APPENDIX F

STATISTICAL ANALYSIS OF DATA

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The statistical analyses conducted were advised by Professor Hariharan K. Iyer, Department of Statistics, Colorado State University (the authors assume responsibility for any misinterpretation of Professor Iyer's advice).

The designations for the statistical analysis techniques used include: (1) trend of relationship between variables, (2) correlation analysis, (3) significance of the association and (4) probability of occurrence. These techniques are described in the following paragraph.

TREND OF VARIABLES

In order to show the trend of the relationship between two variables the median for each variable for each group of points was calculated. The median of the first few points in ascending data, was obtained first. This was followed by obtaining the median of second few points, and so on. These medians thus showed the overall trend in the data. Figure F-1 shows the trend of scattered data by connecting the median points.

Example

Consider Figure F-1. The figure shows a plot of the percentage removal of Giardia cysts, versus percentage removal of turbidity. The total number of data are seventy two points. Picking first 8 points from left to right, compute the medians of these 8 points for turbidity, and also the 8 points for Giardia. Then plot the median. Continue and plot the median for the second group of 8 points of data, and calculate the median for both turbidity and Giardia as before. Continue for third group, the fourth group, etc. Connect these median points by a line to show the general trend of data.

CORRELATION ANALYSIS

Correlation analysis provides a statistical summary of the strength of association between variables. The method, described as the Spearman method, first ranks the data used to calculate the correlation coefficients. The coefficient varies from - 1.0 to + 1.0. A coefficient of 0 always indicates that no monotone relationship exists. A coefficient of 1.0 implies a "perfect" positive monotone relationship. A coefficient of - 1.0 indicates a "perfect" negative monotone relationship. Thus to prove there is positive association between two variables, the coefficient should range from zero to + 1.0.

A computer package (Minitab), available on the CSU Cyber 720 can be used to compute the correlation coefficient, r_s. The Spearman equation for computing r_s is:

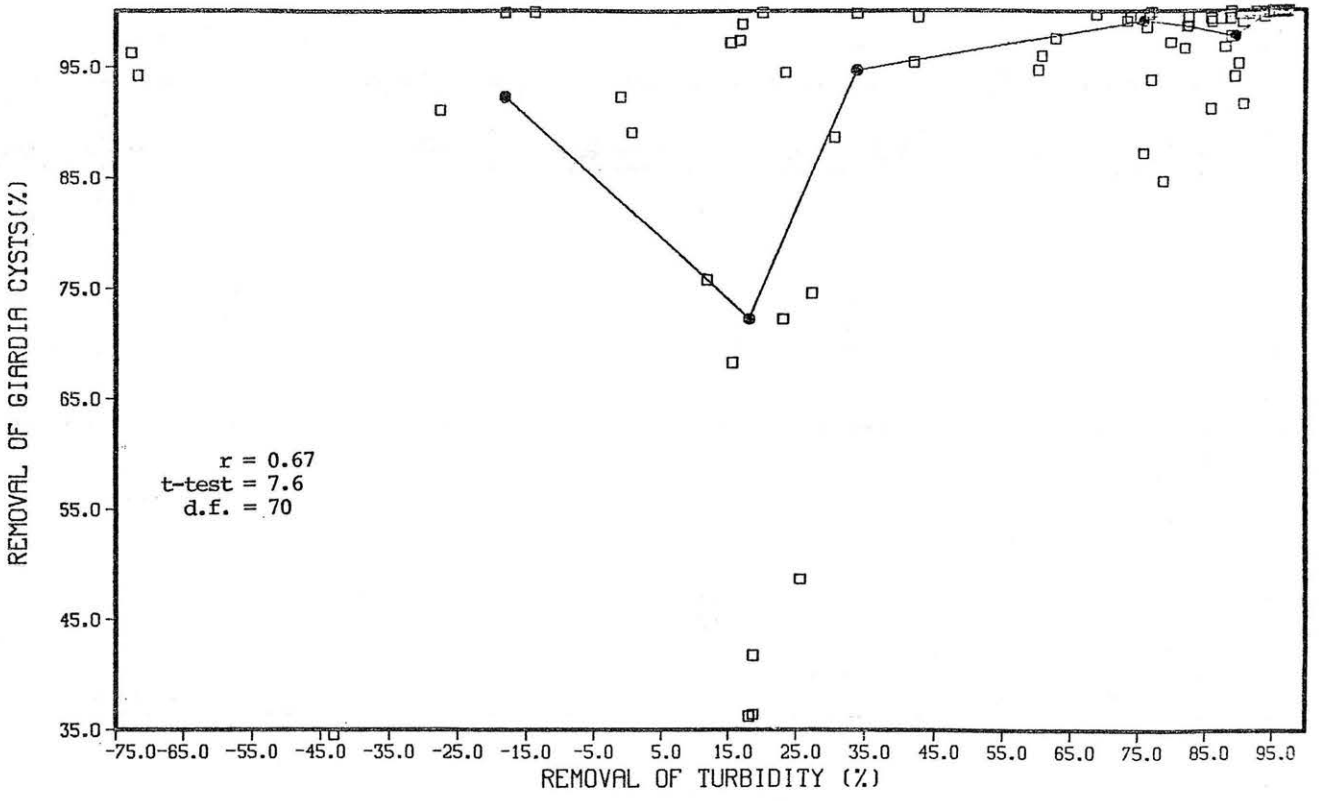


Figure F-1. Observations of percent removal of Giardia cysts with corresponding percent removal of turbidity. Data obtained from Table A-1 and Table A-2. The solid points show the median for each 8 points, connected by line.

$$r_s = 1 - \frac{6 \sum_{i=1}^{n} d_i^2}{N^3 - N}$$

where d = difference between ranks of corresponding pairs of the two variables, and

N = number of observations

The Spearman correlation coefficient for the data shown in Figure F-1 is 0.673. This implies a positive relationship between the percent of turbidity removed and percent of Giardia cysts removed. Thus when a higher percent removal occurs for turbidity, a higher percent removal of Giardia cysts is expected.

SIGNIFICANCE OF ASSOCIATION

The significance of the "association" correlation coefficient, r_s, data is determined by comparing the quantity, t, as defined,

$$t = r_s \frac{N - 2}{1 - r_s^2}^{1/2}$$

with student's t-distribution with N-2 degrees of freedom.

Example

The computed t for data figure 1 is 7.612. This compared with a value 2.65 from t-distribution table (for one tail) at level of significance of 0.005 for 70 degrees of freedom . Therefore we accept the hypothesis of positive association at 0.995 level of confidence.

Probability of Occurrence

A complementary probability method is used to predict the "probability of occurence" of certain levels of removal. Figure F-1 shows the percentage removal of Giardia cysts vs percentages removal of turbidity. The probability of removal of certain level of Giardia cysts due to the removal of certain levels of turbidity is predicted by using a complementary method. To determine the probability of the percent of Giardia removed when 80 percent or more of turbidity removed is predicted as follows. (1) Pick all the points which give 80 percent or more of turbidity removal and put these points in two columns. The right column is percent removal of Giardia cysts, ranked in ascending order; the left column is the percent removal of the associated turbidity values. Table F-1 illustrates, using the data shown in Figure F-1. There are 35 points. Calculate the estimated probability as first point as 35/36, the second point as 34/36, the third point as 33/36 and so on; the last point is 1/36. These probability numbers are in a fourth column in Table F-1, to the right of the Giardia data. Then draw the

Table F-1. Showing an example of calculating an estimated probability.

Point Number	% Removal Turbidity	% Removal Giardia	Estimated Probability
1	86.00	91.250	0.9999
2	90.79	91.690	0.9714
3	89.47	94.150	0.9429
4	90.00	95.320	0.9143
5	82.03	96.720	0.8857
6	88.00	96.850	0.8571
7	80.00	97.240	0.8286
8	88.89	97.770	0.8000
9	82.41	98.700	0.7714
10	90.63	99.060	0.7429
11	86.11	99.130	0.7143
12	87.50	99.370	0.6857
13	88.68	99.380	0.6571
14	85.94	99.390	0.6286
15	82.61	99.470	0.6000
16	93.85	99.480	0.5714
17	91.67	99.740	0.5429
18	19.20	99.750	0.5143
19	94.55	99.810	0.4857
20	95.20	99.870	0.4571
21	97.11	99.900	0.4286
22	96.70	99.900	0.4000
23	94.21	99.910	0.3714
24	92.65	99.920	0.3429
25	96.84	99.940	0.3143
26	95.03	99.950	0.2857
27	95.91	99.950	0.2571
28	88.89	99.960	0.2286
29	97.60	99.970	0.2000
30	96.00	99.970	0.1714
31	95.00	99.970	0.1429
32	94.95	99.970	0.1143
33	95.45	99.980	0.0857
34	97.20	99.990	0.0571
35	96.36	99.990	0.0286

estimated probability of occurence of a given percent removal of Giardia cysts, designated as "estimated probability" in the ordinate, vs Giardia cysts removal percentage on the abcissa, as shown in Figure F-2. To read the graph, consider the question of finding the probability of 95 percent Giardia cysts removal when turbidity removal is 80 percent. The procedure as follows: (1) Enter the abcissa at 95 percent of Giardia cyst removal, (2) using the 80 percent turbidity removal curve, read the corresponding estimated probability of occurence that Giardia removal is 93 percent. Thus if turbidity removal is 80 percent, the estimated probability is 93 percent that Giardia cyst removal is 95 percent.

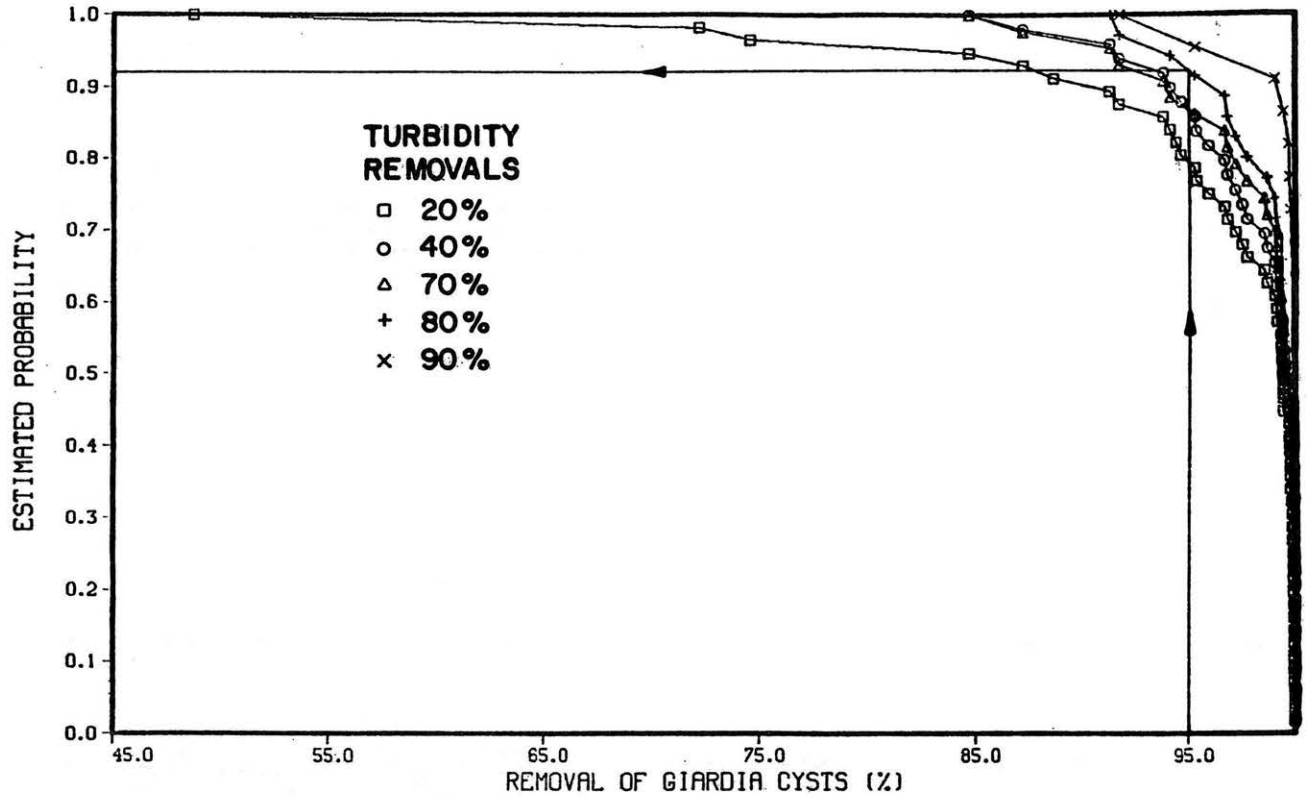


Figure F-2. Probability of a given percent removal of Giardia cysts for specified percent removals of turbidity. Data obtained from Table A-1 and Table A-2 plotted in Figure E-12.

APPENDIX G

SUPPORTING GRAPHS

In analysis of the results, contained in Table A-1, a large number of plots were generated. Because it would be to repititious to include all of them in the text, they are placed here for reference. In addition, some were used as the basis for developing plots and tables to show information in alternative ways, e.g. three-dimensional histograms.

Test Conditions
Run number(s) : 157,158,160
Raw water turbidity : 0.45 NTU
Temperature : 7C
Primary coagulant : Run 157 1.0 mg/l 572C. Run 158 5.4 mg/l alum.
Run 159 4.3 mg/l 572C + 4.3 mg/l alum.
Secondary coagulant : Run 157 9.0 mg/l alum. Run 158 0.5 572C.

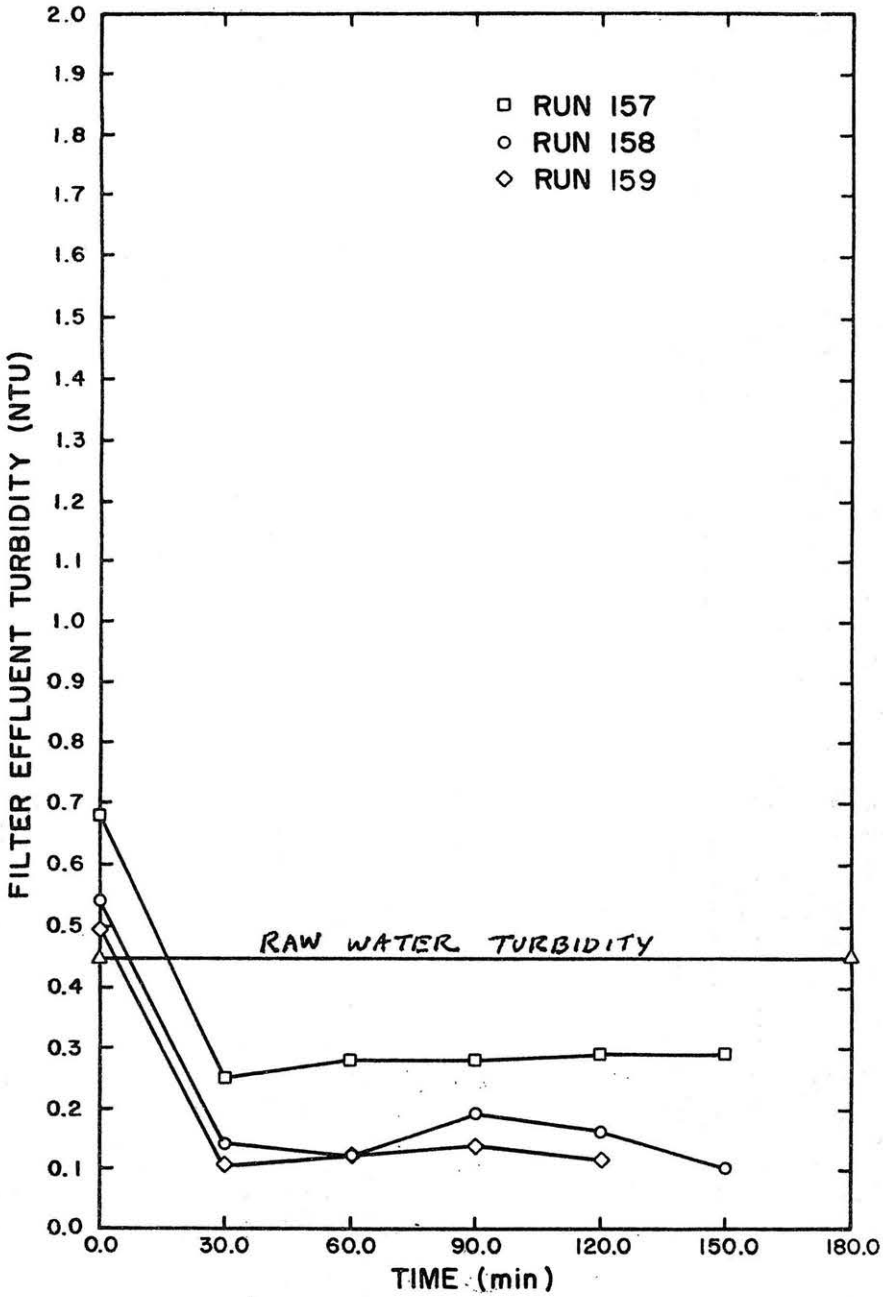


Figure G-1. Effect of sequence of alum-polymer additions on effluent turbidity, laboratory-scale pilot plant using dual media with Fort Collins sand, artificial low turbidity water from Horsetooth Reservoir, and "in-line" filtration.

Test Conditions
Run number(s) : 152,154,155
Raw water turbidity : 0.45 NTU
Temperature : 18C
Primary coagulant : Run 152 0.7 mg/l alum + 1.9 mg/l 572C.
Run 154 0.9 mg/l alum, Run 155 0.5 mg/l 572C.
Secondary coagulant : Run 154 0.5 mg/l 572C, Run 155 1.4 mg/l alum.

