
Metropolitan Water Reclamation District of Greater Chicago

# RESEARCH AND DEVELOPMENT DEPARTMENT

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## VIRUS LEVELS IN THE DES PLAINES RIVER BEFORE AND AFTER CONSTRUCTION OF THE MIDDLE LEG OF THE DES PLAINES TARP SUBSYSTEM

March 2000

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# VIRUS LEVELS IN THE DES PLAINES RIVER BEFORE AND AFTER CONSTRUCTION OF THE MIDDLE LEG OF THE DES PLAINES TARP SUBSYSTEM

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## DISCLAIMER

Mention of proprietary equipment and chemicals in this report does not constitute endorsement by the Metropolitan Water Reclamation District of Greater Chicago.

## SUMMARY AND CONCLUSIONS

A two-year study was conducted to ascertain the effect of the operation of the Middle Leg of the Des Plaines Tunnel and Reservoir Plan (TARP) Subsystem (Middle Leg) on the virus burden in the Des Plaines River, and to document any improvement in water quality with respect to viruses. The Middle Leg is a 6.6 mile segment of TARP running under the Des Plaines River from Fullerton Avenue to Cermak Road. Its function is to intercept and store combined sewer overflows (CSOs) which would otherwise enter the river during storm events.

Virus levels were measured monthly at two stations (Roosevelt Road, designated as Station 43, and Ogden Avenue, designated as Station 46) for the same five-month period (January through May) in 1993, just prior to the Middle Leg becoming operational, and in 1994, the first year after the Middle Leg became operational.

Stations 43 and 46 were selected for this study for the following reasons: 1) these stations are on the downstream end of the stretch of the Des Plaines River served by the Middle Leg, and thus would be expected to show the greatest effect; 2) there are numerous CSOs located upstream of these stations; 3) samples are routinely collected from these stations as part of the Metropolitan Water Reclamation District of Greater Chicago (District) waterways monitoring program; 4) these stations are easily accessible for sampling personnel.

Information provided by the District's Maintenance and Operations Department indicates that virtually all CSO discharges which might have entered the portion of the Des Plaines River served by the Middle Leg were captured by this portion of TARP from the end of May 1993 through the end of May 1994.

The findings of this study were as follows.

1. The number of virus types isolated was smaller in 1994 (post-TARP period) than in 1993 (pre-TARP period). Only 8 virus types were isolated in 1994 compared to 13 virus types isolated in 1993.
2. The mean (arithmetic) virus levels were numerically lower in the post-TARP period compared to the pre-TARP period. The mean virus level at Station 43 in 1993 was 0.088 PFU/L compared to 0.048 in 1994 ($p = >0.05$). The mean virus level at Station 46 in 1993 was 0.080 PFU/L compared to 0.024 in 1994 ($p = >0.05$).
3. As a corollary to this study, routinely collected fecal coliform data for these same two stations were reviewed. Fecal coliform levels were also numerically lower in 1994 compared to the four preceding years, 1990 through 1993. Results of statistical analyses indicated that the fecal coliform densities ob-

served in 1994 (post TARP) were significantly lower at both Stations 43 ($p = 0.035$) and 46 ($p = 0.013$) when compared to the pre-TARP period. These data indicate a reduction in fecal pollution to the Des Plaines River due to TARP operation.

This two-year study indicated that the virus burden in the Des Plaines River, as measured at Stations 43 and 46, was reduced due to the operation of the Middle Leg. Both the number of virus types isolated and the arithmetic mean virus levels were lower in the post-TARP period as compared to the pre-TARP period.

The reductions found in both fecal coliform and virus measurements demonstrate that the implementation of TARP has had a beneficial impact on the Des Plaines River.

# INTRODUCTION

Combined sewers in the Chicagoland area carry both storm-water runoff and sanitary sewage directly into the waterways in the service area of the District. The District designed the TARP system to capture CSOs which would otherwise enter these waterways during storm events, and store the CSOs for treatment after the storm event ends. As part of TARP, huge underground tunnels have been excavated beneath the Chicagoland area to intercept the CSOs and ultimately convey them to three large surface reservoirs for storage. Following a storm, the captured CSOs are pumped out of the tunnels and reservoirs to a WRP for treatment.

The TARP system is divided into four main subsystems: Mainstream, Calumet, Upper Des Plaines, and Des Plaines. Construction of the first TARP tunnel began in 1975 and construction of the entire system is still in progress as of today (2000).

This study was conducted to evaluate the operation of the Middle Leg of the Des Plaines TARP subsystem. The Middle Leg, which runs 6.6 miles from Fullerton Avenue, on the north, to Cermak Road, on the south, was completed and placed in operation in late May 1993. There are 21 CSO discharge points on the stretch of the Des Plaines River served by the Middle Leg. As previously mentioned, District operating records indicate

that the Middle Leg was successful in capturing all CSO discharges from the 21 points during the period of this study.

Station 43 (Roosevelt Road) is located on the Des Plaines River 3 miles south of Fullerton Avenue and Station 46 (Ogden Avenue) is located on the Des Plaines River just south of the end of the Middle Leg. Figure 1 shows the location of these two stations on the Des Plaines River. It was anticipated that the virus load in the Des Plaines River at these stations would be lower in 1994 than in 1993 due to the operation of the Middle Leg. This study was conducted to verify this expectation.

