E+03	copies per 100ml
SM23D07009	BC Pfeiffer	4/6/2023		Cow_CowM2(EPA1)	32.51	32.73	3.05E+02	DNQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07010	Cynthiana	4/6/2023		Cow_CowM2(EPA1)	32.98	34.19	1.74E+02	DNQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07011	McAdoo	4/6/2023		Pig_Pig2Bac	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM23D07012	Little Creek	4/6/2023		Pig_Pig2Bac	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM23D07013	BC New Harmony	4/6/2023		Pig_Pig2Bac	36.93	36.7	9.05E+01	DNQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07014	BC Pfeiffer	4/6/2023		Pig_Pig2Bac	30.59	31.11	4.82E+03	ROQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07015	Cynthiana	4/6/2023		Pig_Pig2Bac	34.46	33.6	6.10E+02	ROQ	1.50E+02	5.00E+02	copies per 100ml
SM23D07016	McAdoo	4/6/2023		Poultry_CL	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM23D07017	Little Creek	4/6/2023		Poultry_CL	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM23D07018	BC New Harmony	4/6/2023		Poultry_CL	33.76	35.08	1.01E+03	DNQ	1.50E+03	5.00E+03	copies per 100ml
SM23D07019	BC Pfeiffer	4/6/2023		Poultry_CL	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml
SM23D07020	Cynthiana	4/6/2023		Poultry_CL	ND	ND	0.00E+00	ND	1.50E+02	5.00E+02	copies per 100ml

Reported Results Authorized By: Anda Quintero, Quality Manager

Results reported herein apply only to the sample matrices as received.
Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Revision 2.2
Effective Date: 11/11/2021

LUMINULTRA

15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016

ACCREDITED CERT# 4126.01

Fecal Host Quantification ID Test Results Report

Sample Processing and Analysis Information

Submitter: Posey County Soil and Water Conservation D
Report Generated: 4/20/2023

SM #	Sample ID	Analysis Requested	Sample Type	Processed Date	Extraction Date	Analysis Date	Amount Processed	Amount Processed Unit	Extracted DNA/RNA Volume (ul)	PCR Input Volume (ul)	PCR Plate ID	Sample Comments
SM23D07001	McAdoo	Human_HF183	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q01	
SM23D07002	Little Creek	Human_HF183	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q01	
SM23D07003	BC New Harmony	Human_HF183	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q01	
SM23D07004	BC Pfeiffer	Human_HF183	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q01	
SM23D07005	Cynthiana	Human_HF183	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q01	
SM23D07006	McAdoo	Cow_CowM2(EPA1)	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q02	
SM23D07007	Little Creek	Cow_CowM2(EPA1)	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q02	
SM23D07008	BC New Harmony	Cow_CowM2(EPA1)	Water	4/7/2023	4/18/2023	4/19/2023	100	ml	100	0.2	20230419_q01	
SM23D07009	BC Pfeiffer	Cow_CowM2(EPA1)	Water	4/7/2023	4/18/2023	4/19/2023	100	ml	100	2	20230419_q01	
SM23D07010	Cynthiana	Cow_CowM2(EPA1)	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q02	
SM23D07011	McAdoo	Pig_Pig2Bac	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q02	
SM23D07012	Little Creek	Pig_Pig2Bac	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q02	
SM23D07013	BC New Harmony	Pig_Pig2Bac	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q02	
SM23D07014	BC Pfeiffer	Pig_Pig2Bac	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q02	
SM23D07015	Cynthiana	Pig_Pig2Bac	Water	4/7/2023	4/18/2023	4/19/2023	100	ml	100	2	20230419_q01	
SM23D07016	McAdoo	Poultry_CL	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q03	
SM23D07017	Little Creek	Poultry_CL	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q03	
SM23D07018	BC New Harmony	Poultry_CL	Water	4/7/2023	4/18/2023	4/19/2023	100	ml	100	0.2	20230419_q01	
SM23D07019	BC Pfeiffer	Poultry_CL	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q03	
SM23D07020	Cynthiana	Poultry_CL	Water	4/7/2023	4/18/2023	4/18/2023	100	ml	100	2	20230418_q03	