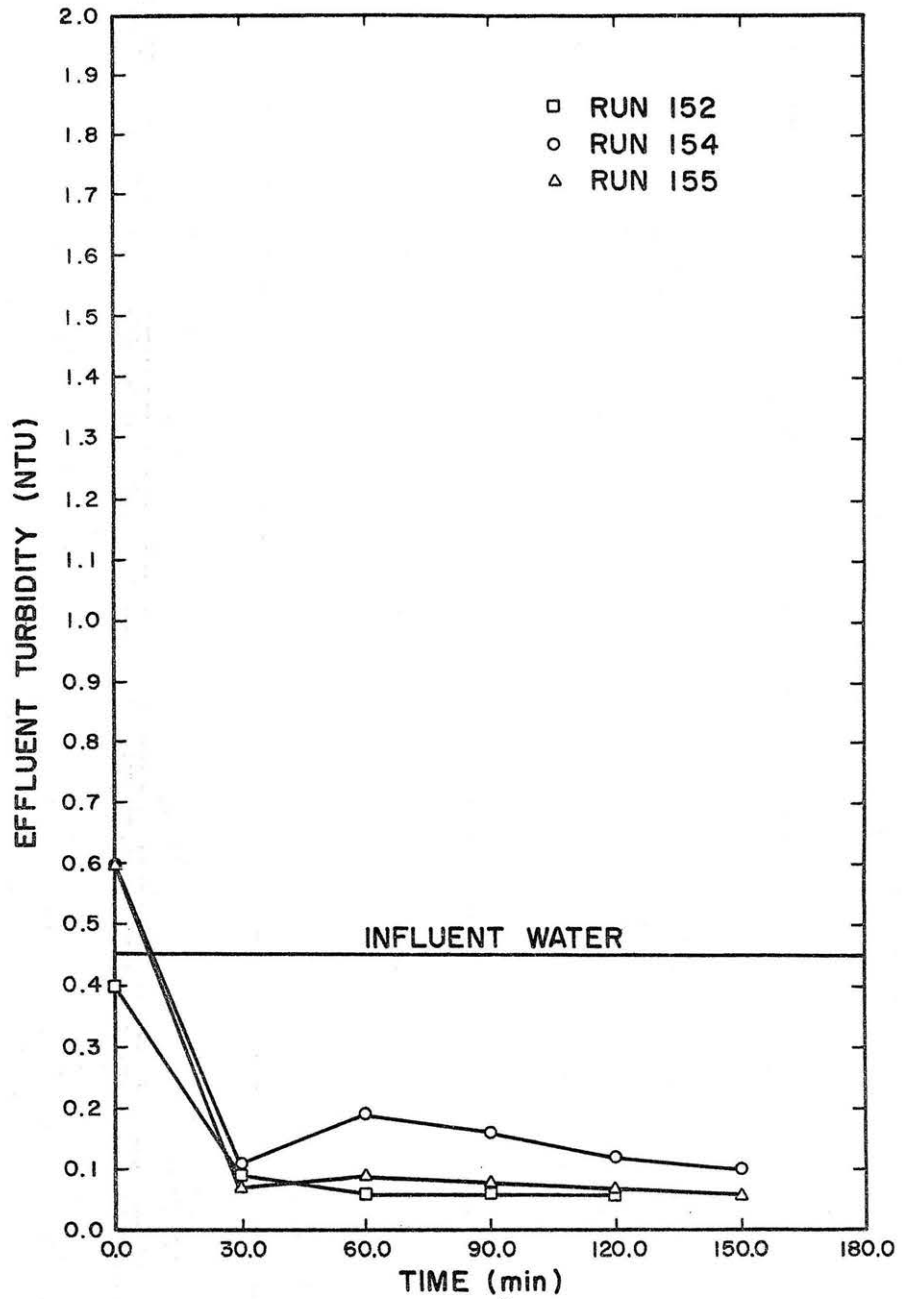


Figure G-2. Effect of sequence of alum-polymer additions on effluent turbidity, laboratory-scale pilot plant using dual media with Fort Collins sand, artificial low turbidity water from Horsetooth Reservoir and "in-line" filtration.

Test Conditions
Run number(s) : 146 "in-line", 147 direct filtration
Raw water turbidity : 0.89 NTU
Temperature : 3.5C
Primary coagulant : Run 146, and Run 147 9.32 mg/l alum.
Secondary coagulant : Run 146, and Run 147 0.35 mg/l 572C

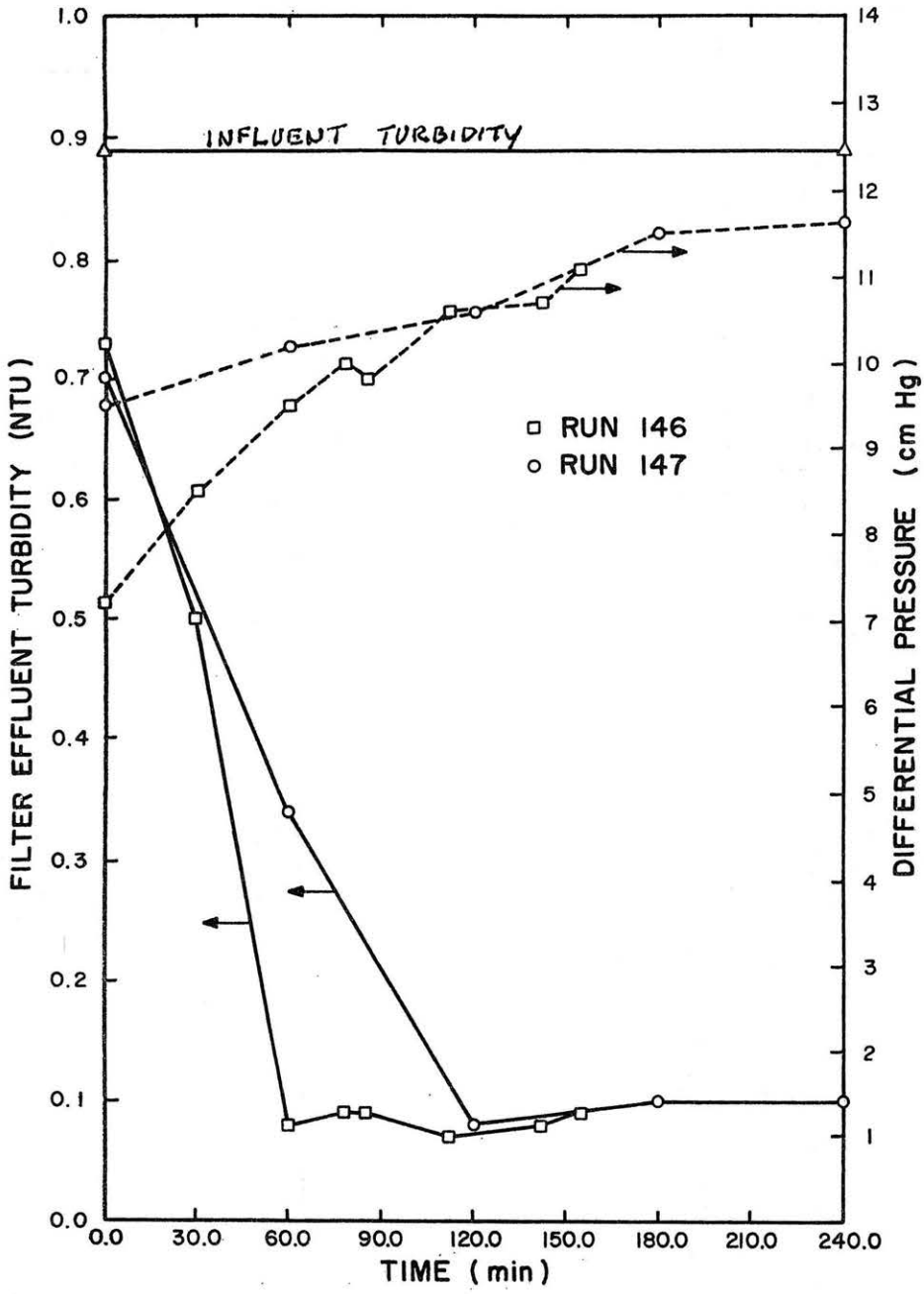


Figure G-3. Comparison of turbidity and headloss for "in-line" and "direct" filtration laboratory-scale pilot plant using dual media Fort Collins sand, artificial low turbidity water from Horsetooth Reservoir.

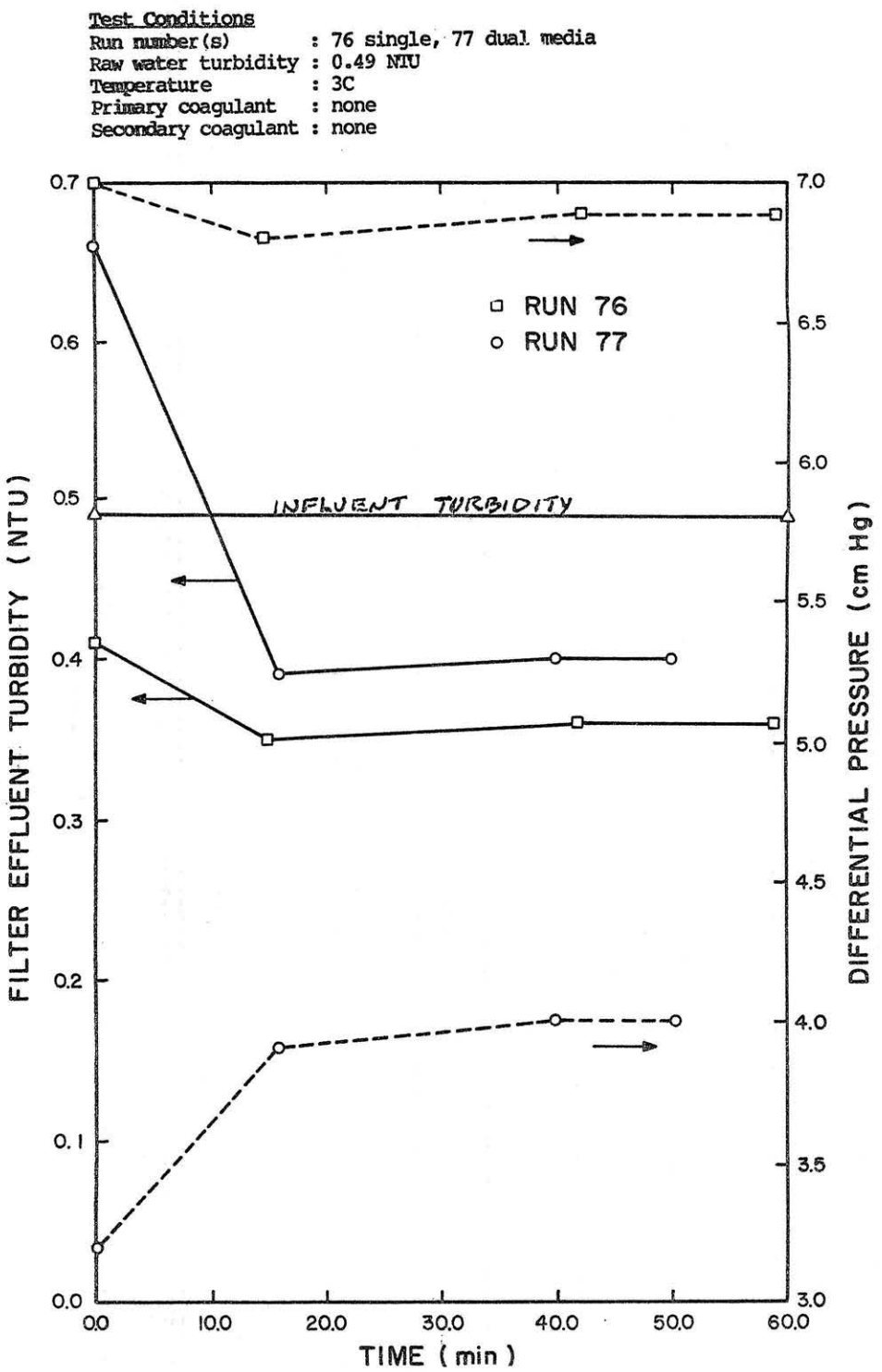


Figure G-4. Comparison of turbidity and headloss for single and dual media of Loveland sand, wth no chemical pretreatment. Laboratory-scale using artificial low turbidity water from Horsetooth Reservoir, and "in-line" filtration.

Test Conditions
Run number(s) : 71 sand, 72 dual media.
Raw water turbidity : 0.96 NTU
Temperature : 3C
Primary coagulant : Run 71 15 mg/l alum. Run 72 16.6 mg/l alum.
Secondary coagulant : Run 71 1.3 mg/l 572C. Run 72 1.3 mg/l 572C.

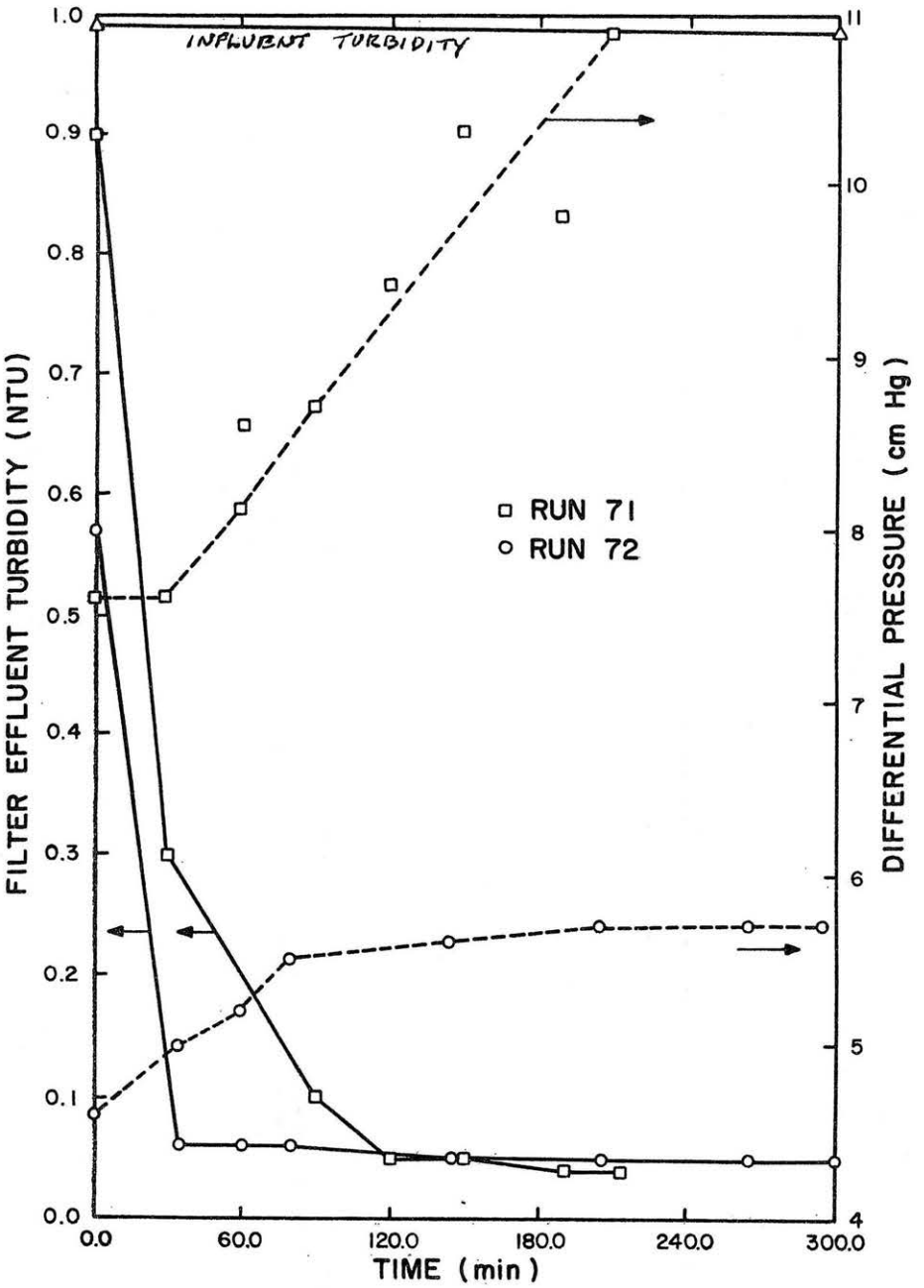


Figure G-5. Comparison of turbidity and headloss for single and dual media of Loveland sand, with optimum chemical pretreatment. Laboratory-scal pilot plant, using artificial low turbidity water from Horsetooth Reservoir and "in-line" filtration.

Test Conditions
Run number(s) : 98 sand, 99 dual media
Raw water turbidity : 2.4 NTU
Temperature : 3C
Primary coagulant : Run 98 8.79 mg/l 573C
Run 99 7.46 mg/l 573C
Secondary coagulant : Run 98 none. Run 99 none.

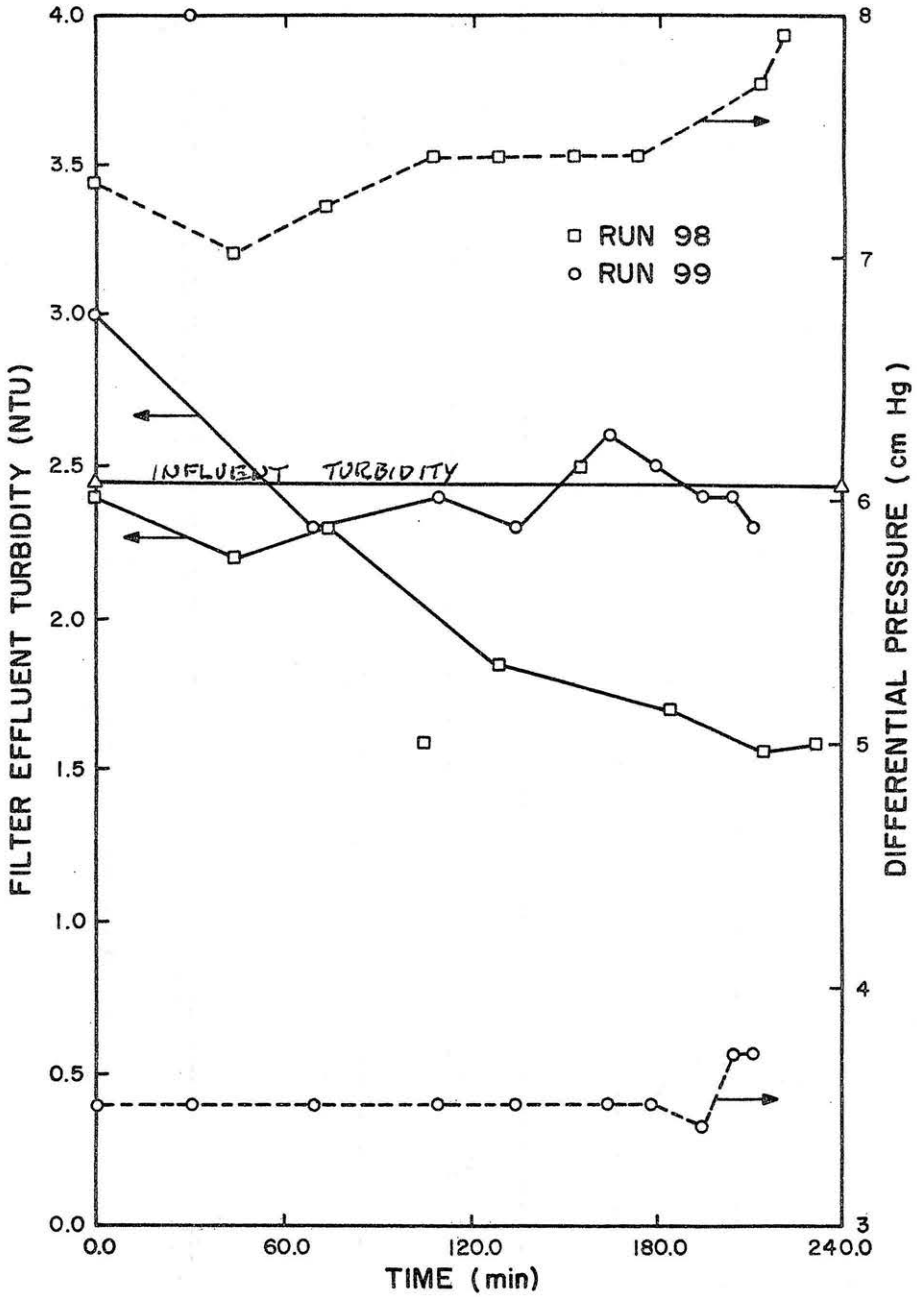


Figure G-6. Comparison of turbidity and headloss for single and dual media of Loveland sand, with alum pretreatment. Laboratory-scale pilot plant using artificial low turbidity water from Horsetooth Reservoir and "in-line" filtration.