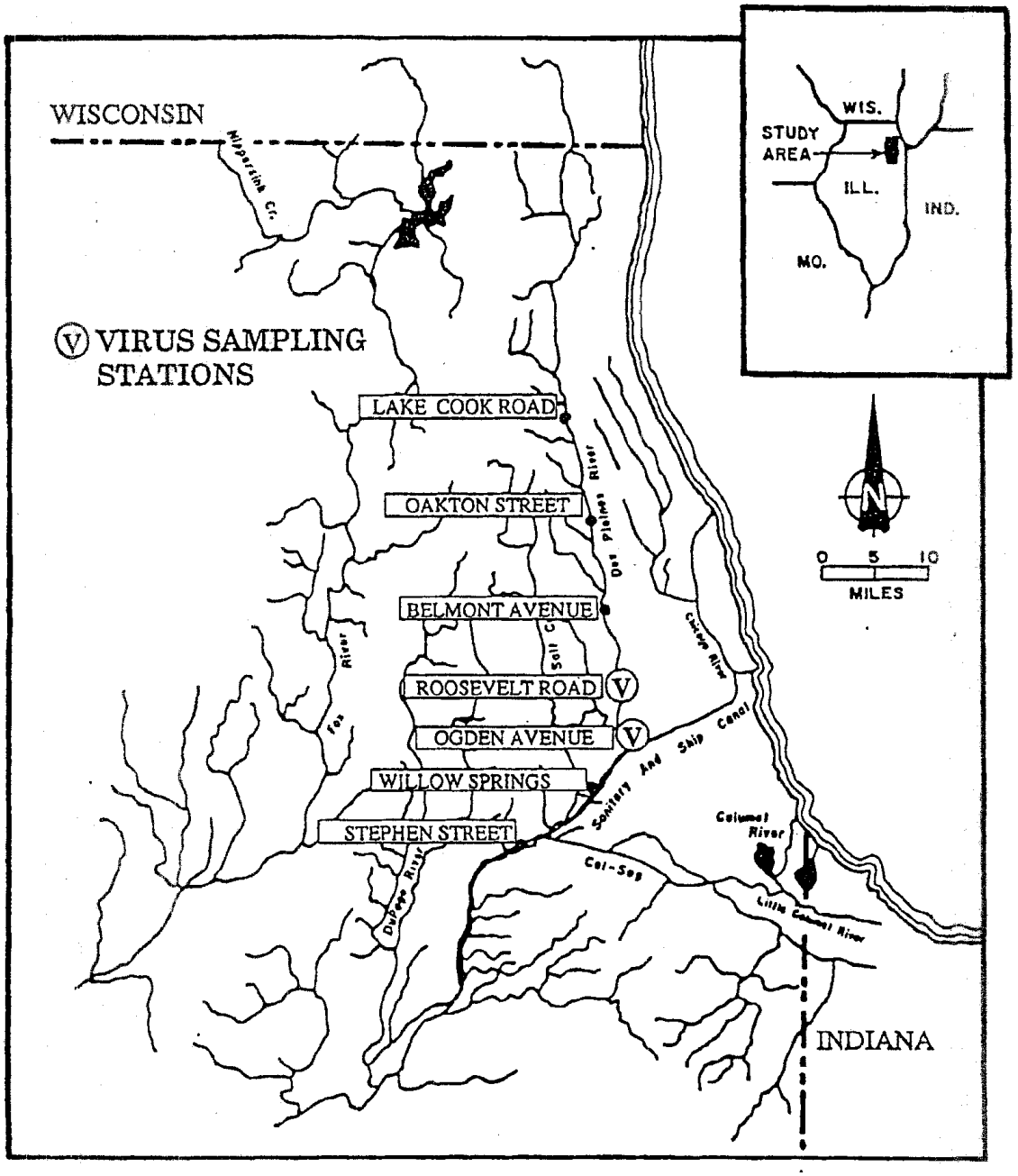
Figure 2 presents a schematic of the entire TARP system, and indicates the approximate locations of the two sampling stations. Please note that the TARP tunnels lie approximately 200 feet below the river, and that samples were collected from the river, not the tunnels.

Also note that the North Leg of the Des Plaines System, which is upstream of the Middle Leg, was not completed until 1999. There are 28 CSOs on this portion of the Des Plaines River and an unknown number of discharges from these CSOs to the Des Plaines River occurred during the period of this study (1993-94). The West and South Legs of the Des Plaines System are both downstream of the study area, and had no impact on this study.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