Reported Results Authorized By: Anda Quintero, Quality Manager

Results reported herein apply only to the sample matrices as received.
Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

LUMINULTRA

15280 NW 79th Court, Suite 107 Miami Lakes, Florida 33016

ACCREDITED CERT#4126.01

Fecal Host Quantification ID Test Results Report

qPCR Analysis QAQC information

Submitter: Posey County Soil and Water Conservatior
Report Generated: 4/20/2023

Analysis Requested	PCR Plate ID	Y-intercept	Slope	R^2	Efficiency %	NTC1 (no template control)	NTC2 (no template control)	NTC3 (no template control)	Positive control Ct (if applicable)	Comments
Human_HF183	20230418_q01	37.09	-3.45	0.999	94.90	ND	ND	ND		
Cow_CowM2(EPA1)	20230418_q02	35.27	-3.34	0.999	99.34	ND	ND	ND		
Pig_Pig2Bac	20230418_q02	37.71	-3.47	1.000	94.25	ND	ND	ND		
Poultry_CL	20230418_q03	35.24	-3.24	0.999	103.77	ND	ND	ND		
Cow_CowM2(EPA1)	20230419_q01	35.17	-3.26	0.998	102.84	ND	ND	ND		
Pig_Pig2Bac	20230419_q01	37.77	-3.50	0.999	93.01	ND	ND	ND		
Poultry_CL	20230419_q01	35.27	-3.28	0.999	101.83	ND	ND	ND		

Reported Results Authorized By: Anda Quintero, Quality Manager

Results reported herein apply only to the sample matrices as received.
Results reported herein relate to the genetic material extracted from the sample matrix processed and included in the analysis.

Laboratory Comments

Submitter: Posey County Soil and Water Conservation District
Report Generated: 4/20/2023

Non-Detect (ND) Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification (LOQ, see below). This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations, and the confidence of such quantification will be lower than that declared by the definition of LOQ.

Quantifiable Results (ROQ)
Sample results are within the range of quantification of calibration curves (standard curves) of a validation qPCR method. For most qPCR assays, the range is 1E1 to 1E5 copies/reaction. Copy number measurements reported are relative, not absolute, quantification.

LOD (Limit of Detection, lower)
A general consensus was reached around the definition of the LOD as the lowest amount of analyte, which can be detected with more than a stated percentage of confidence (95%), but, not necessarily quantified as an exact value. It must be noted that LOD is not a limiting value and therefore, that Ct vlaues below the LOD cannot automatically be considered as negative. From the definition of LOD, it is evident that values below LOD are absolutely valid in terms of microornanism prescence. However, the probabality of their repeated detection is lower than 95%.

LOQ (Limit of Quantification, lower)
The LOQ was defined as the smallest amount of analyte, which can be measured and quantified with defined precision and accuracy under the experimental conditions by the method under validation. Numerically, the LOQ is defined as the lowest concentration of analyte, which gives a predefined variability (coeffecient of variation, CV) of under 25%.

Inhibition check
A 1:10 dilution of the original sample is analyzed togther each time with the undiluted sample to evaluate the effect of PCR inhibition. If the sample is inhibited, where 1:10 dilution produces a high signal than undiluted sample, the 1:10 dilution results will be used for quantification. The use of 1:10 dilution sample results will be reflected in Analytical Volume(ul). For example, if the analytical volume for undiluted sample is 2ul, the analytical volume for 1:10 dilution will be 0.2ul.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. The most reliable way to accurately test for contamination is to combine genetic testing with scientifically sound and adequate study design appropriate for the environmental quality questions to be answered or issues to be resolved.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination.

Qualitification Assay Results (Detected/Non-Detected only)
Such results are only reported as Detected or Non-Detected without quantification. Non-Detected results are defined as stated above, and Detected results are defined as detected Ct in both replicate qPCR reactions.

DNA Analytical Method Explanation
Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for and the DNA extracted per kit manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.