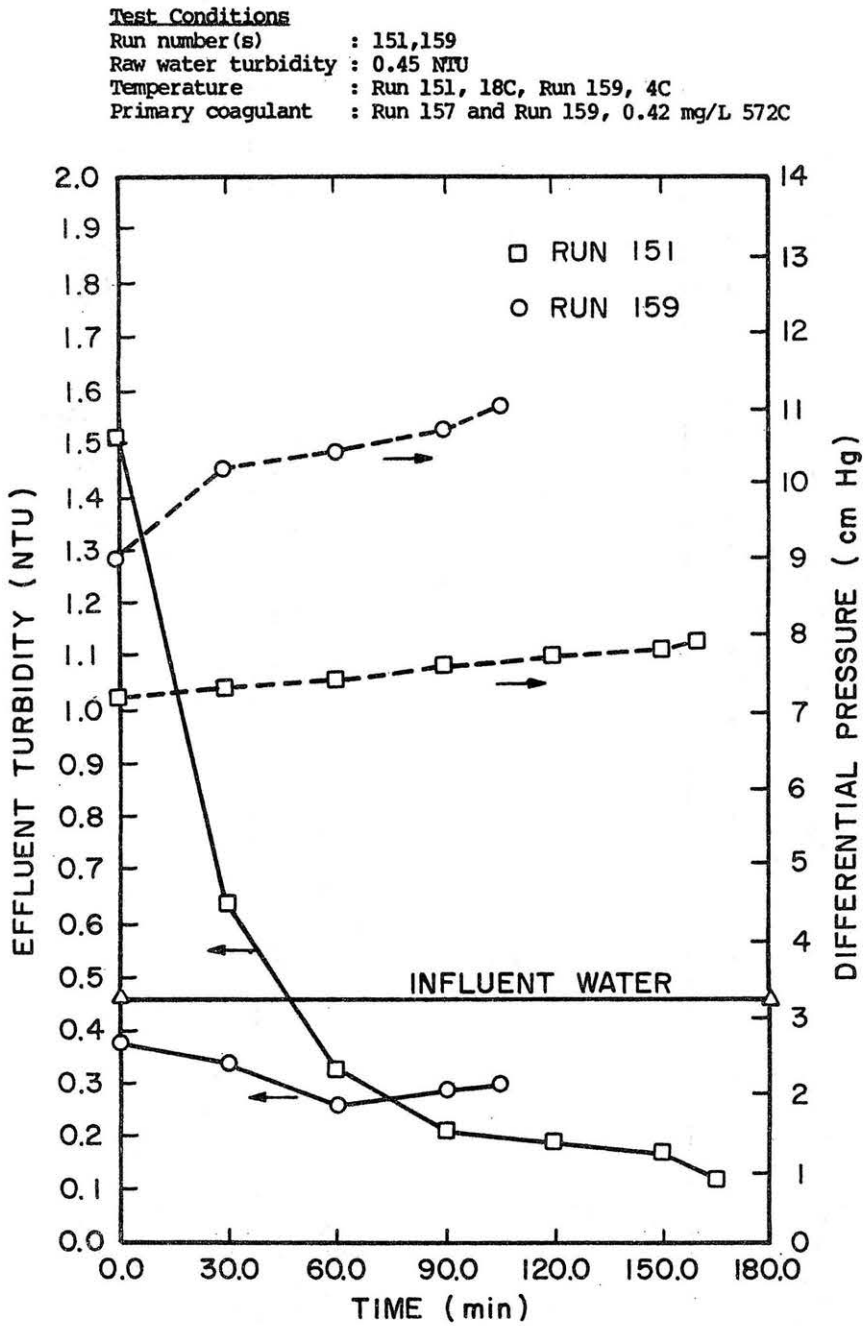


Figure G-7. Effluent turbidity and headloss versus time, showing the effect of temperature on turbidity reduction, and headloss.

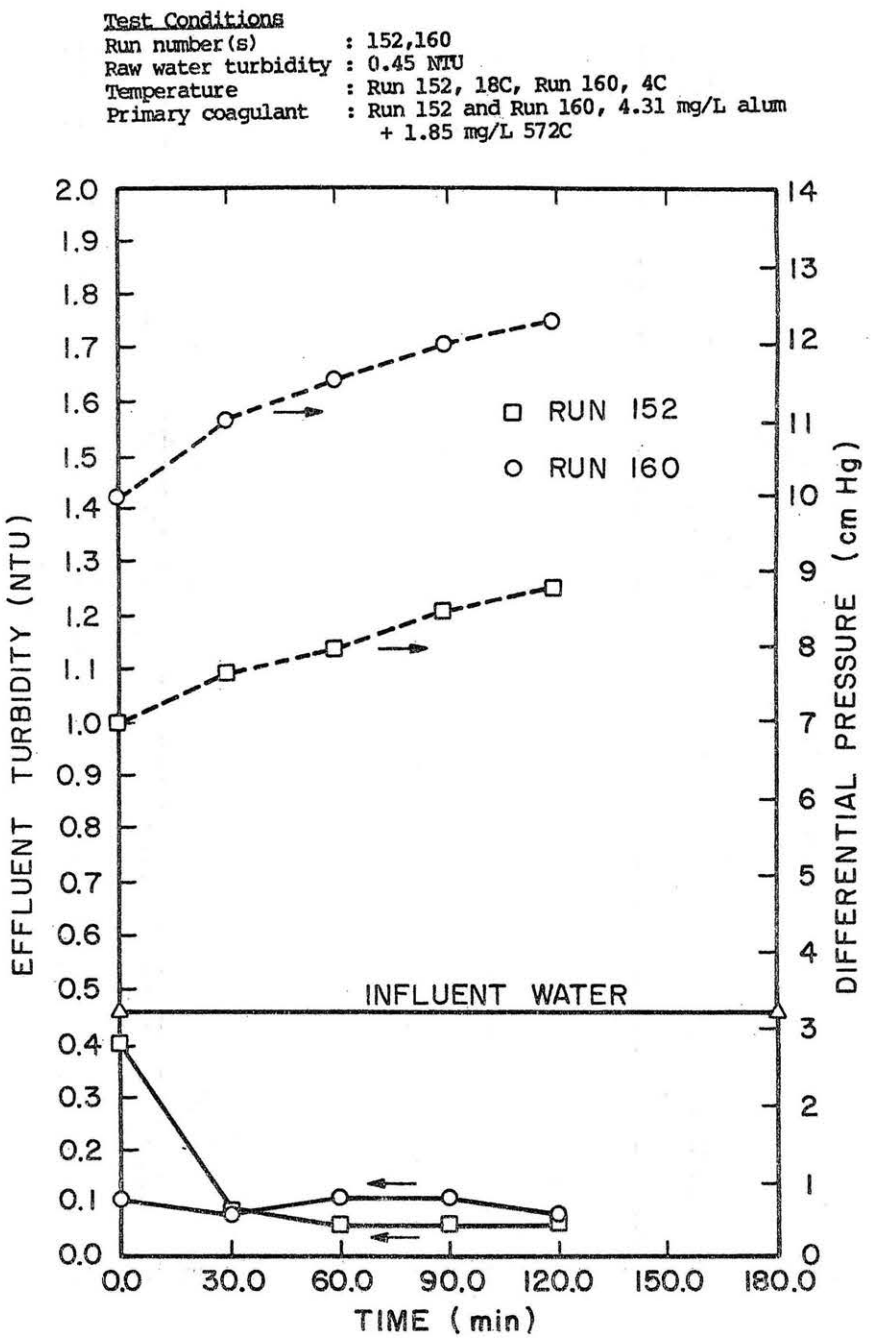


Figure G-8. Effluent turbidity and headloss versus time, showing the effect of temperature on turbidity reduction and headloss for adding alum and Magnifloc 572C in the same basin.

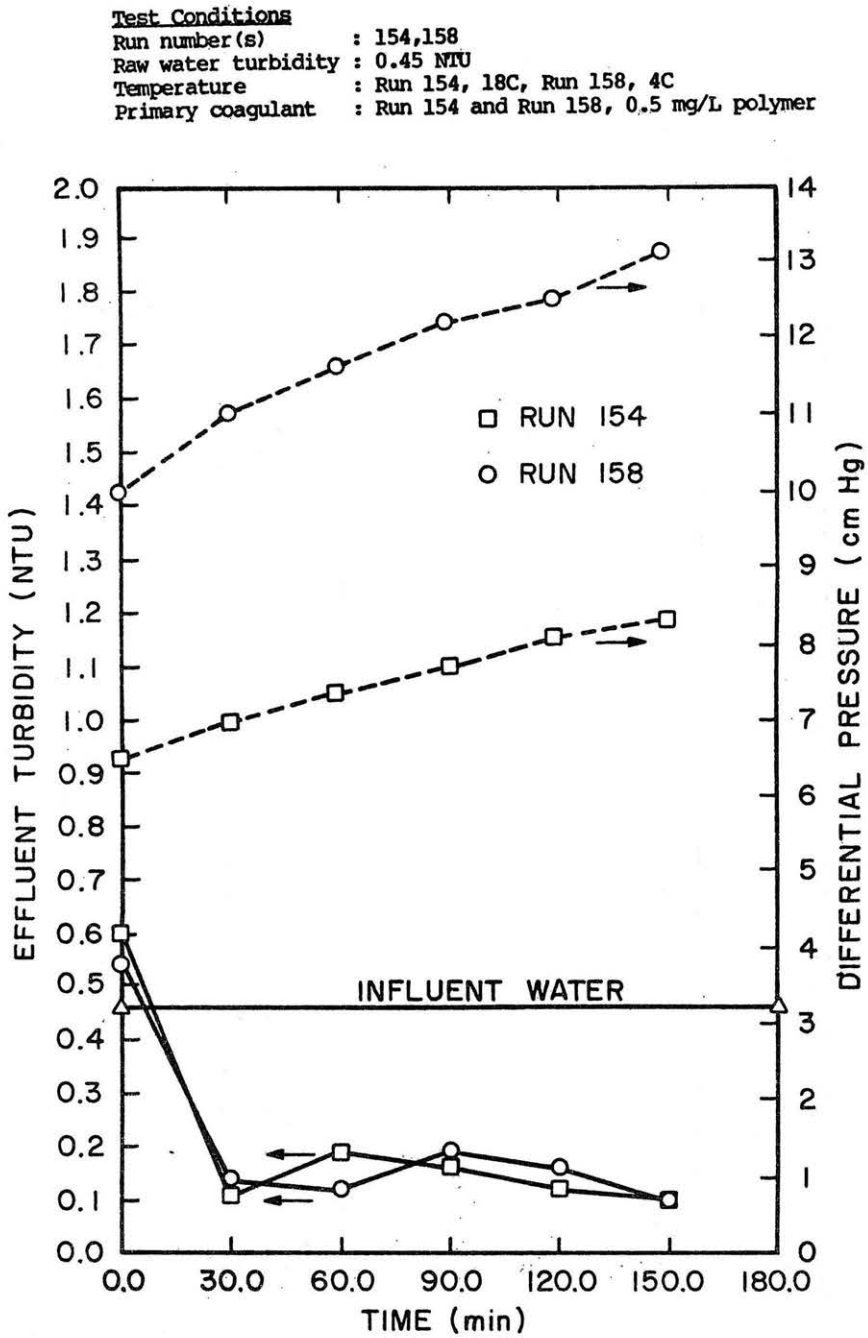


Figure G-9. Effluent turbidity and headloss versus time, showing the effect of temperature on turbidity reduction and headloss when alum was added and then Magnifloc 572C in two coagulant basins.

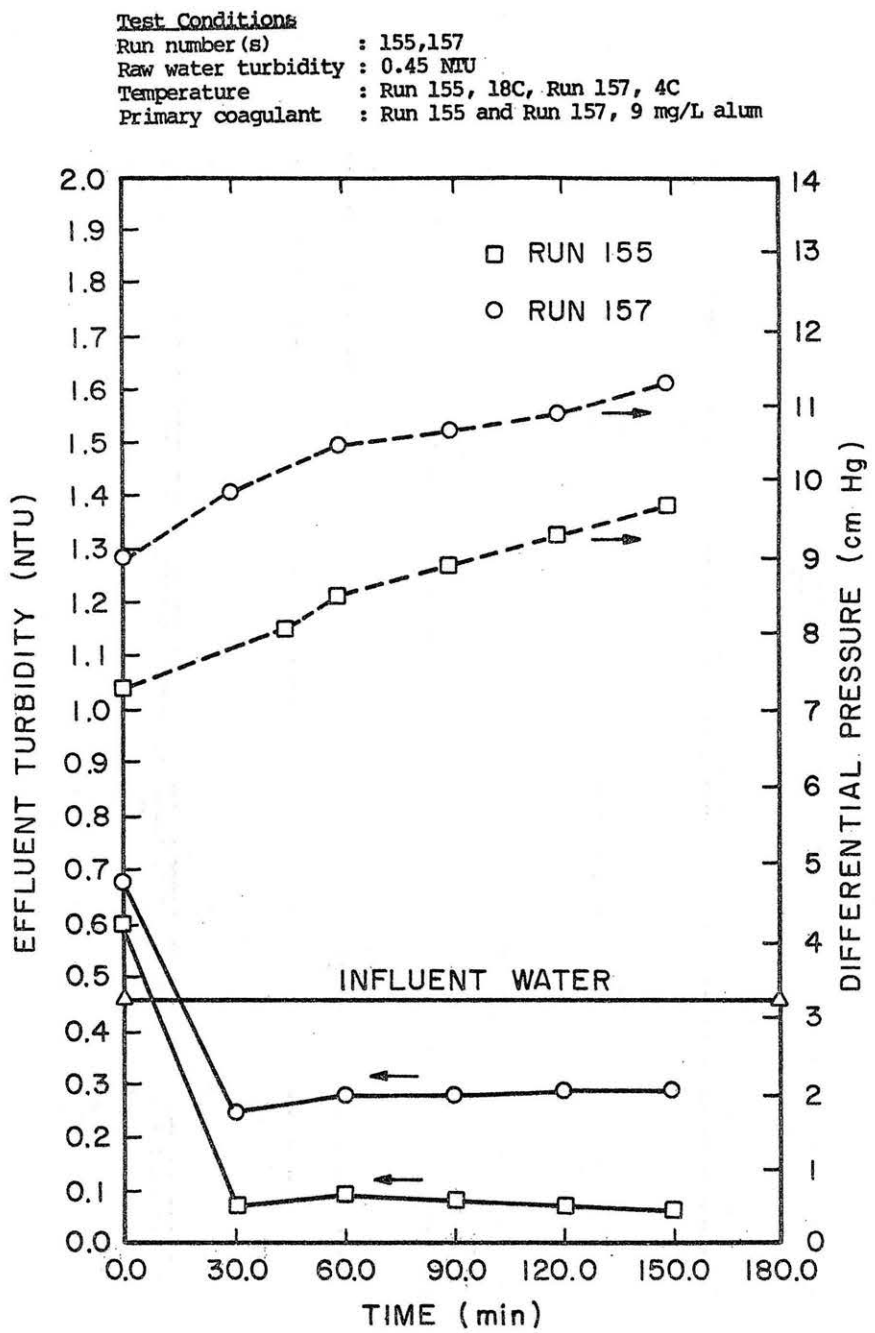


Figure G-10. Effluent turbidity and headloss versus tim, showing the effect of temperature on turbidity reduction and headloss when Magnifloc 572C was added before alum in two coagulant basins.

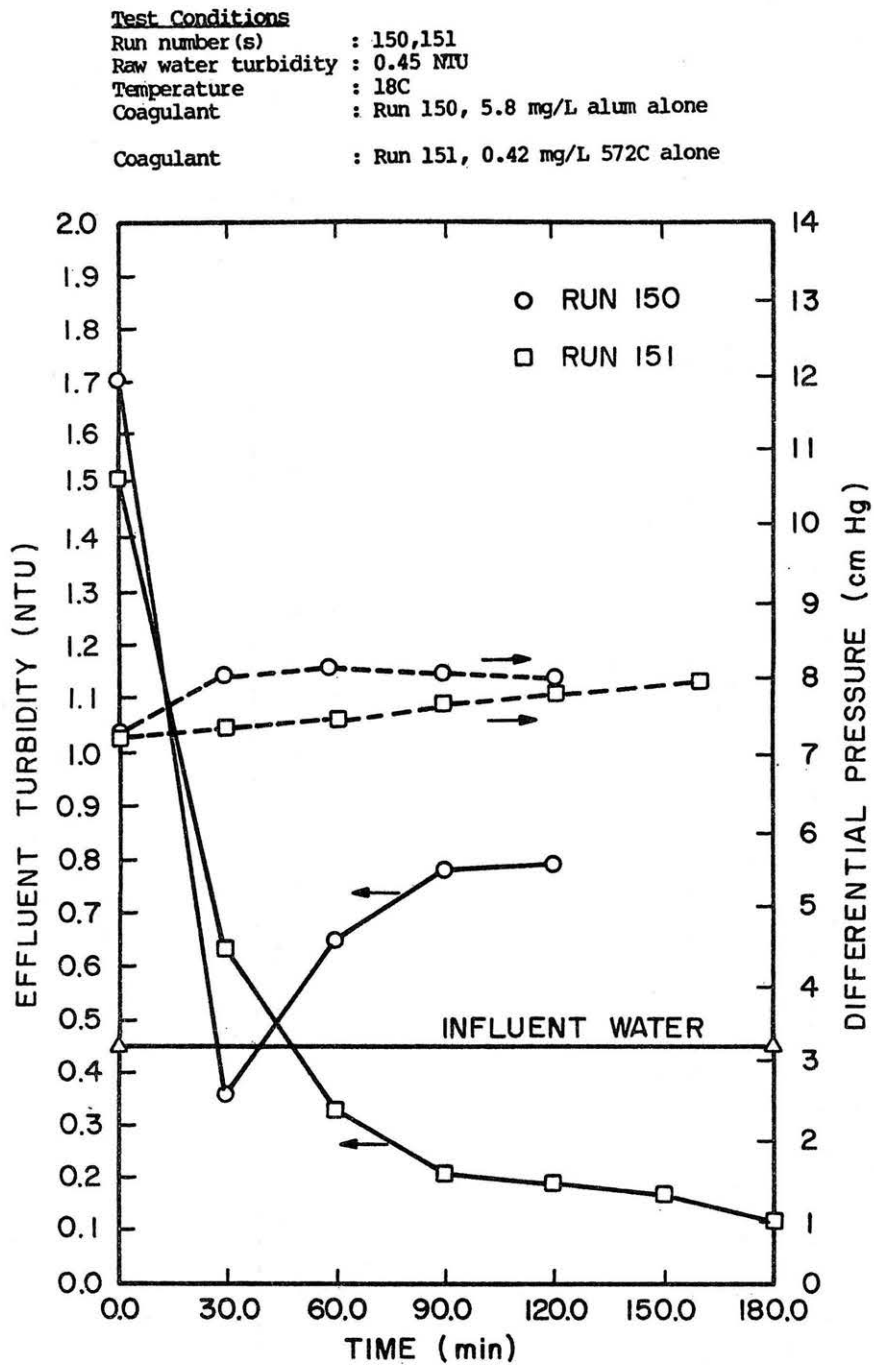


Figure G-11. Effluent turbidity versus time, showing the effect of turbidity reduction and headloss of using alum alone or Magnifloc 572C alone.