FIGURE 1

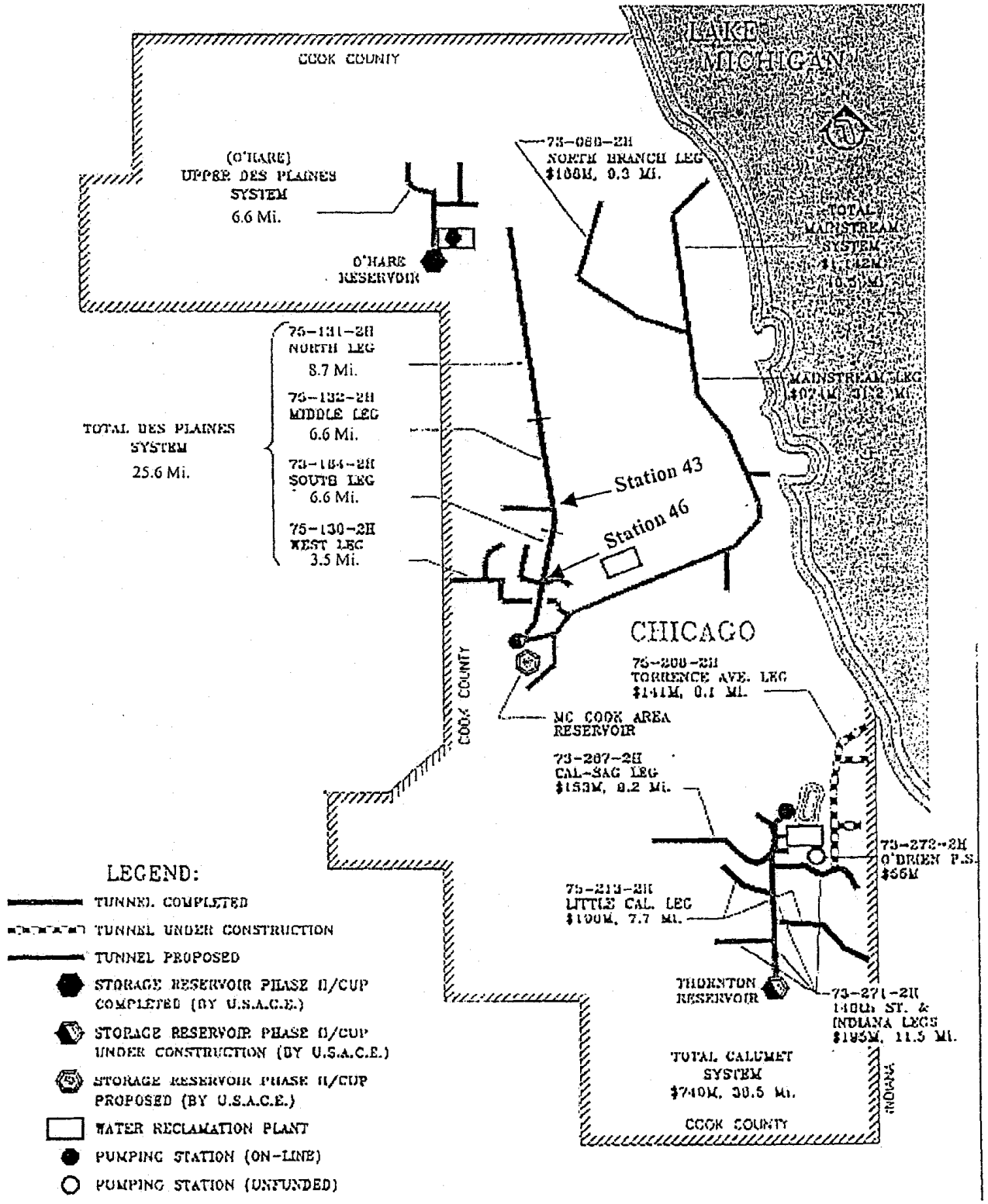
DES PLAINES RIVER WATERWAY SAMPLING STATIONS



METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

FIGURE 2

TARP SYSTEM SCHEMATIC



## MATERIALS AND METHODS

### Collection of Water Samples from the Des Plaines River

Samples for virological analysis were collected at the stations listed in Table 1. The locations of these stations are shown in Figure 1. Samples were collected once per month at each station from January through May in 1993 and 1994. For each sampling event, a stainless steel bucket was lowered into the center of the river on the upstream side of each bridge to draw water to fill two large carboys, each containing 16 milliliters (mL) of a 10 percent solution of sodium thiosulfate. These sample containers were placed on ice and transported directly to the laboratory.

Separate samples were collected for fecal coliform analysis as described by Sedita et al. (1987)

### Aluminum Hydroxide Continuous Flow Centrifugation Procedure

Viruses were concentrated by the aluminum hydroxide-continuous flow centrifuge technique (Bertucci and Patterson, 1978). Immediately upon receipt in the laboratory, each sample was transferred to a 55-liter Nalgene™ cylindrical tank, and the temperature, pH, and volume were recorded. The sample (38 to 43 liters) was adjusted to pH 6.0 with 50 percent HCl, and 5 mL of preformed aluminum hydroxide floc were added per liter of sample. Preformed floc for 38 to 43 liters of water

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TABLE 1

SAMPLING OF THE DES PLAINES RIVER FOR VIROLOGICAL ANALYSIS: JANUARY THROUGH MAY 1993 AND 1994

| Station Number | Station Location[1] | Sample Volume (L) | Sampling Frequency |
|---|---|---|---|
| 43 | Roosevelt Road | 38 - 43 | Monthly |
| 46 | Ogden Avenue | 40 - 43 | Monthly |

[1]See Figure 1.

samples used in this study was prepared by adding 2 M $Na_2CO_3$ to 400 mL of 25 M $AlCl_3 \cdot 6H_2O$ to achieve a pH of 7.2, and initiate floc formation. The floc was collected by centrifugation for ten minutes at 3,000 x g and the supernatant discarded. Two hundred mL of 0.75 percent saline were added to the pellet and mixed. The pH of the sample was readjusted to 6.0, and the sample was stirred with a magnetic stirrer in the cold room overnight.

Sedimentable material was collected by centrifugation at 27,000 x g with a KSB continuous flow system (Dupont Instruments/Sorvall™, Newtown, CT) at 5°C. The sample was pumped through the system at a rate of 200 mL per minute with a Cole-Parmer peristaltic pump.

The sediment collected was washed into a polypropylene beaker with 25 mL of elution medium, and sonified with a Bronson Model 450 sonifier equipped with a microtip set at 50 percent duty cycle. Elution medium consisted of 0.05 M glycine, containing 3 percent disodium ethylenediamine tetraacetic acid (EDTA), and 10 percent heat inactivated fetal bovine serum (HIFBS) at pH 9.0 (heat inactivation is to remove complement). The pH was adjusted to 9.0 with 1 M NaOH, and the suspension was sonicated for an additional five seconds before centrifugation at 27,000 x g, at 5°C. The supernatant was collected, the pH was adjusted to 7.2, and 2.5 mL of 50 percent $MgCl_2$ were added. The sedimented material was eluted as above a

second time, and the first and second eluates were combined and brought up to a volume of 150 mL with dilution medium. Dilution medium consisted of 1 percent Bacto™ gelatin, and 3 percent Bacto™ beef extract. Five aliquots of the concentrate were stored frozen at -66°C until assayed.

## Cell Cultures and Media

The African green monkey continuous cell line designated BGM-K was purchased in its 70th passage from M. A. Bioproducts (Walkersville, MD). The medium used for routine cultivation of the BGM-K cell line consisted of Eagle's minimum essential medium with Earle's salts (E-MEM) 0.225 percent in sodium bicarbonate, and 0.01 M in HEPES buffer containing 10 percent (v/v) fetal bovine serum (FBS) Sigma™, and 100 units penicillin G/mL, and 100 µg streptomycin sulfate/mL, or 100 µg gentamicin/mL, and 10 µg Fungizone™/mL. All media components and antibiotics were purchased from Gibco BRL (Grand Island, NY). Ampoules of the 73rd passage of the BGM-K cells were stored in liquid nitrogen for use in this study. When needed, ampoules of the BGM-K cells were taken out of liquid nitrogen storage, thawed in a waterbath at 45°C, and seeded into disposable polystyrene tissue culture ware.

A sufficient number of BGM-K cells for seeding up to 300, 100 x 20 mm tissue culture dishes were grown in 175 $cm^2$ tissue culture flasks (Nunclon™), and 490 $cm^2$ (small), and 850 $cm^2$

(large) roller bottles. Tissue culture dishes and roller bottles were purchased from Corning Glass Works, Corning, NY. BGM-K cells, taken from storage in liquid nitrogen, were seeded into the flasks, passaged after three days, and used to seed small roller bottles, and to continue the cell line in flasks. The cells in small roller bottles were passaged after four days, and used to seed large roller bottles. The cells were grown in the large roller bottles for three days, passaged, and seeded into the dishes. Tissue culture dishes, and flasks with loose fitting caps were incubated at 36.5 ± 0.5°C in an atmosphere of 5 percent $CO_2$ with 95 percent humidity. Roller bottles were tightly capped and rolled at a rate of approximately one revolution per minute on a cell production roller apparatus (Bellco Glass Inc., Vineland, NJ) in an environmental chamber at 36.5 ± 0.5°C.

Tissue culture plates were seeded on a routine basis every Friday morning. The cells were allowed to grow to confluency over the weekend for use in virus detection assays (plaque assays) which were set up on Mondays.

Agar Overlay Medium for Plaque Assays

Agar overlay medium for plaque assays was prepared by combining equal volumes of 3 percent agar and 2X E-MEM for overlays. Agar overlay medium for plaque assays consisted of the following: E-MEM; 1.5 percent purified agar; 0.225 per-

cent $NaHCO_3$; 0.01 M HEPES buffer; 0.01 M nonessential amino acids; 0.5 percent $MgCl_2$; 2 percent heat inactivated fetal bovine serum (HIFBS); 250 units penicillin G/mL; 250 μg streptomycin sulfate/mL; 125 μg gentamicin/mL; and 12.5 μg Fungizone™ /mL. Flasks containing 3 percent agar and 2X E-MEM for overlays were held in a waterbath at 45°C before the contents were combined to prepare the agar overlay medium. This agar overlay medium was used for the first overlay. Agar overlay medium for the second overlay was prepared by adding 36 mL of a 0.333 percent neutral red solution (Gibco BRL, Grand Island, NY) to a liter of the agar overlay medium described above.

THREE PERCENT AGAR

Three grams of purified agar (Sigma™ A7921) (plant tissue culture tested) was dissolved per 100 ml of milli-Q purified water by autoclaving for 25 minutes. Quantities of 1.5 to 2.0 L were usually prepared.

2X E-MEM FOR OVERLAYS

2X E-MEM for overlays consisted of the following: 2X E-MEM; 0.45 percent $NaHCO_3$; 0.02 M HEPES buffer; 0.02 M nonessential amino acids; 1.0 percent $MgCl_2$; 4 percent HIFBS; 500 units penicillin G/mL; 500 μg streptomycin sulfate/mL; 250 μg gentamicin/mL; and 25 μg Fungizone™/mL.

## Plaque Assays

Plaque assays were performed with BGM-K cells in the 77th through 99th passage. Monolayers of BGM-K cells in tissue culture dishes (from which the growth medium had been poured off) were inoculated with 1 mL of virus suspension in dilution medium (positive controls), undiluted or diluted sample concentrate, or dilution medium (negative controls). Inocula were spread uniformly over the monolayers by means of a manual back-and-forth, gentle, rocking motion of the tissue culture plates every 20 minutes for two hours. This two-hour virus adsorption step was carried out at room temperature. The monolayers were washed twice with Hank's balanced salt solution and overlaid with 15 mL of agar overlay medium. Agar overlay medium was held in a waterbath at 45°C prior to being overlaid on the cell monolayers. After solidification of the agar, the plates were inverted and incubated at 36.5 ± 0.5°C in an atmosphere of 5 percent $CO_2$ and 95 percent humidity.

Forty-eight hours (±2 hours) after application of the first agar overlay, 10 mL of a second purified agar overlay containing neutral red and the same constituents as the first overlay was added to the plaque assay plates. After solidification of the agar, the plates were inverted and incubated at 36.5 ± 0.5°C in an atmosphere of 5 percent $CO_2$, and 95 percent humidity. Plates were observed after three and four days, respectively, for plaque formation.

## Plaque Confirmation Assays

All plaques were confirmed to be of viral origin as described by Bertucci et al., (1979). The characteristic viral cytopathogenic effect (CPE) was scored on days two through seven after inoculation.

## Identification of Enterovirus Isolates

All enterovirus isolates were identified with a microneutralization test performed essentially as described by Egbertson and Mayo (1986). Lim Benyesh-Melnick (LBM) pools for enterovirus typing were obtained from the World Health Organization Collaborating Center for Virus Reference and Research, Copenhagen, Denmark.

Type-specific antisera obtained from the National Institute of Health (NIH) and American Type Culture Collection (ATCC) were used to confirm results obtained with the LBM pools.

## Laboratory Virus Strains

The Sabin strain of poliovirus 1 was used as control.

## Fecal Coliform Analysis

Samples were analyzed for fecal coliforms using the membrane filtration technique and m-FC agar as outlined in the 14th edition of Standard Methods for the Examination of Water and Wastewater (APHA, 1975).

## Statistical Analysis

Virological data were analyzed for normality using the Shapiro-Wilk Test (SAS Institute, 1990b). Virus levels in pre-TARP and post-TARP periods were compared using the Kruskal-Wallis Test (Chi-square Approximation) (Walpole and Myers, 1985) and the Wilcoxon Rank-Sum Test (Walpole and Myers, 1985) (SAS Institute, 1990a). Statistical analysis of the data showed that the differences were not statistically significant.

Pre- and post-TARP fecal coliform data were log transformed and tested for normality using the Shapiro-Wilk Test (SAS Institute, 1995). The variances in the pre- and post-TARP log transformed fecal coliform data were tested for equality with the F-test (Hogg and Craig, 1978a). Log transformed pre- and post-TARP fecal coliform data were compared using an exact T-test (Hogg and Craig, 1978b).

## RESULTS AND DISCUSSION

### Virus Populations and Levels in Pre-TARP and Post-TARP Samples

The complete plaque assay data are shown in Appendix AI. Toxicity of concentrates of the river water samples to the BGM cell line was not a problem. It was not necessary to treat any of the sample concentrates to reduce toxicity to the BGM-K cell line.

The virus levels measured in the Des Plaines River at Stations 43 and 46 from January through May 1993 (pre-TARP) are shown in Tables 2 and 3, respectively. These levels ranged from 0.19 PFU/L to less than the detection limit of 0.02 PFU/L. The virus levels measured in the Des Plaines River at Stations 43 and 46 from January through May 1994 (post-TARP) are shown in Tables 4 and 5, respectively. These levels ranged from 0.24 PFU/L to less than the detection limit of 0.02 PFU/L. Fourteen of the 15 viruses isolated in 1994 were from the samples collected in March.

Thirty-four viruses were isolated from the samples collected in 1993 (pre-TARP), and all were successfully identified. These identities are shown in Tables 2 and 3. The viruses isolated were: poliovirus P2 (32.4 percent); coxsackievirus B3 (20.6 percent); coxsackievirus B1 (11.8 percent); coxsackievirus B5 (5.9 percent); echovirus 27 (5.9 percent); coxsackievirus A16 (2.9 percent); coxsackievirus B4 (2.9 percent); echovirus 7 (2.9 percent); echovirus 9 (2.9 percent);

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TABLE 2

VIRUS LEVELS IN THE DES PLAINES RIVER AT STATION 43 (ROOSEVELT ROAD), JANUARY THROUGH MAY 1993, BEFORE TARP OPERATION

| Sampling Date | Sample Volume (L) | PFU/L[1,2] | Viruses Isolated[3,4] |
|---|---|---|---|
| January 6 | 38 | 0.16 | CB1 (1); CB5 (1); P2 (1); CB3 (3) |
| February 9 | 42 | 0.07 | E27 (1); P2 (1); CB3 (1) |
| March 22 | 42 | 0.02 | CB3 (1) |
| April 1 | 42 | 0.19 | CA16(1); CB4(1); P1 (1); P2 (5) |
| May 6 | 43 | <0.02 | None |

[1]Confirmed plaque forming units/Liter.