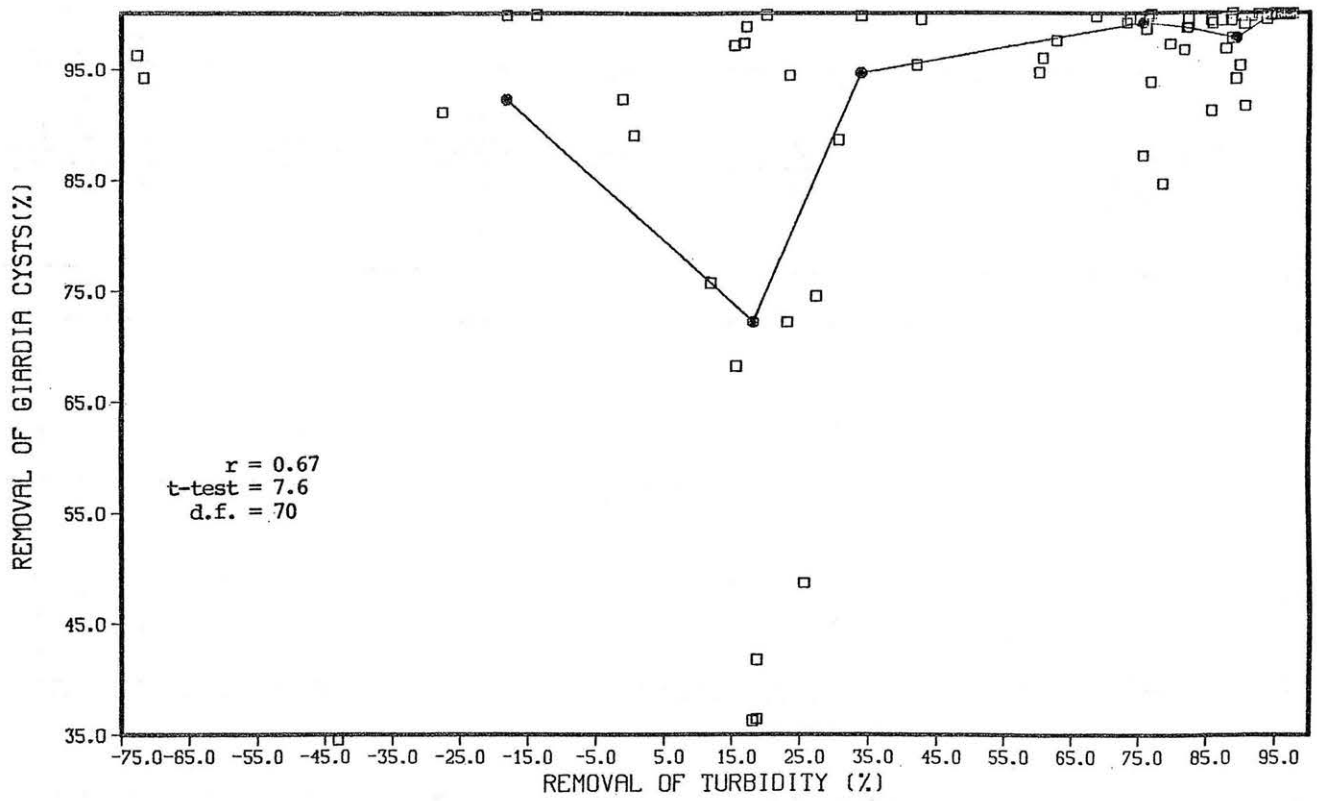


Figure G-12. Observations of percent removal of Giardia cysts with corresponding percent removal of turbidity. Data obtained from Table A-1 and Table A-2. The solid points show the median for each 8 points, connected by line.

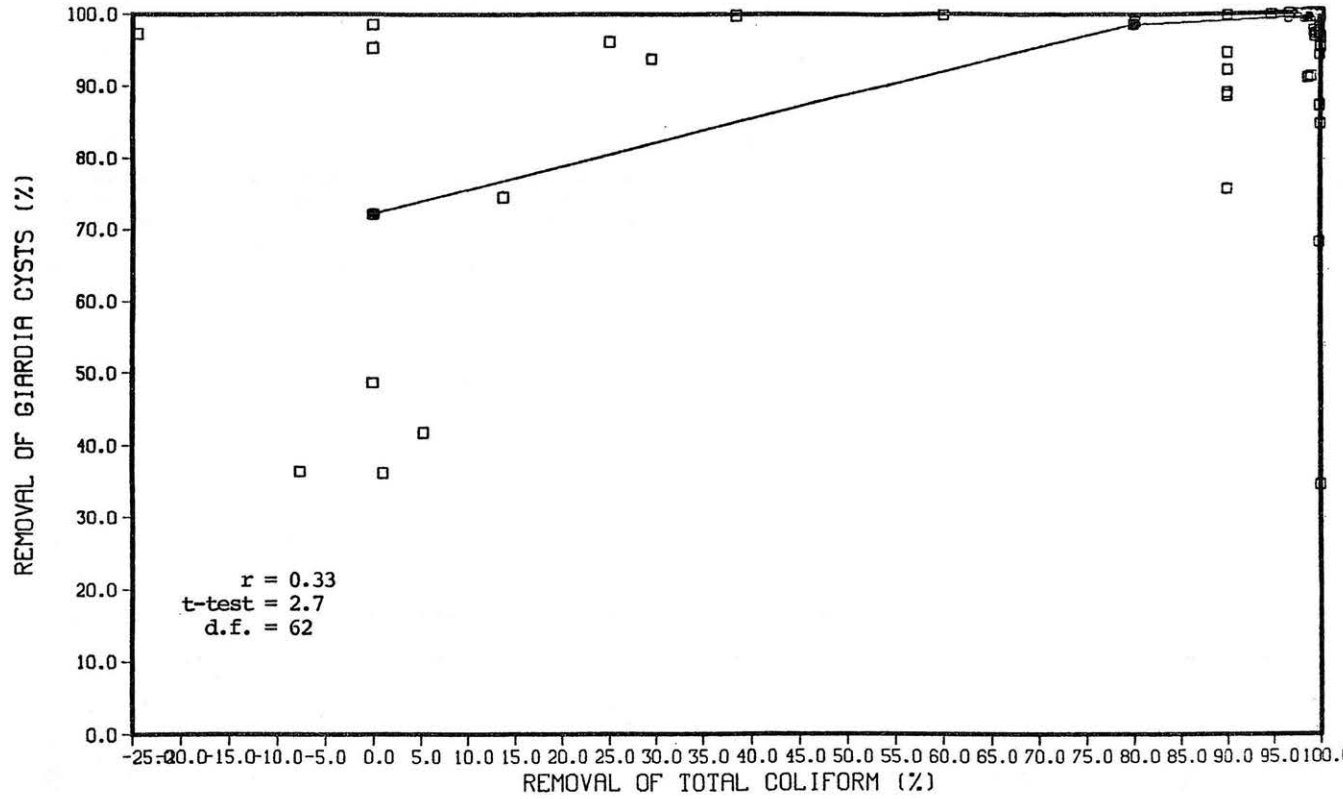


Figure G-13. Observations of percent removal of Giardia cysts with corresponding percent removal of total coliform bacteria. Data obtained from Table A-1 and Table A-2. The solid points show the median for each 8 points, conneccted by a line.

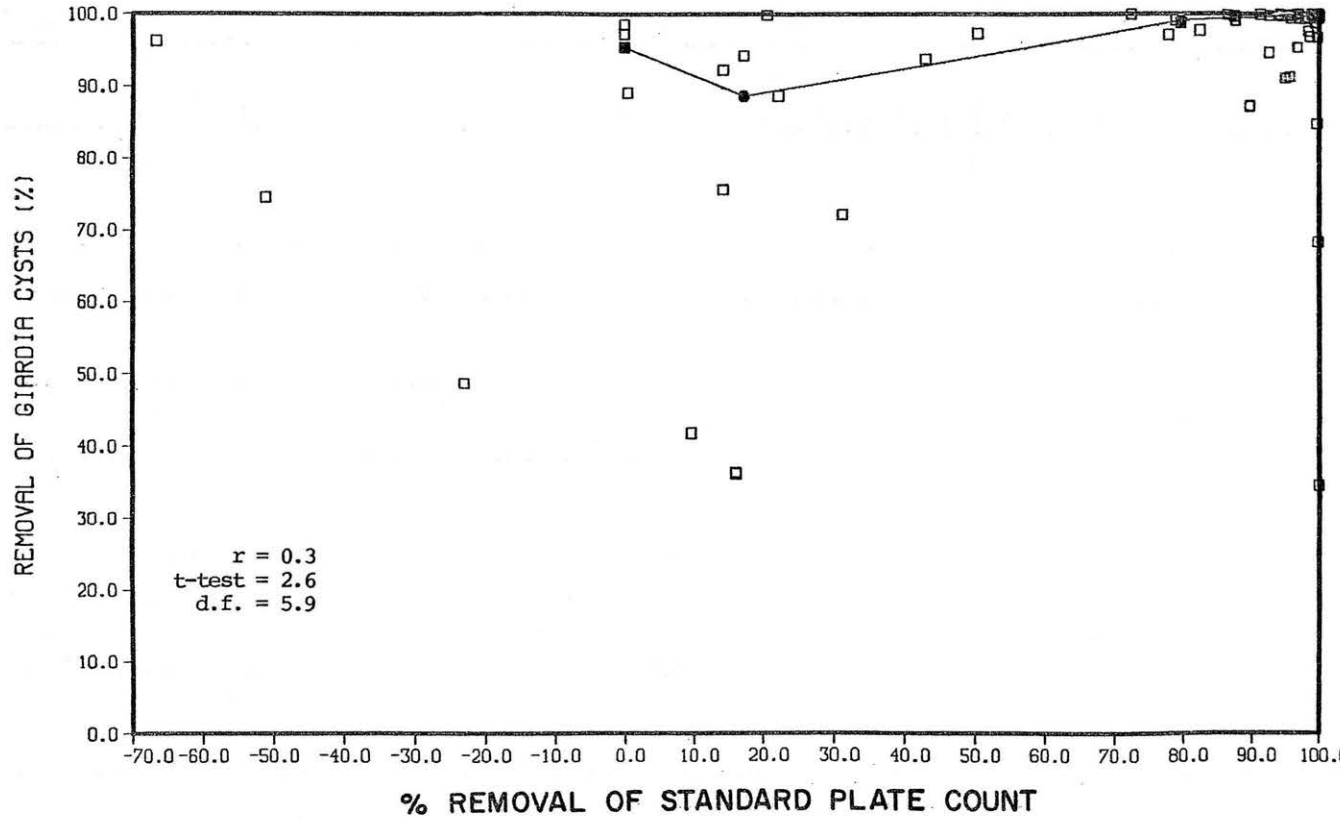


Figure G-14. Observations of percent removal of Giardia cysts with corresponding percent removal of standard plate count bacteria. Data obtained from Table A-1 and Table A-2. The solid points show the median for each 8 points connected by a line.

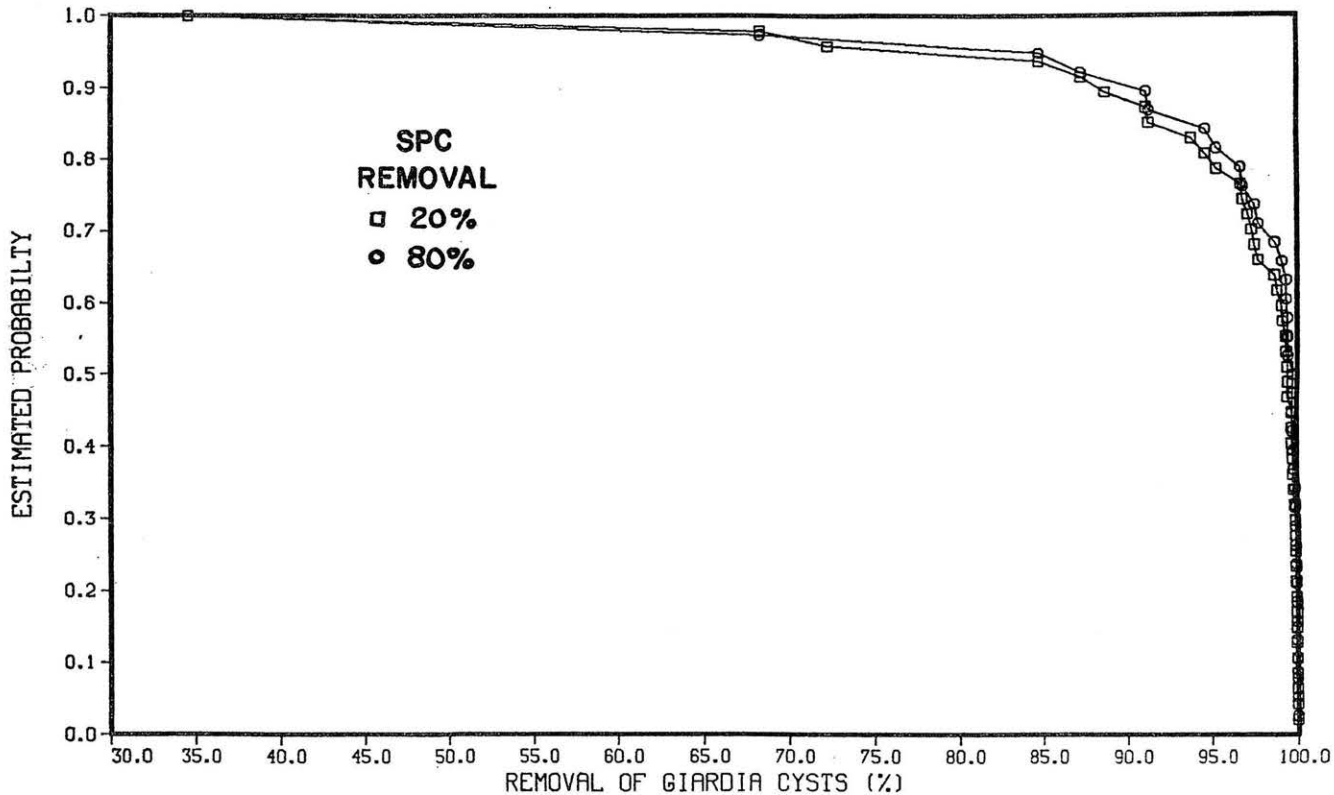


Figure G-15. Probability of a given percent removal of Giardia cysts for specific percent removal of standard plate count bacteria. Data from Table A-1 and plotted in Figure G-14.

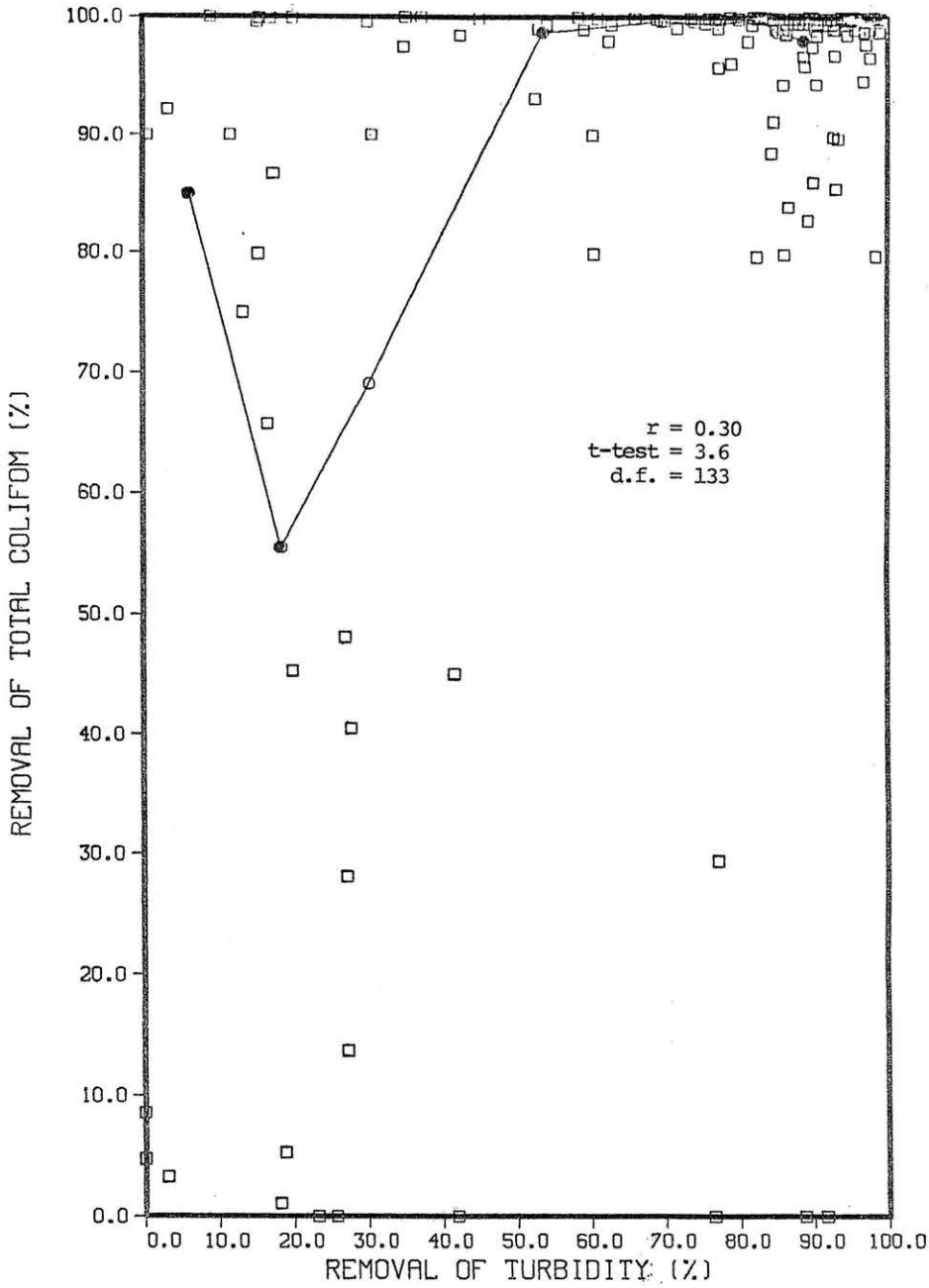


Figure G-16. Observation of percent removal of total coliform bacteria with corresponding percent removal of turbidity. Data obtained from Table A-1. The solid points show time median for each 10 points connected by a line.