[2]Failure to detect viruses in river water concentrates is recorded as less than (<) the limit of test sensitivity.

[3]CA16 = coxsackievirus A16; CB1 = coxsackievirus B1; CB3 = coxsackievirus B3; CB4 = coxsackievirus B4; CB5 = coxsackievirus B5; E27 = echovirus 27; P1 = poliovirus 1; P2 = poliovirus 2.

[4]The numbers in parentheses indicate the number of virus isolates.

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TABLE 3

VIRUS LEVELS IN THE DES PLAINES RIVER AT STATION 46 (OGDEN AVENUE), JANUARY THROUGH MAY 1993, BEFORE TARP OPERATION

| Sampling Date | Sample Volume (L) | PFU/L[1,2] | Viruses Isolated[3,4] |
|---|---|---|---|
| January 6 | 40 | 0.18 | CB1 (3); CB5 (1); E7 (1); E27 (1); P2 (1) |
| February 9 | 42 | 0.05 | E9 (1); CB3 (1) |
| March 22 | 43 | 0.07 | E11 (1); E20 (1); CB3 (1) |
| April 1 | 41 | 0.10 | P2 (3); P3 (1) |
| May 6 | 43 | <0.02 | None |

[1]Confirmed plaque forming units/Liter.

[2]Failure to detect viruses in river water concentrates is recorded as less than (<) the limit of test sensitivity.

[3]CB1 = coxsackievirus B1; CB3 = coxsackievirus B3; CB5 = coxsackievirus B5; E7 = echovirus 7; E9 = echovirus 9; E11 = echovirus 11; E20 = echovirus 20; E27 = echovirus 27; P2 = poliovirus 2; P3 = poliovirus 3.

[4]The numbers in parentheses indicate the number of virus isolates.

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TABLE 4

VIRUS LEVELS IN THE DES PLAINES RIVER AT STATION 43 (ROOSEVELT ROAD), JANUARY THROUGH MAY 1994, AFTER TARP OPERATION

| Sampling Date | Sample Volume (L) | PFU/L[1,2] | Viruses Isolated[3,4] |
|---|---|---|---|
| January 5 | 42 | <0.02 | None |
| February 7 | 41 | <0.02 | None |
| March 1 | 42 | 0.24 | CA7 (1); CA9 (1); CB3 (5); E13 (1); E21 (1); P1 (1) |
| April 4 | 42 | <0.02 | None |
| May 23 | 42 | <0.02 | None |

[1] Confirmed plaque forming units/Liter.

[2] Failure to detect viruses in river water concentrates is recorded as less than (<) the limit of test sensitivity.

[3] CA7 = coxsackievirus A7; CA9 = coxsackievirus A9; CB3 = coxsackievirus B3; E13 = echovirus 13; E21 = echovirus 21; P1 = poliovirus 1.

[4] The numbers in parentheses indicate the number of virus isolates.

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TABLE 5

VIRUS LEVELS IN THE DES PLAINES RIVER AT STATION 46 (OGDEN AVENUE), JANUARY THROUGH MAY 1994, AFTER TARP OPERATION

| Sampling Date | Sample Volume (L) | PFU/L[1,2] | Viruses Isolated[3,4] |
|---|---|---|---|
| January 3 | 42 | 0.02 | CB4 (1) |
| February 22 | 41 | <0.02 | None |
| March 3 | 42 | 0.10 | CA16 (1); CB3 (3) |
| April 6 | 42 | <0.02 | None |
| May 24 | 43 | <0.02 | None |

[1]Confirmed plaque forming units/Liter.
[2]Failure to detect viruses in river water concentrates is recorded as less than (<) the limit of test sensitivity.
[3]CA16 = coxsackievirus A716, CB3 = coxsackievirus B3; CB4 = coxsackievirus B4.
[4]The numbers in parentheses indicate the number of virus isolates.

echovirus 11 (2.9 percent); echovirus 20 (2.9 percent); poliovirus 1 (2.9 percent); and poliovirus 3 (2.9 percent).

Fifteen viruses were isolated from the samples collected in 1994, and all were successfully identified. These identities are shown in Tables 4 and 5. The viruses isolated were: coxsackievirus B3 (53.3 percent); coxsackievirus A7 (6.7 percent); coxsackievirus A9 (6.7 percent); coxsackievirus A16 (6.7 percent); coxsackievirus B4 (6.7 percent); echovirus 13 (6.7 percent); echovirus 21 (6.7 percent); poliovirus 1 (6.7 percent).

The mean (arithmetic) virus levels measured at Stations 43 and 46 in 1993 and 1994 are compared in Figure 3. In 1993 the mean of the virus levels measured at Station 43 was 0.088 PFU/L (five samples), and the mean of the virus levels measured at Station 46 was 0.080 PFU/L (five samples). In 1994 the mean of the virus levels measured at Station 43 was 0.048 PFU/L (five samples), and the mean of the virus levels measured at Station 46 was 0.024 PFU/L (five samples). Viruses were recovered from only three of ten samples collected in 1994, while eight of ten samples collected in 1993 were positive for viruses (Table 6). The total number of viruses isolated in 1994 was less than half of the number isolated in 1993.

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FIGURE 3

VIRUS LEVELS (ARITHMETIC MEAN) IN THE DES PLAINES RIVER BEFORE (1993), AND AFTER (1994) OPERATION OF THE MIDDLE LEG OF THE DES PLAINES TARP SUBSYSTEM

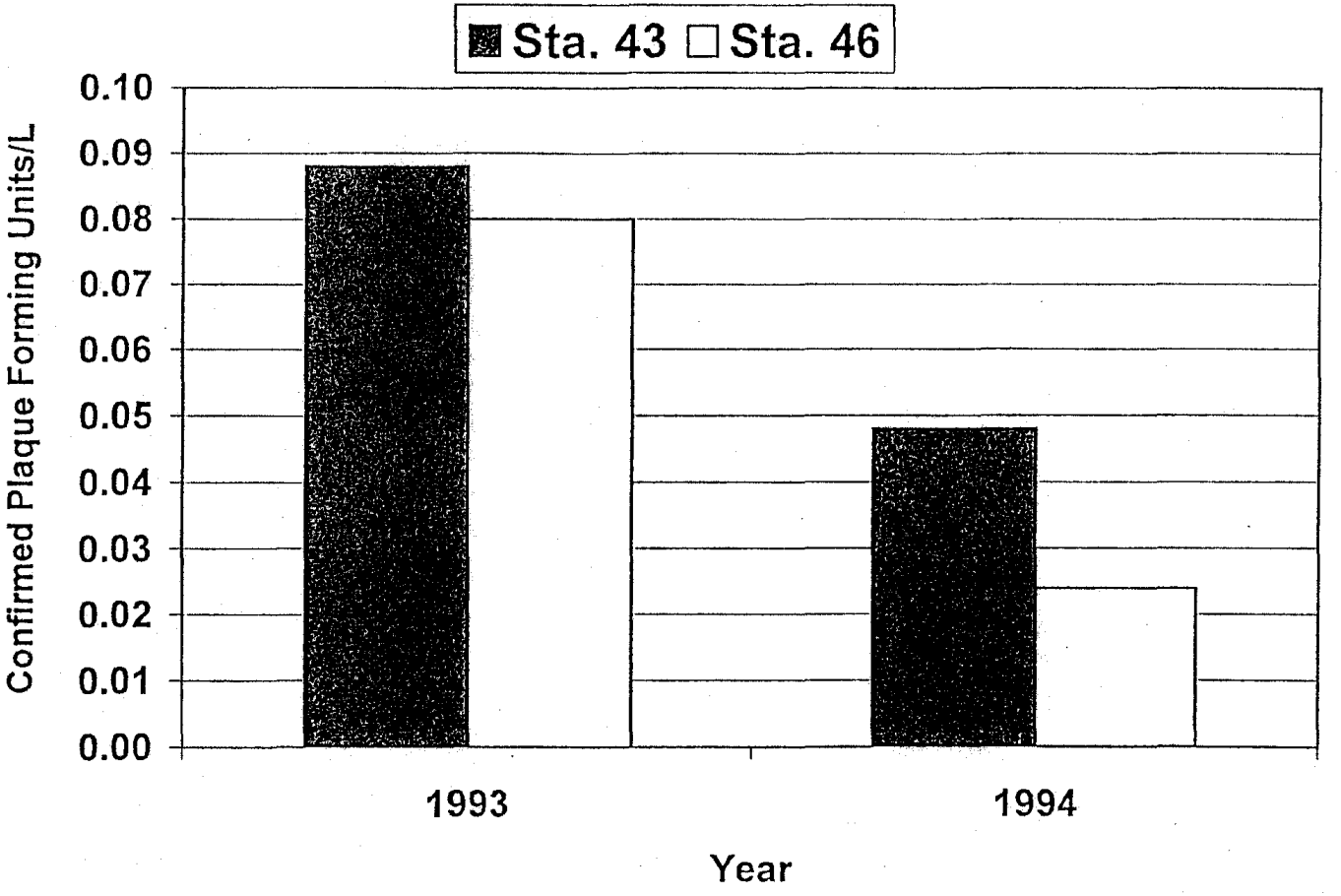

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TABLE 6

RESULTS OF VIROLOGICAL ANALYSIS CONDUCTED ON SAMPLES COLLECTED FROM THE DES PLAINES RIVER: NUMBER OF POSITIVE SAMPLES/NUMBER OF SAMPLES

| Station | 1993 (Pre-TARP) | 1994 (Post-TARP) |
|---|---|---|
| 43 | 4/5 | 1/5 |
| 46 | 4/5 | 2/5 |

The mean (arithmetic) virus levels appeared to be lower post-TARP compared to the pre-TARP period. However, statistical analysis of the data showed that the differences were not significant (Appendix AII). The mean virus level at Station 43 in 1993 was 0.088 PFU/L compared to 0.048 in 1994 ($p = >0.05$). The mean virus level at Station 46 in 1993 was 0.080 PFU/L compared to 0.024 in 1994 ($p = >0.05$). Unfortunately, the number of samples collected for this study was small.

The number of virus types isolated was smaller in 1994 (post-TARP period) than in 1993 (pre-TARP period). Only eight virus types were isolated in 1994 compared to 13 virus types isolated in 1993.

Fecal Coliform Levels in Pre-TARP and Post-TARP Samples

Table 7 shows the fecal coliform densities at Stations 43 and 46 on the Des Plaines River determined from five samples separately collected from the virological samples, on a monthly basis, from January through May, 1990 through 1993. Fecal coliform density is an indicator of the sanitary quality of water. Samples from January through May (1990 through 1993) were used for fecal coliform densities, because this is the same five-month period which was used in 1993 and 1994 for virus analyses. In the four years prior to the operation of the tunnel, i.e., 1990 through 1993, the geometric means of fecal coliform levels at Stations 43 and 46 ranged from 604 to 7,092 colony forming units (CFUs)/100 mL, and from 791 to

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TABLE 7

FECAL COLIFORM DENSITY[1] IN THE DES PLAINES RIVER AT STATION 43 (ROOSEVELT ROAD) AND STATION 46 (OGDEN AVENUE), JANUARY THROUGH MAY, 1990 THROUGH 1993, BEFORE TARP OPERATION

| Year | Station 43 | Station 46 |
|---|---|---|
| 1990 | 2,234 | 1,642 |
| 1991 | 7,092 | 5,916 |
| 1992 | 604 | 791 |
| 1993 | 1,796 | 1,512 |

[1]Colony forming units/100 mL. Values shown are the geometric mean of results of analyses of samples collected monthly from January through May each year.

5,916 CFUs/100 mL, respectively (Table 7). After the Middle Leg of the Des Plaines TARP Subsystem became operational in 1994, these levels dropped to 478 CFUs/100 mL at Station 43, and to 323 CFUs/100 mL at Station 46 (Table 8). Results of statistical analyses indicated that the fecal coliform densities observed in 1994 (post-TARP) were significantly lower at both Stations 43 ($p = 0.035$) and 46 ($p = 0.013$) when compared to the pre-TARP period. These lower fecal coliform levels in 1994 indicated that fecal pollution of the Des Plaines River was lower in 1994 than in the four previous years.