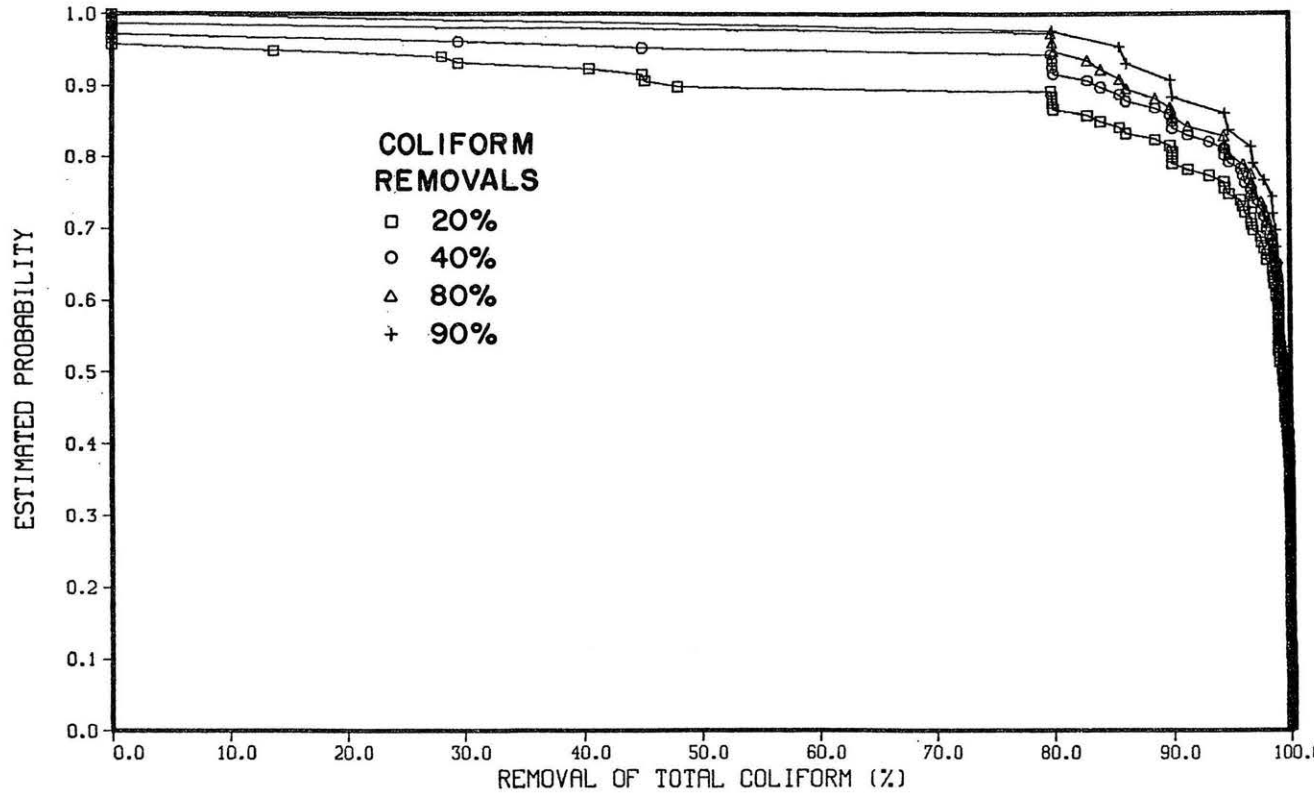


Figure G-17. Probability of a given percent removal of total coliform bacteria for specific percent removal of turbidity. Data from Table A-1 and plotted in Figure G-13.

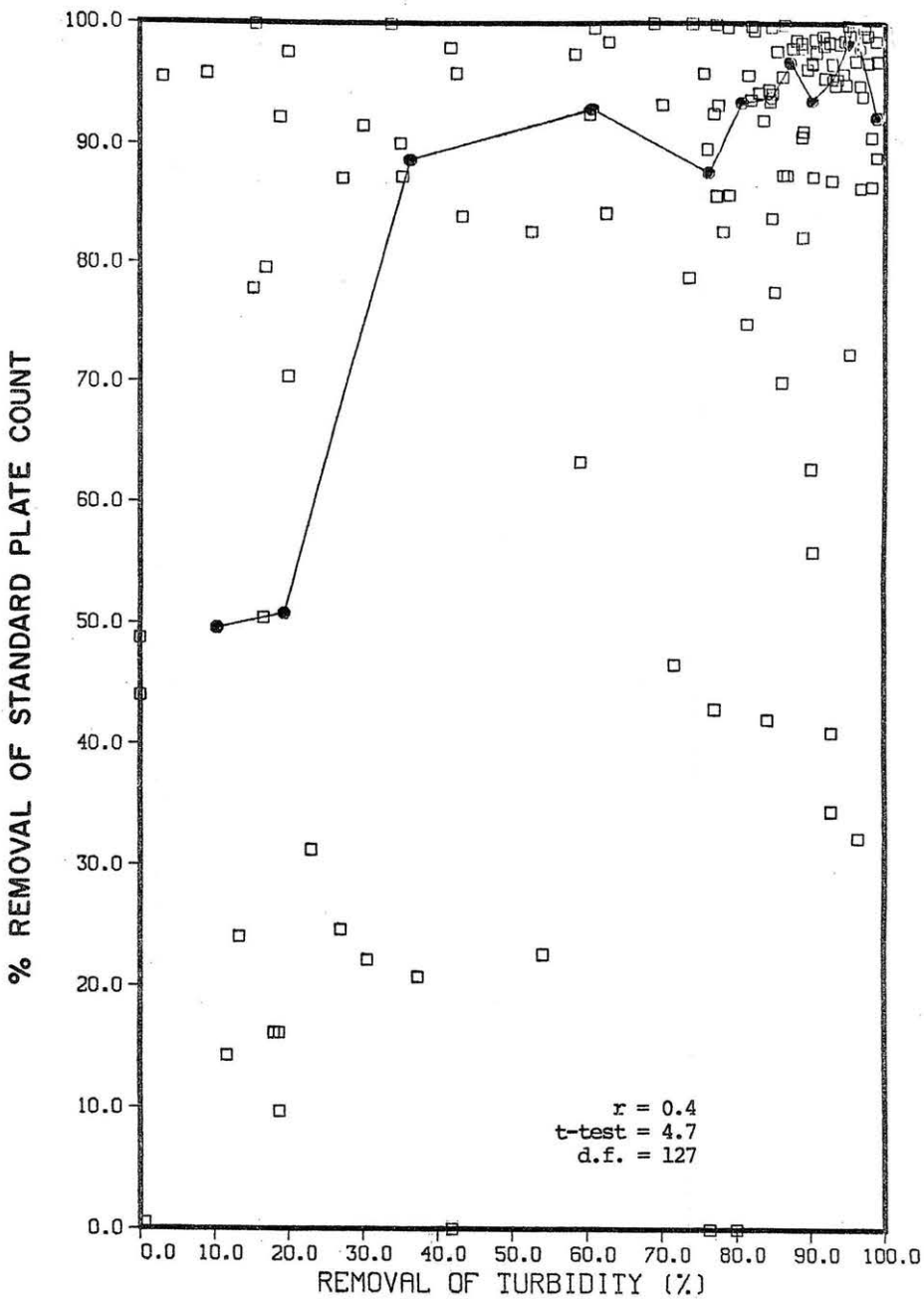


Figure G-18. Observations of percent removal of standard plate count with corresponding percent removal of turbidity. Data obtained from Table A-1. The solid points show the median for each 10 points connected by a line.

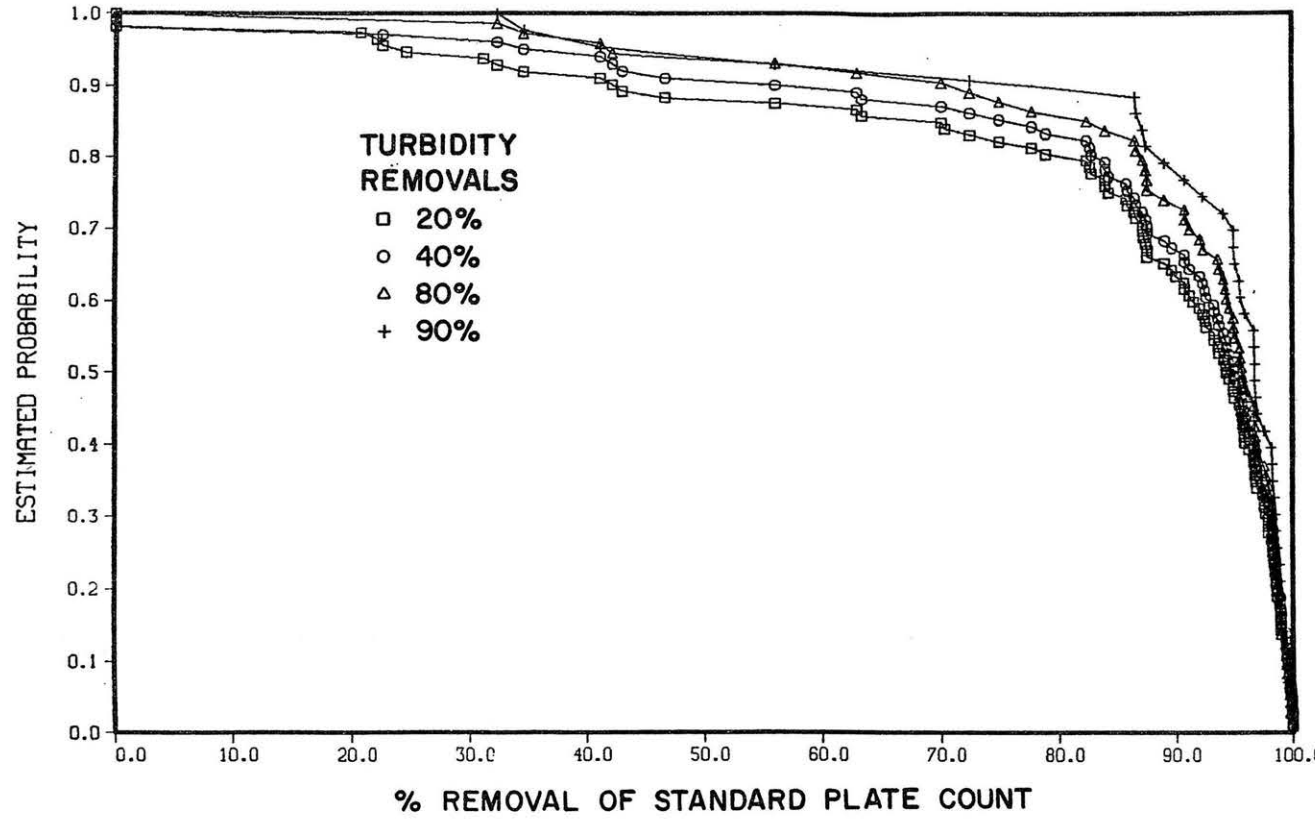


Figure G-19. Probability of a given percent removal of standard plate count for specific percent removal of turbidity. Data from Table A-1 and plotted in Figure G-18.

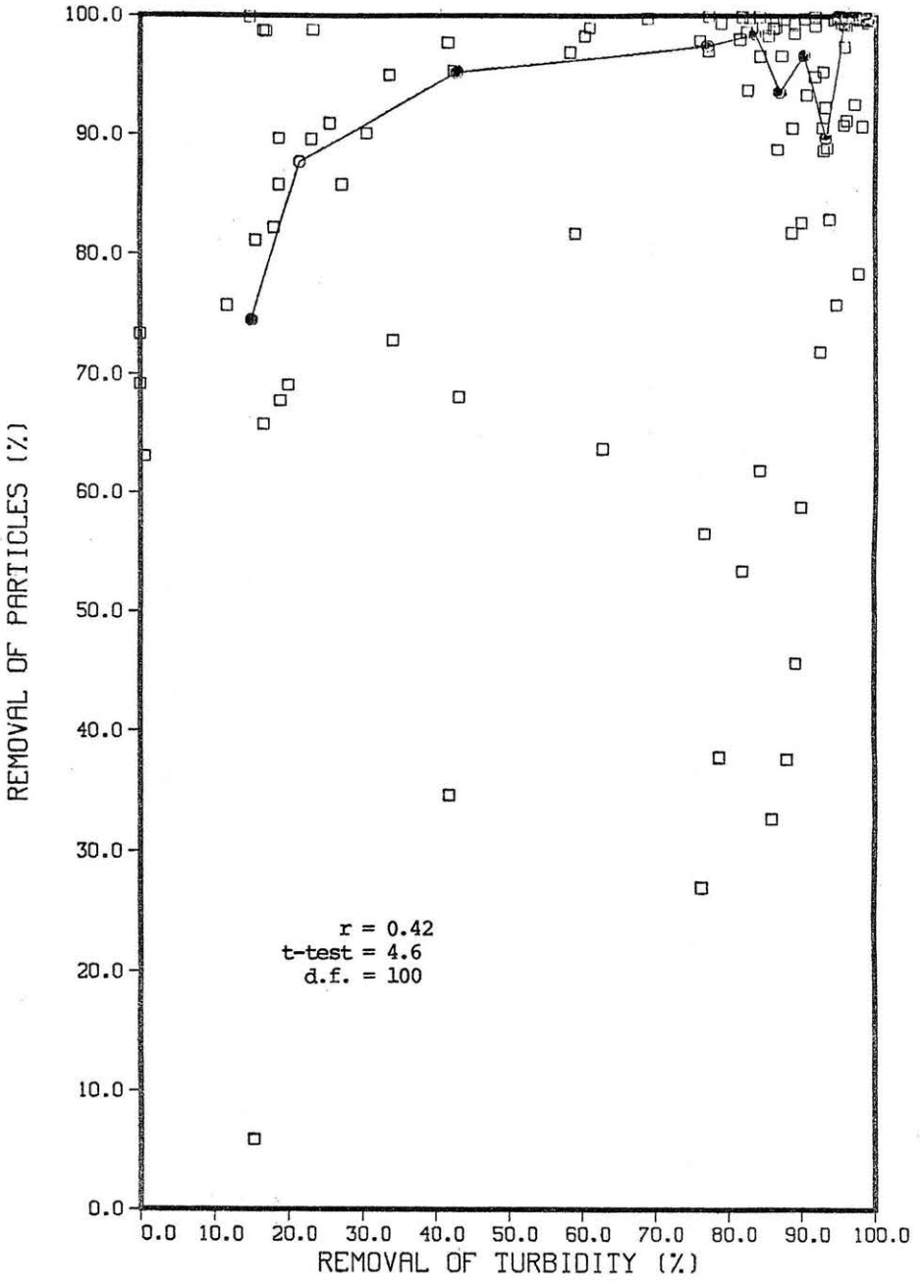


Figure G-20. Observations of percent removal of particles with corresponding percent removal of turbidity. Data were obtained from Table A-1. The solid points show the median for each 10 points connected by a line.

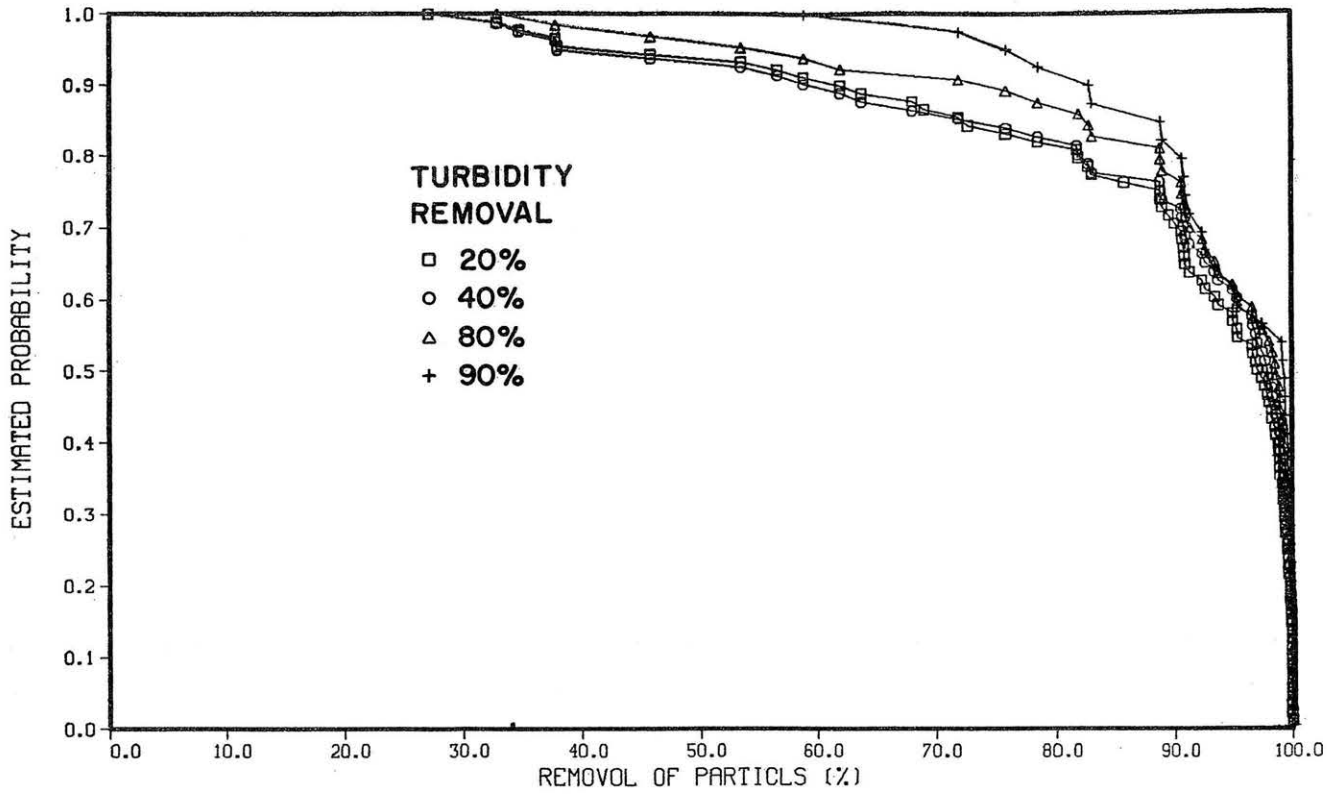


Figure G-21. Probability of a given percent removal of particles for specific percent removal of turbidity. Data from Table A-1 and plotted in Figure G-20.

APPENDIX H

RESULTS FROM FAMILIARIZATION TESTING, HORSETOOTH TESTING AND SPRING RUNOFF TESTING

FAMILIARIZATION TESTING

The first group of figures, Figures H-1 to H-12, show turbidity vs time relationships developed during a "familiarization" testing period. These curves show that within about one-hour the effluent turbidity was "stabilized", i.e. did not change significantly with time. From these results the run time of one-hour was used as an index time for sampling in subsequent testing. Horsetooth Reservoir water was used for this purpose. The purposes of the familiarization phase were to become familiar with the pilot plant, and to size and calibrate chemical feed tanks, pumps, etc. Some 22 test runs were completed during the familiarization phase. The testing was conducted during the period 11/9/82 to 2/9/83. The pilot plant was located on the floor of the Hydraulics Laboratory at the Engineering Research Center, which can withdraw water from the adjacent Horsetooth Reservoir.

HORSETOOTH TESTING

Sixty eight tests were performed using Horsetooth Reservoir water, exclusive of the familiarization testing. These 68 tests included 46 effluent turbidity vs chemical dose tests. From these data typical U-shaped curves were developed. Based on these curves, three categories of chemical dosages were designed: "zero" dose, "optimum" dose, and "nonoptimum" dose. These dose categories were used to evaluate the effectiveness of coagulant dosage on removal of Giardia cysts and coliform bacteria. The results are summarized in Table H-1. Figures H-13 to Figure H-16 show the effectiveness of different chemicals on turbidity removal. Figures H-17, H-18, H-19 show various associations between parameters, while Figures H-20 and H-21 show the effect of run time on parameters.

SPRING RUNOFF TESTING

When the 1983 spring runoff started the WATER BOY was located at Fort Collins Water Treatment Plant No. 1 on the Cache La Poudre River. Turbidity levels at this time ranged from 12 to 44 NTU. The results illustrate the contrust in treating high turbidity water compared with low turbidity water, the focus of the research.