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TABLE 8

FECAL COLIFORM DENSITY[1] IN THE DES PLAINES RIVER AT STATION 43 (ROOSEVELT ROAD) AND STATION 46 (OGDEN AVENUE), JANUARY THROUGH MAY 1994, AFTER TARP OPERATION

| Year | Station 43 | Station 46 |
|---|---|---|
| 1994 | 478 | 323 |

[1]Colony forming units/100 mL. Values shown are the geometric mean of results of analyses of samples collected monthly from January through May 1994.

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# APPENDIX AI

## PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES FROM THE DES PLAINES RIVER

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-1

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (PRE-TARP)
FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1993

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| 46 | 1/6/93 | 3/22/93 | Undiluted | 5 | 1 | 1 | CB5 |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 4/5/93 | Undiluted | 40 | 2 | 0 | |
| | | 5/3/93 | Undiluted | 21 | 3 | 3 | CB1(2), P2 |
| | | 5/10/93 | Undiluted | 40 | 3 | 2 | CB1,E27 |
| | | 5/17/93 | Undiluted | 40 | 1 | 1 | E7 |
| 43 | 1/6/93 | 3/22/93 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 1 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 3/29/93 | Undiluted | 21 | 3 | 1 | CB3 |
| | | 3/29/93 | Undiluted | 40 | 2 | 1 | CB3 |
| | | 4/5/93 | Undiluted | 40 | 9 | 3 | CB5,P2,CB3 |
| | | 5/3/93 | Undiluted | 40 | 1 | 1 | CB1 |
| 46 | 2/9/93 | 3/22/93 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-1 (Continued)

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (PRE-TARP)
FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1993

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| | | | 1:15 | 5 | 0 | 0 | |
| 46 | 2/9/93 | 3/29/93 | Undiluted | 40 | 2 | 1 | E9 |
| | | 4/5/93 | Undiluted | 40 | 1 | 1 | CB3 |
| | | 5/3/93 | Undiluted | 21 | 0 | 0 | |
| | | 5/17/93 | Undiluted | 40 | 1 | 0 | |
| 43 | 2/9/93 | 3/22/93 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 3/29/93 | Undiluted | 40 | 2 | 1 | P2 |
| | | 5/3/93 | Undiluted | 40 | 2 | 2 | E27,CB3 |
| | | 5/17/93 | Undiluted | 40 | 0 | 0 | |
| | | 5/24/93 | Undiluted | 21 | 0 | 0 | |
| 46 | 3/22/93 | 3/29/93 | Undiluted | 17 | 0 | 0 | |
| | | | 1.2 | 6 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | 5/3/93 | Undiluted | 40 | 1 | 1 | E20 |
| | | 5/10/93 | Undiluted | 40 | 2 | 1 | E11 |
| | | 5/17/93 | Undiluted | 40 | 1 | 1 | CB3 |
| | | 5/24/93 | Undiluted | 9 | 0 | 0 | |

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-1 (Continued)

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (PRE-TARP)
FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1993

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| 43 | 3/22/93 | 3/29/93 | Undiluted | 17 | 0 | 0 | |
| | | | 1:2 | 6 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | 5/3/93 | Undiluted | 40 | 0 | 0 | |
| | | 5/10/93 | Undiluted | 40 | 0 | 0 | |
| | | 5/17/93 | Undiluted | 9 | 0 | 0 | |
| | | 5/24/93 | Undiluted | 40 | 1 | 1 | CB3 |
| 46 | 4/1/93 | 4/5/93 | Undiluted | 17 | 1 | 0 | |
| | | | 1:2 | 6 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | 5/10/93 | Undiluted | 9 | 0 | 0 | |
| | | 5/24/93 | Undiluted | 40 | 3 | 3 | P2(2),P3 |
| | | 6/7/93 | Undiluted | 40 | 0 | 0 | |
| | | 6/14/93 | Undiluted | 40 | 1 | 1 | P2 |
| 43 | 4/1/93 | 4/5/93 | Undiluted | 17 | 0 | 0 | |
| | | | 1:2 | 6 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | 5/10/93 | Undiluted | 9 | 2 | 2 | CA16,P2 |
| | | 5/24/93 | Undiluted | 40 | 0 | 0 | |
| | | 6/7/93 | Undiluted | 40 | 2 | 2 | P2(2) |
| | | 6/14/93 | Undiluted | 40 | 4 | 4 | P2(2),P1 CB4 |

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-1 (Continued)

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (PRE-TARP)
FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1993

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| 46 | 5/6/93 | 5/10/93 | Undiluted | 17 | 0 | 0 | |
| | | | 1:2 | 6 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | 5/17/93 | Undiluted | 9 | 0 | 0 | |
| | | 5/24/93 | Undiluted | 40 | 0 | 0 | |
| | | 6/7/93 | Undiluted | 40 | 0 | 0 | |
| | | 6/21/93 | Undiluted | 40 | 0 | 0 | |
| 43 | 5/6/93 | 5/10/93 | Undiluted | 17 | 0 | 0 | |
| | | | 1:2 | 6 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | 6/7/93 | Undiluted | 9 | 0 | 0 | |
| | | 6/14/93 | Undiluted | 40 | 0 | 0 | |
| | | 6/21/93 | Undiluted | 40 | 0 | 0 | |
| | | 6/28/93 | Undiluted | 40 | 1 | 0 | |

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-2

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (POST-TARP) FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1994

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| 46 | 1/3/94 | 1/10/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 1 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 1/18/94 | Undiluted | 40 | 0 | 0 | |
| | | 1/24/94 | Undiluted | 40 | 0 | 0 | |
| | | 1/31/94 | Undiluted | 40 | 1 | 0 | |
| | | 2/7/94 | Undiluted | 21 | 1 | 1 | CB4 |
| 43 | 1/5/94 | 1/10/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 1/18/94 | Undiluted | 40 | 0 | 0 | |
| | | 1/24/94 | Undiluted | 40 | 0 | 0 | |
| | | 1/31/94 | Undiluted | 40 | 0 | 0 | |
| | | 2/7/94 | Undiluted | 21 | 1 | 0 | |
| 43 | 2/7/94 | 2/14/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 2 | 0 | |

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-2 (Continued)

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (POST-TARP) FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1994