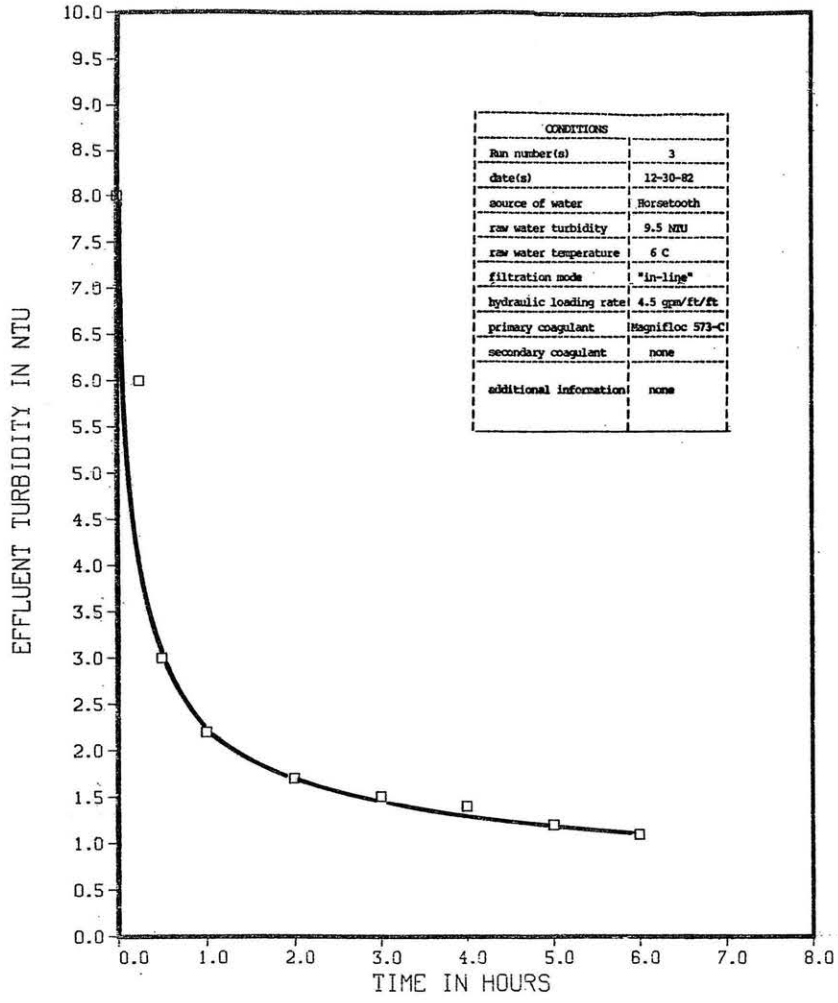


Figure H-1. Effluent-turbidity vs time using 0.5 mg/L of Magnifloc 573-C. Familiarization testing.

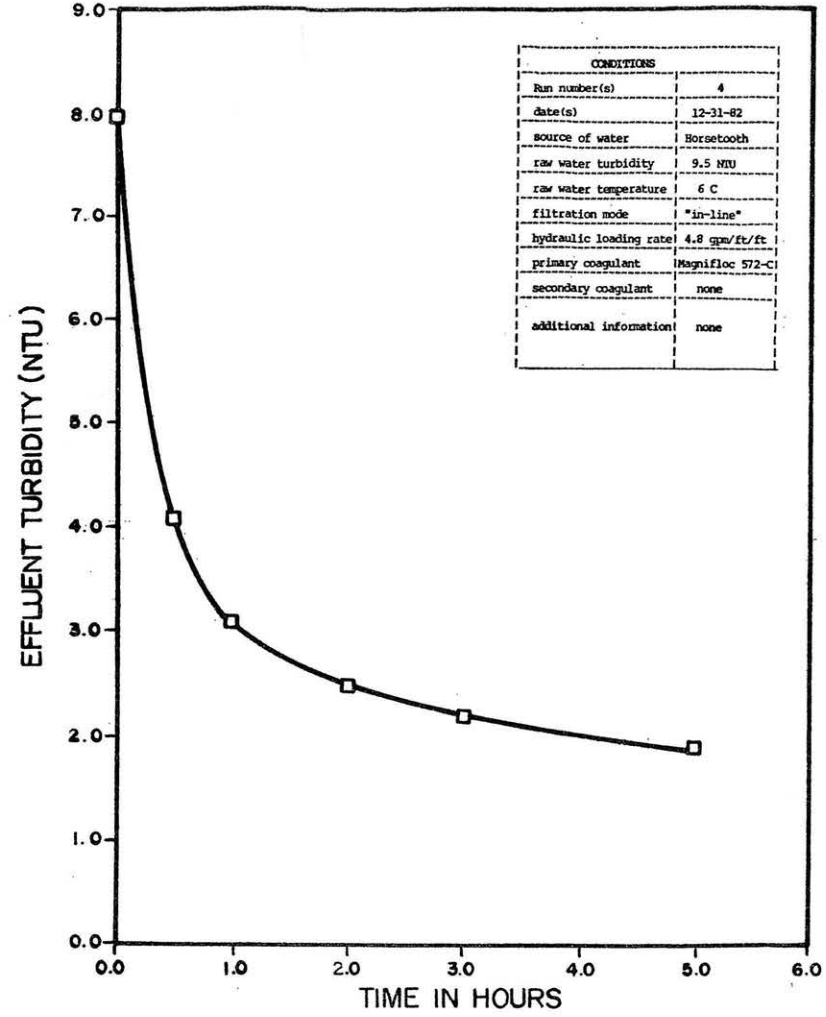


Figure H-2. Effluent-turbidity vs time using 0.5 mg/L of Magnifloc 572-C. Familiarization testing.

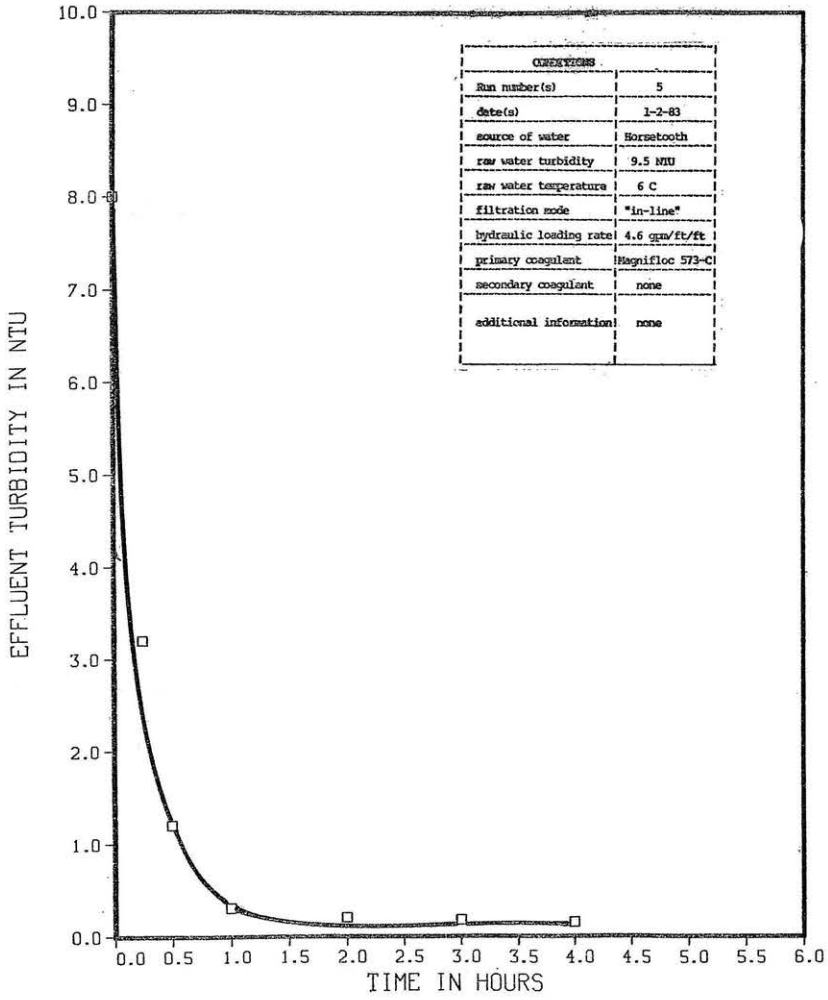


Figure H-3. Effluent-turbidity vs time using 1.5 mg/L of Magnifloc 573-C. Familiarization testing.

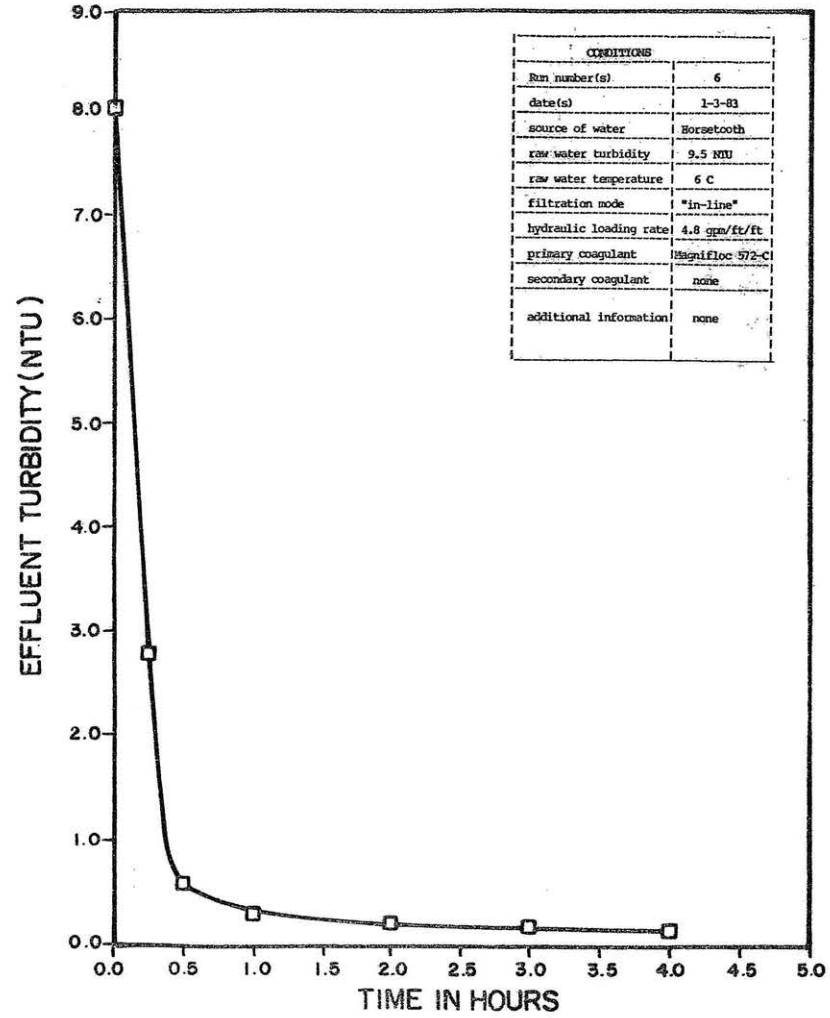


Figure H-4. Effluent-turbidity vs time using 1.5 mg/L of Magnifloc 572-C. Familiarization testing.

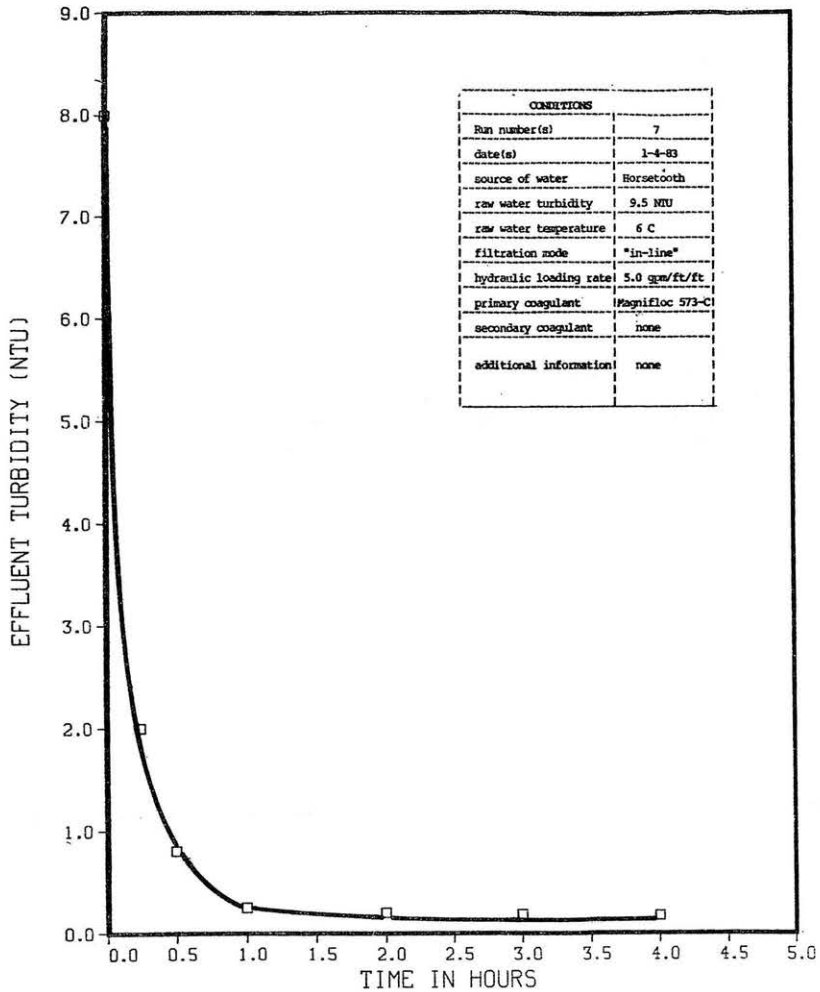


Figure H-5. Effluent-turbidity vs time using 3.8 mg/L of Magnifloc 573-C. Familiarization testing.

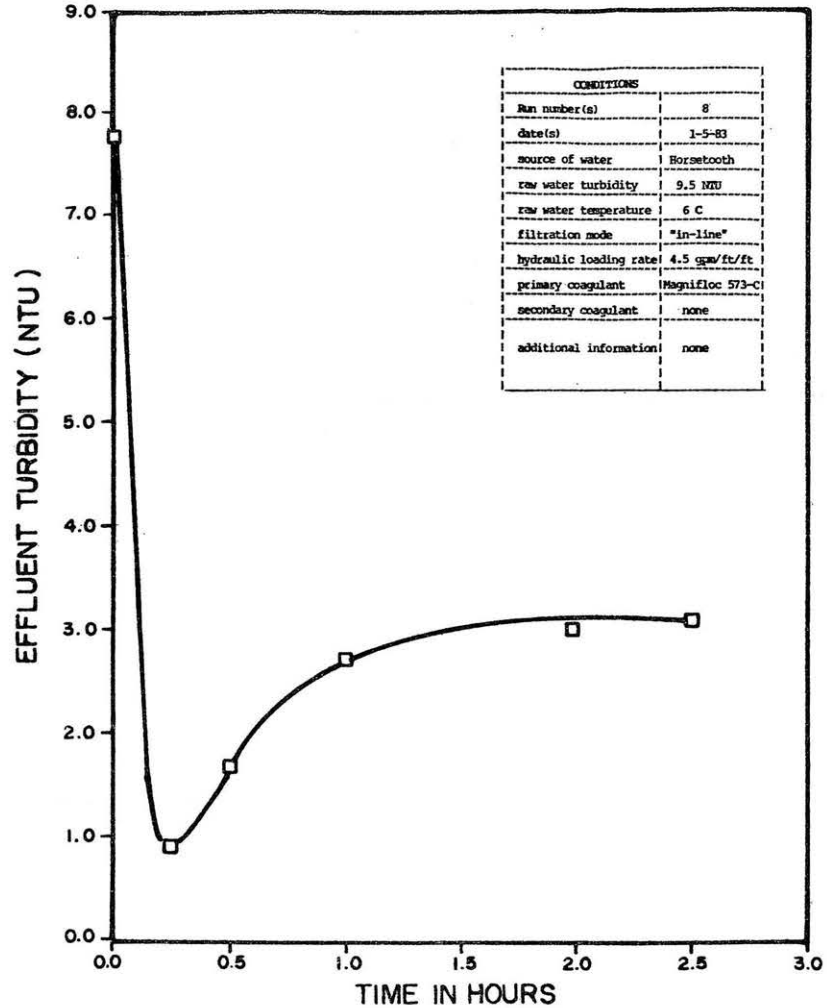


Figure H-6. Effluent-turbidity vs time using 3.8 mg/L of Magnifloc 573-C. Familiarization testing.

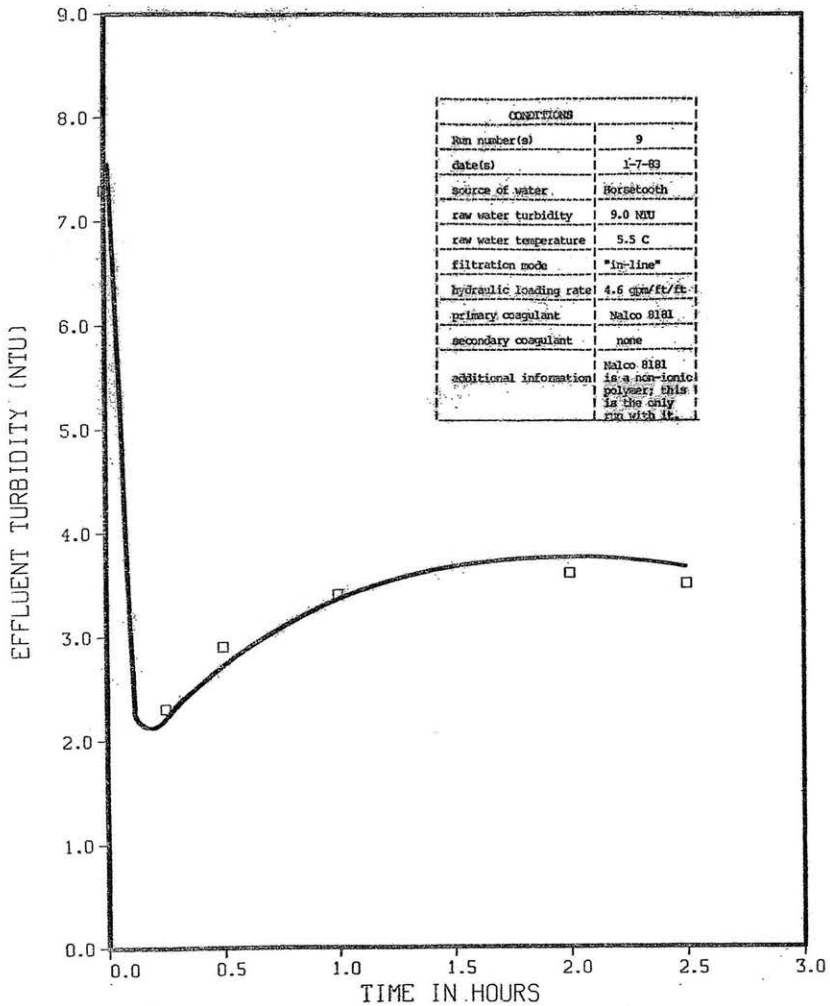


Figure H-7. Effluent-turbidity vs time using 6.7 mg/L of Nalco 8181 (non-ionic polymer). Familiarization testing.