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| 43 | 2/7/94 | | 1:5 | 5 | 0 | 0 | |
| | | 3/28/94 | Undiluted | 40 | 0 | 0 | |
| | | 4/25/94 | Undiluted | 21 | 1 | 0 | |
| | | 5/2/94 | Undiluted | 40 | 0 | 0 | |
| | | 5/9/94 | Undiluted | 40 | 0 | 0 | |
| 46 | 2/22/94 | 3/28/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 1 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 4/4/94 | Undiluted | 40 | 1 | 0 | |
| | | 4/25/94 | Undiluted | 21 | 0 | 0 | |
| | | 5/2/94 | Undiluted | 40 | 5 | 0 | |
| | | 5/9/94 | Undiluted | 40 | 7 | 0 | |
| 43 | 3/1/94 | 3/28/94 | Undiluted | 5 | 1 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 4/4/94 | Undiluted | 40 | 6 | 3 | P1, CA7 CB3 |
| | | 4/25/94 | Undiluted | 40 | 6 | 6 | CA9, E13 |

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-2 (Continued)

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (POST-TARP)
FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1994

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| | | | | | | | E21, CB3(3) |
| 43 | 3/1/94 | 5/2/94 | Undiluted | 21 | 0 | 0 | |
| | | 5/9/94 | Undiluted | 40 | 5 | 1 | CB3 |
| 46 | 3/3/94 | 4/4/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 1 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 4/25/94 | Undiluted | 40 | 2 | 2 | CA16, CB3 |
| | | 5/2/94 | Undiluted | 21 | 0 | 0 | |
| | | 5/9/94 | Undiluted | 40 | 3 | 2 | CB3(2) |
| | | 6/6/94 | Undiluted | 40 | 1 | 0 | |
| 43 | 4/4/94 | 4/25/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1.5 | 5 | 0 | 0 | |
| | | 5/2/94 | Undiluted | 40 | 0 | 0 | |
| | | 5/9/94 | Undiluted | 40 | 3 | 0 | |
| | | 6/6/94 | Undiluted | 40 | 0 | 0 | |
| | | 6/27/94 | Undiluted | 21 | 1 | 0 | |

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-2 (Continued)

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (POST-TARP)
FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1994

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| 46 | 4/6/94 | 4/25/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 6/6/94 | Undiluted | 40 | 0 | 0 | |
| | | 6/27/94 | Undiluted | 40 | 0 | 0 | |
| | | 7/11/94 | Undiluted | 40 | 0 | 0 | |
| | | 8/1/94 | Undiluted | 21 | 0 | 0 | |
| 43 | 5/23/94 | 6/6/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |
| | | | 1:5 | 5 | 0 | 0 | |
| | | 6/27/94 | 1:3 | 120 | 2 | 0 | |
| | | 7/11/94 | 1:3 | 120 | 3 | 0 | |
| | | 8/1/94 | 1:3 | 120 | 1 | 0 | |
| | | 8/8/94 | 1:3 | 63 | 0 | 0 | |
| 46 | 5/24/94 | 6/6/94 | Undiluted | 5 | 0 | 0 | |
| | | | 1:2 | 2 | 0 | 0 | |
| | | | 1:3 | 3 | 0 | 0 | |
| | | | 1:4 | 4 | 0 | 0 | |

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AI-2 (Continued)

PLAQUE ASSAY DATA: ASSAYS OF CONCENTRATES OF SAMPLES (POST-TARP)
FROM THE DES PLAINES RIVER, JANUARY THROUGH MAY 1994

| Station Number | Date Sample Collected | Date of Assay | Dilution of Concentrate Tested | Number of Plates | Number of Plaques Picked | Number of Confirmed Plaques | Identity of Virus(es) |
|---|---|---|---|---|---|---|---|
| 46 | 5/24/94 | | 1:5 | 5 | 2 | 0 | |
| | | 8/1/94 | 1:3 | 63 | 0 | 0 | |
| | | 8/8/94 | 1:3 | 120 | 1 | 0 | |
| | | 8/8/94 | 1:3 | 32 | 0 | 0 | |
| | | 8/15/94 | 1:3 | 88 | 0 | 0 | |
| | | 8/22/94 | 1:3 | 120 | 3 | 0 | |

# APPENDIX AII

## STATISTICAL ANALYSIS OF VIROLOGICAL AND FECAL COLIFORM DATA

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-1

COMPARISON OF MEAN VIRUS LEVELS AT STATION 43
IN 1993 (PRE-TARP), AND IN 1994 (POST-TARP)

| Year | Mean PFU/L | Kruskal-Wallis Test (Chi-square Approximation) Statistics | Significance Probability | Wilcoxon Rank-Sum Test (T-test on Ranked Data) Statistics | Significance Probability |
|---|---|---|---|---|---|
| 1993 | 0.088 | 0.010909 | 0.91681[1] | 0.099136 | 0.92347[1] |
| 1994 | 0.048 | | | | |

[1]Not significant.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-2

COMPARISON OF MEAN VIRUS LEVELS AT STATION 46
IN 1993 (PRE-TARP), AND IN 1994 (POST-TARP)

| Year | Mean PFU/L | Kruskal-Wallis Test (Chi-square Approximation) Statistics | Significance Probability | Wilcoxon Rank-Sum Test (T-test on Ranked Data) Statistics | Significance Probability |
|---|---|---|---|---|---|
| 1993 | 0.08 | 1.32 | 0.25059[1] | 1.26596 | 0.24114[1] |
| 1994 | 0.024 | | | | |

[1]Not significant.

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-3

COMPARISON OF LOG TRANSFORMED GEOMETRIC MEANS OF FECAL COLIFORM LEVELS AT STATION 43 FROM 1990 THROUGH 1993 (PRE-TARP), AND IN 1994 (POST-TARP)

| Years(s) | Log Transformed Geometric Mean Fecal Coliform Level | F | T-Statistic | Significance Probability |
|---|---|---|---|---|
| 1990 through 1993 | 7.61896 | 0.52221 | 1.90216 | 0.034873[1] |
| 1994 | 6.16955 | | | |

[1]Significant

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

TABLE AII-4

COMPARISON OF LOG TRANSFORMED GEOMETRIC MEANS OF FECAL COLIFORM LEVELS AT STATION 46 FROM 1990 THROUGH 1993 (PRE-TARP), AND IN 1994 (POST-TARP)

| Years(s) | Log Transformed Geometric Mean Fecal Coliform Level | F | T-Statistic | Significance Probability |
|---|---|---|---|---|
| 1990 through 1993 | 7.52069 | 0.13627 | 2.38347 | 0.013105[1] |
| 1994 | 5.77770 | | | |

[1]Significant