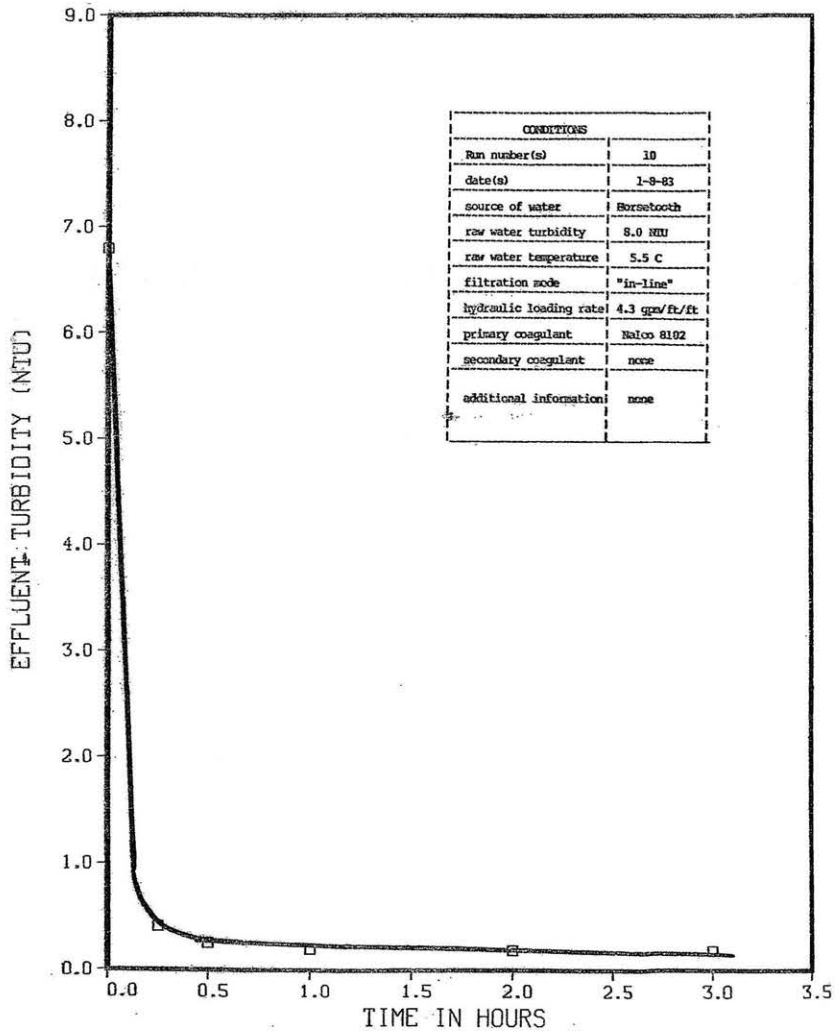


Figure H-8. Effluent-turbidity vs time using 4.3 mg/L of Nalco 8102. Familiarization testing.

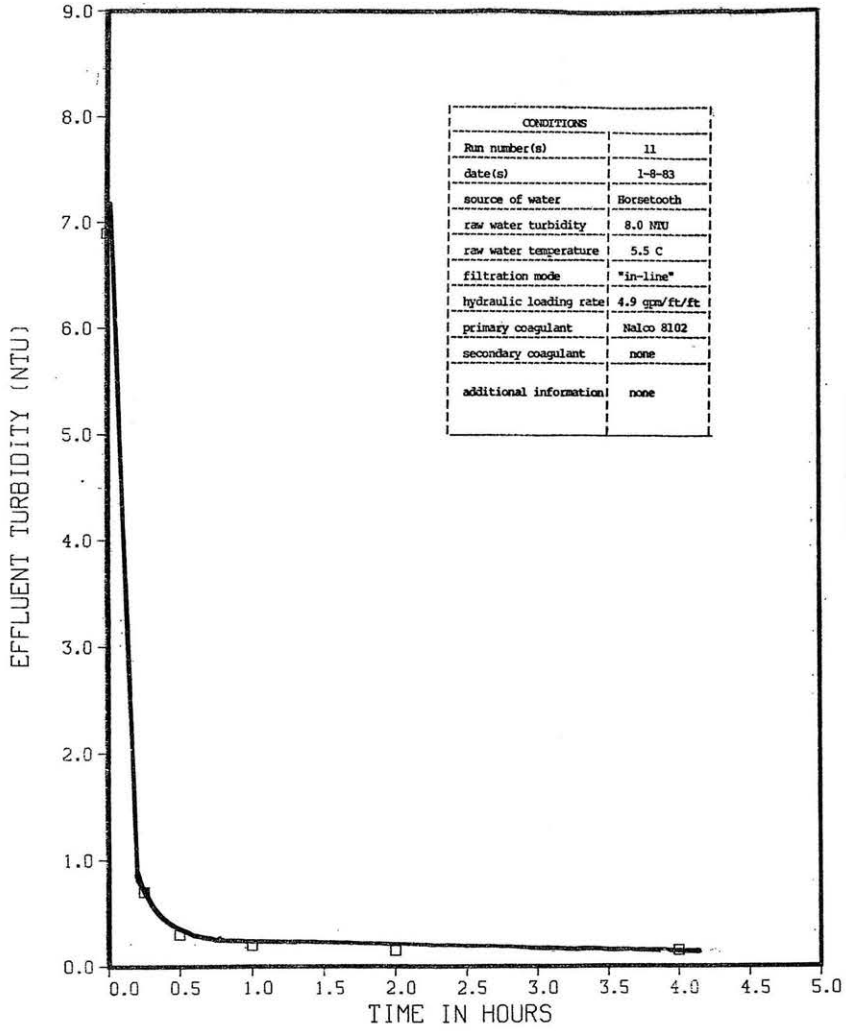


Figure H-9. Effluent-turbidity vs time using 8.0 mg/L of Nalco 8102. Familiarization testing.

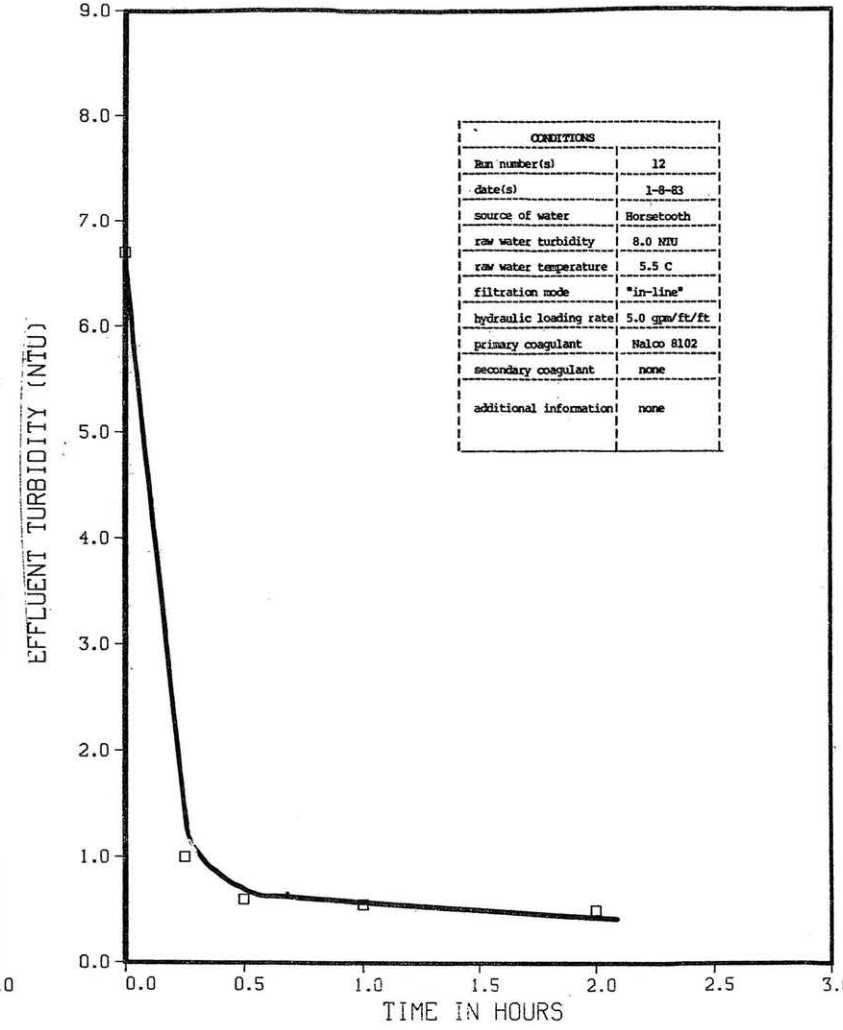


Figure H-10. Effluent-turbidity vs time using 10.5 mg/L of Nalco 8102. Familiarization testing.

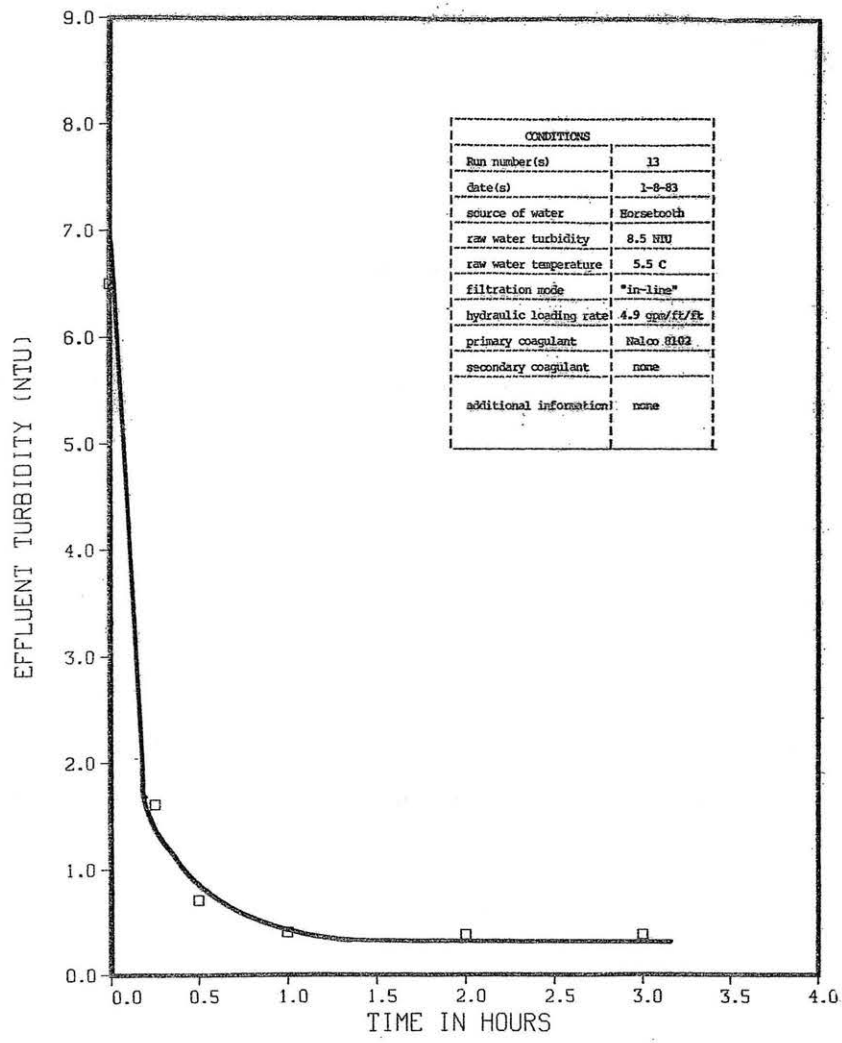


Figure H-11. Effluent-turbidity vs time using 0.9 mg/L of Nalco 8101. Familiarization testing.

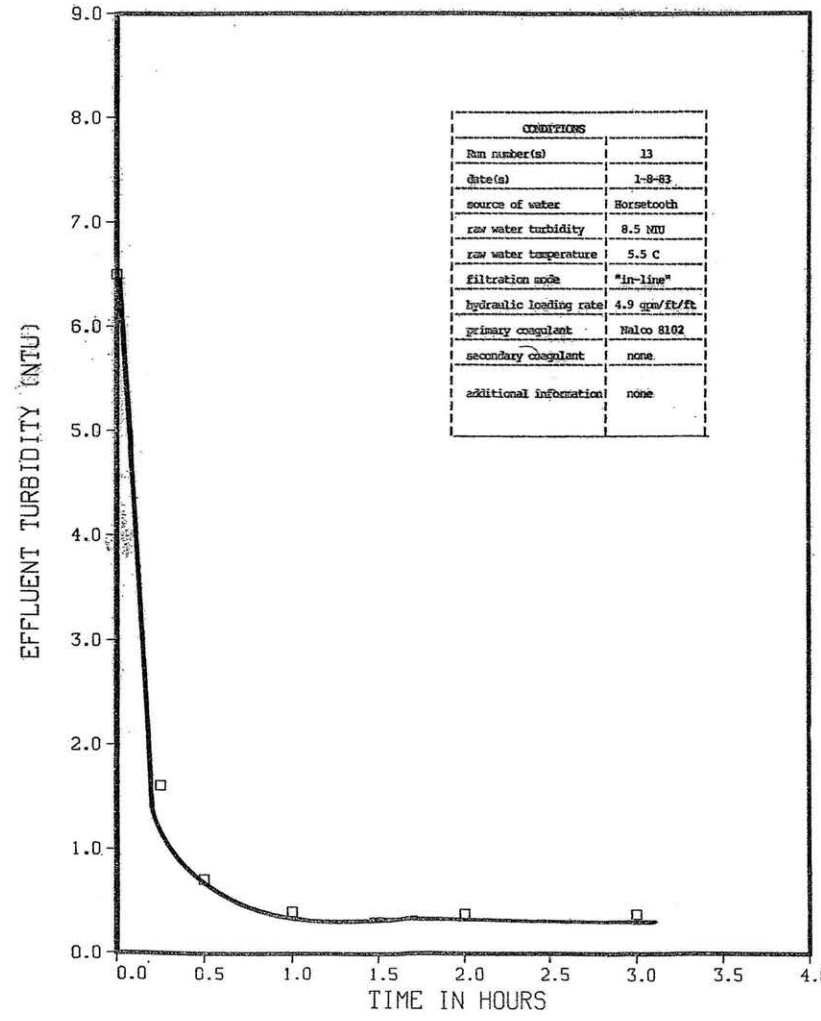


Figure H-12. Effluent-turbidity vs time using 6.7 mg/L of Magnifloc 572-C. Familiarization testing.

Table H-1. Effect of coagulant dosages on removals of coliform bacteria and *Giardia* cysts for Horsetooth water.[1/11]

Run #	Coagulant Dosage Category[2/3/]	Coagulant Used: Chemical Species[6/]	Coagulant Used: Chemical Dosage[5/] (mg/L)	Percent Removals[10/]: Turbidity	Coliforms[8/12/]	*Giardia* Cysts[4/7/13/]
50	None	None	0	+	*	>99
57	None	None	0	14	*	<1
60	None	None	0	14	*	84
61	None	None	0	11	*	<1
62	None	None	0	13	*	<1
74	None	None	0	9	33	**
99	None	None	0	3	27	41
49	optimum	573-C	2.5	93	*	>99
52	optimum	8102	2.5	+	95	>99
58	optimum	8102	5.5	94	*	>99
73	optimum	572-C	2.5	93	97	**
76	optimum	alum	25	97	96	**
90	optimum	8102	4.0	94	>99	>99
92	optimum	alum	12	76	98	97
98	optimum	alum	15	84	98	94
105	optimum	alum/8102	15/4	89	98	93
106	optimum	alum/8102	15/4	90	96	>99
51	nonoptimum	573-C	8.0	+	>99	>99
59	nonoptimum	8102	11.0	86	98	>99
75	nonoptimum	572-C	1.0	76	81	**
91	nonoptimum	8102	1.0	83	96	>99

1/ Abstracted from Table B-1
2/ Three dosage categories are: i) no coagulants used, indicated as "none"; ii) "optimum" coagulant dosage is with respect to turbidity removal; iii) "nonoptimum" coagulant dosage is a dosage greater than or less than "optimum."
3/ "Optimum" and "nonoptimum" are dosages based on Figures 3-1 to 3-4
4/ Based on detected influent cyst concentration
5/ Alum dose reported is mg/L as $Al_2(SO_4)_3 \cdot 14H_2O$
6/ Nalco 8102, Magnifloc 572-C, Magnifloc 573-C
7/ Double asterisk indicates cysts used were of questionable viability
8/ Asterisk indicates no data; missed dilution range
9/ No effluent turbidity sample taken obtained
10/ Data represents removals after one hour of filtration
11/ Influent turbidity levels were 7.0 NTU for all tests and water temperatures ranged between 10 and 12°C
12/ Influent coliform concentrations ranged from 220/100 ml to 30000/100 mL; median was 8700/100 mL
13/ Influent detected *Giardia* cyst concentrations ranged between 10/L to 2000/L; median was 110/L

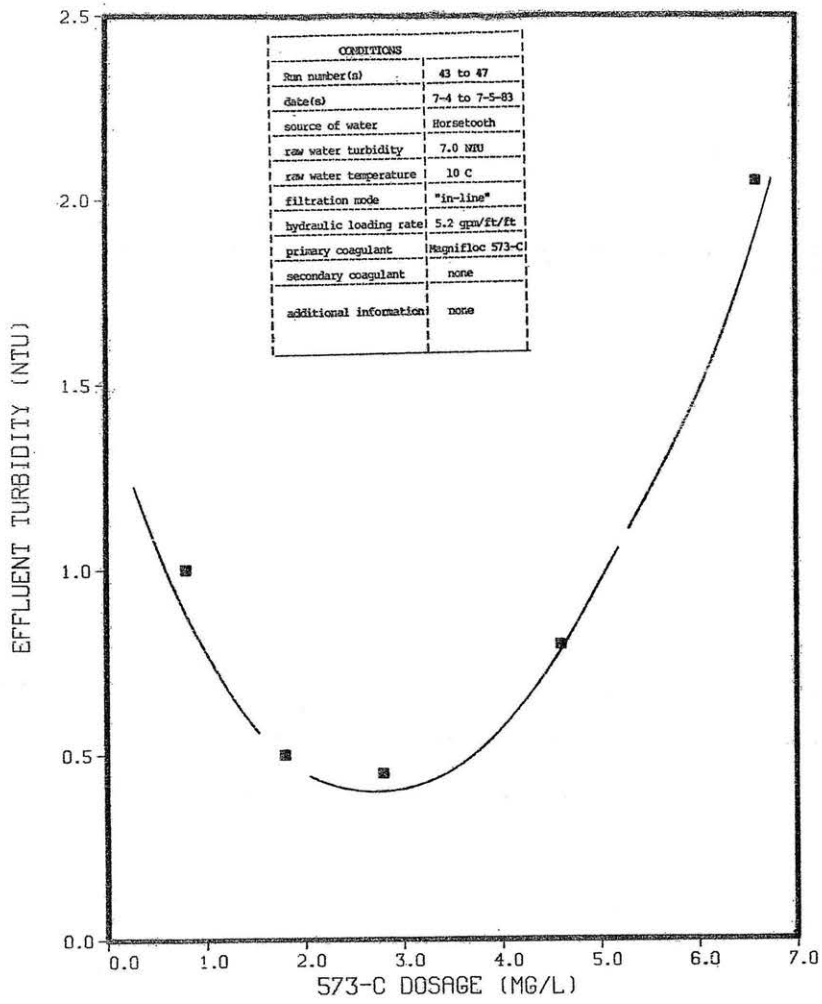


Figure H-13. Effluent turbidity vs dose of Magnifloc 573-C. Horsetooth water.

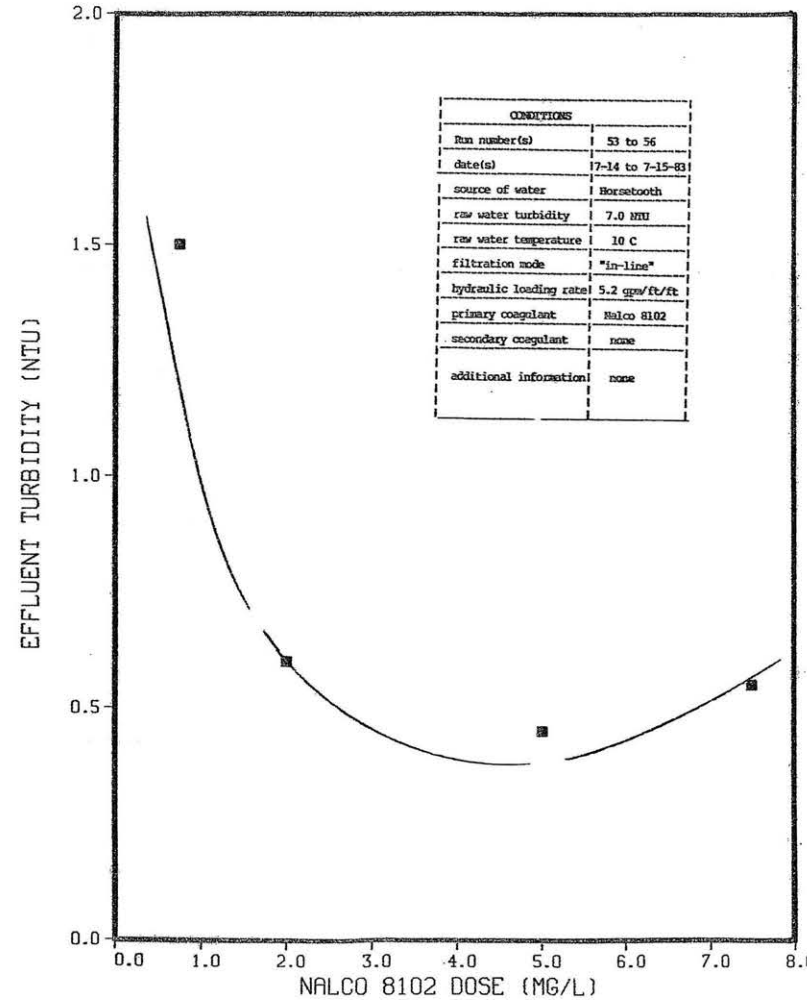


Figure H-14. Effluent turbidity vs dose of Nalco 8102. Horsetooth water.

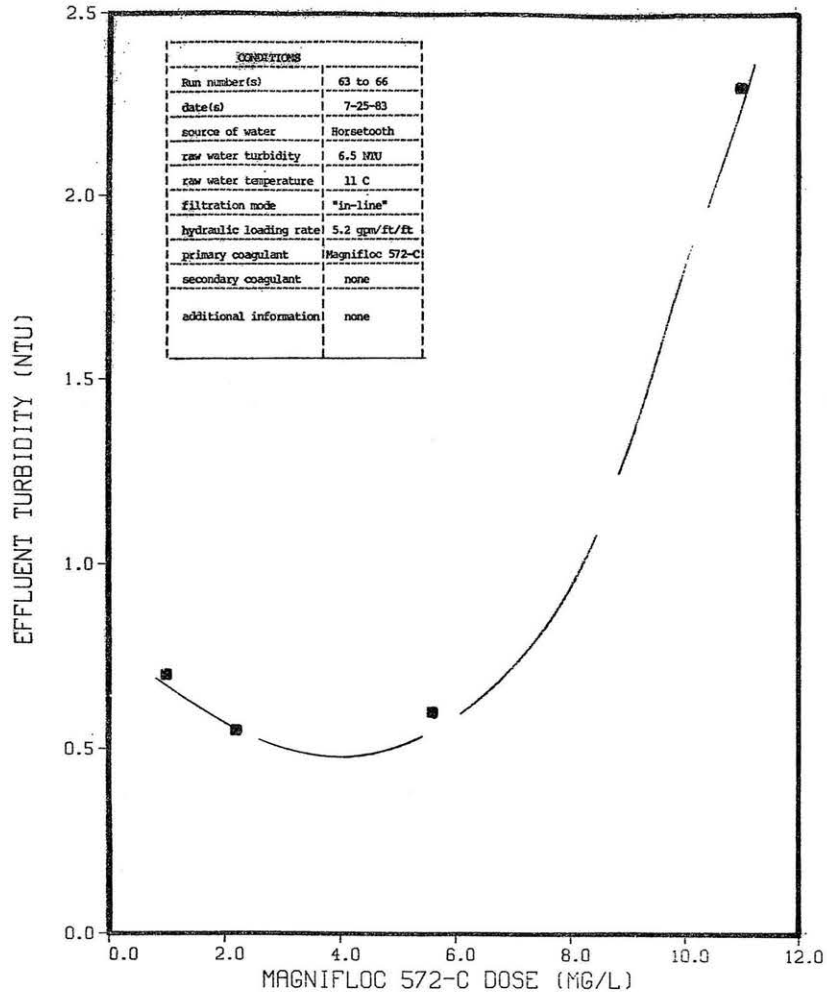


Figure H-15. Effluent turbidity vs dose of Magnifloc 572-C. Horsetooth water.

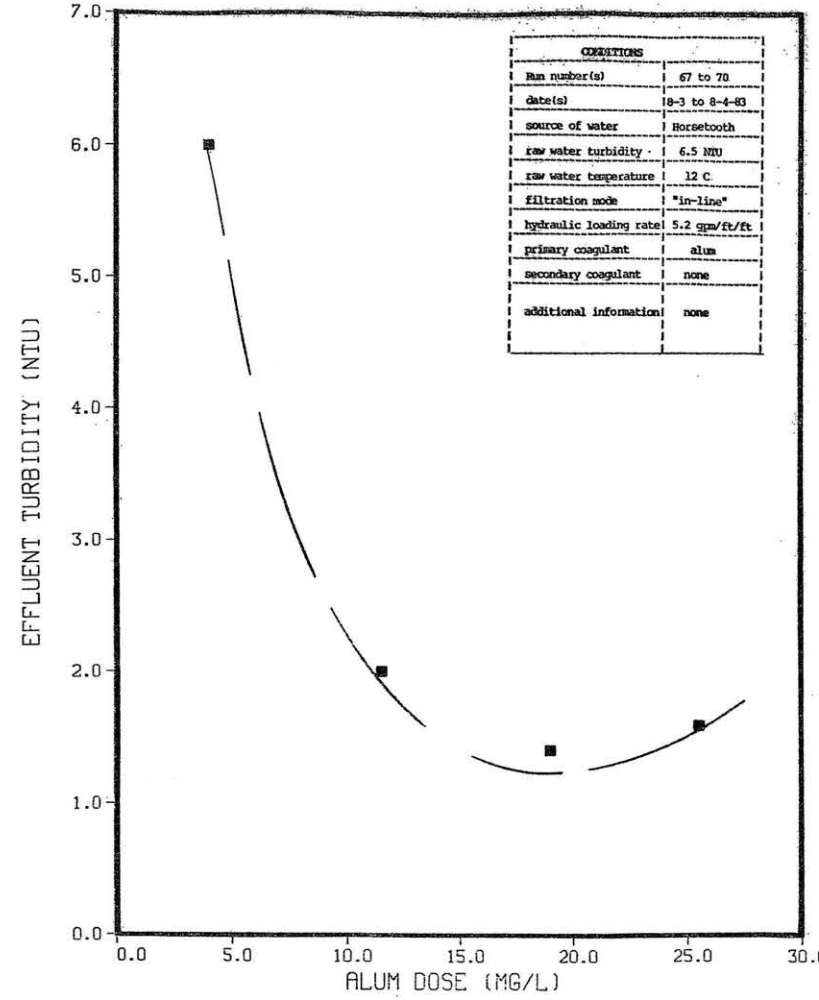


Figure H-16. Effluent turbidity vs dose of alum as $Al_2(SO_4)_3\ 14H_2O$. Horsetooth water.

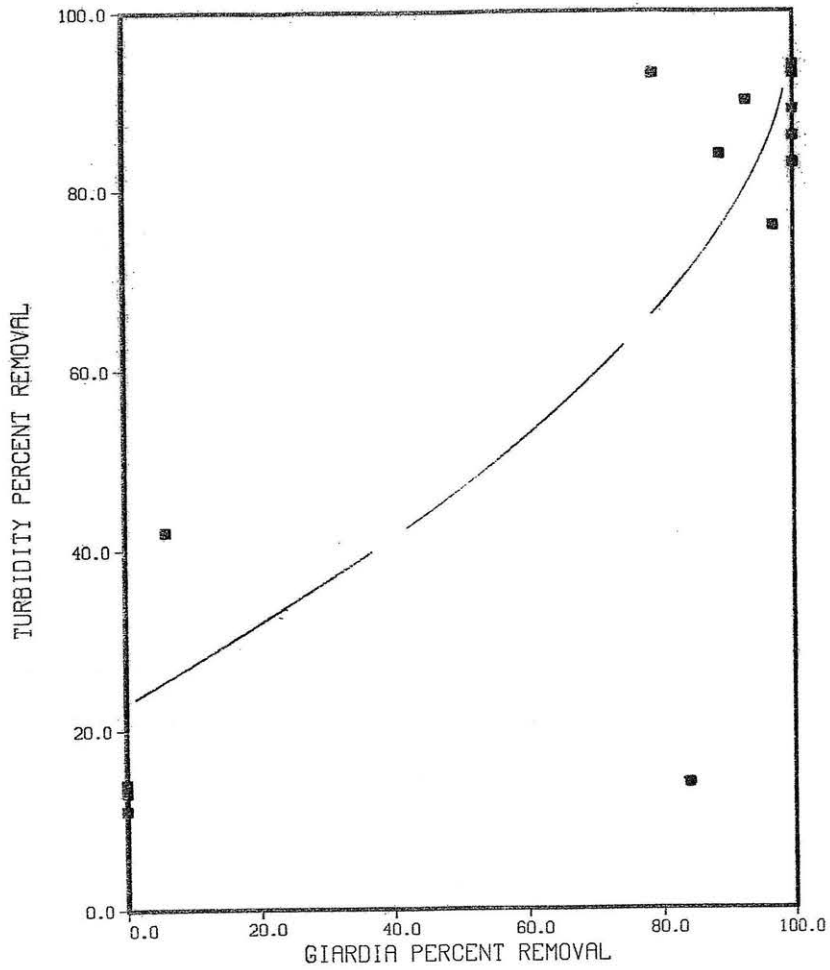


Figure H-17. Percent removal of turbidity vs percent removal of Giardia cysts. Horsetooth water. Raw water characteristics were 7.0 NTU and temperature 10-12°C.

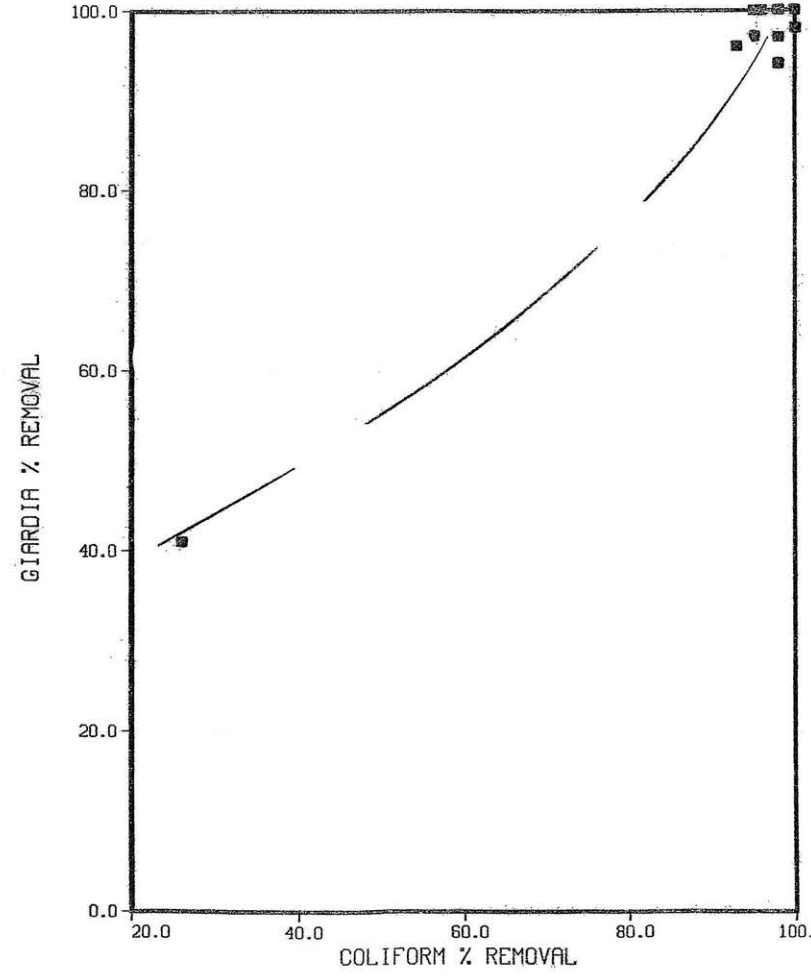


Figure H-18. Percent removal of coliform bacteria vs percent removal of Giardia cysts. Horsetooth water. Raw water characteristics were 7.0 NTU and 10-12°C.

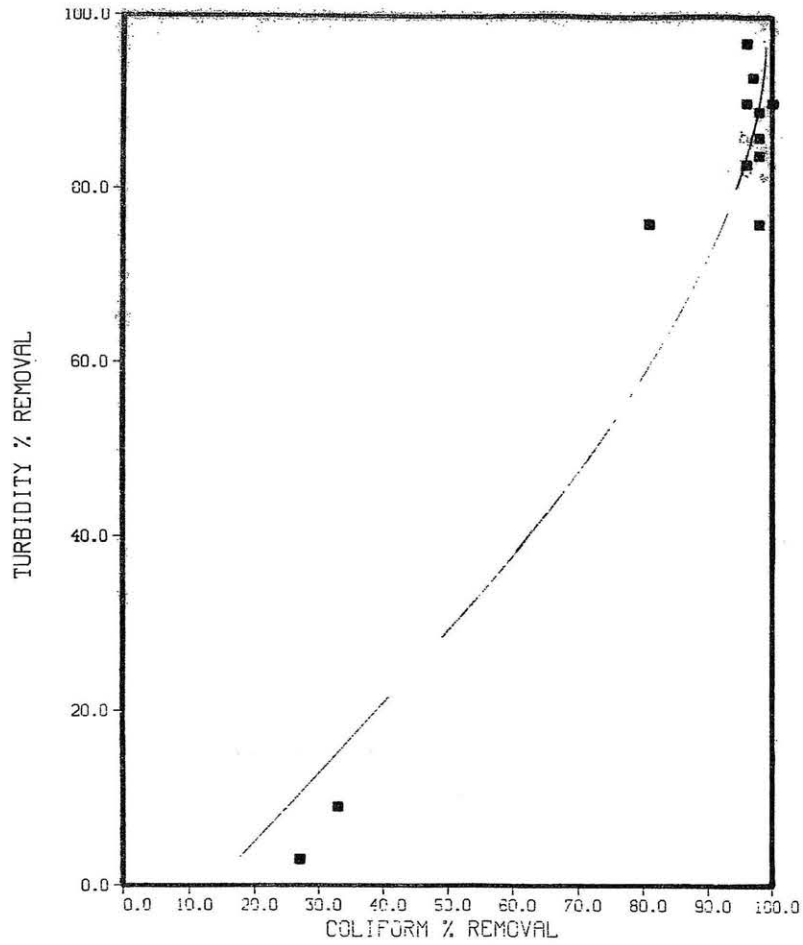


Figure H-19. Percent removal of turbidity vs percent removal of coliform bacteria. Horsetooth water. Raw water characteristics were 7.0 NTU and 10-12°C.

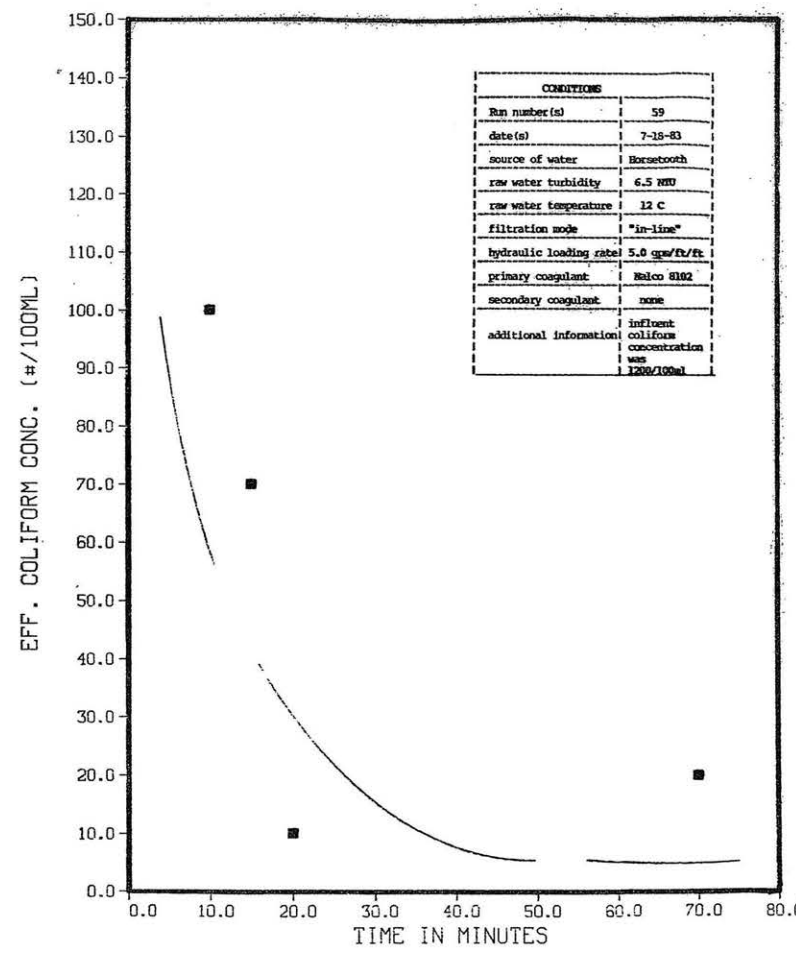


Figure H-20. Effluent coliform bacteria concentrations vs time. Horsetooth water.

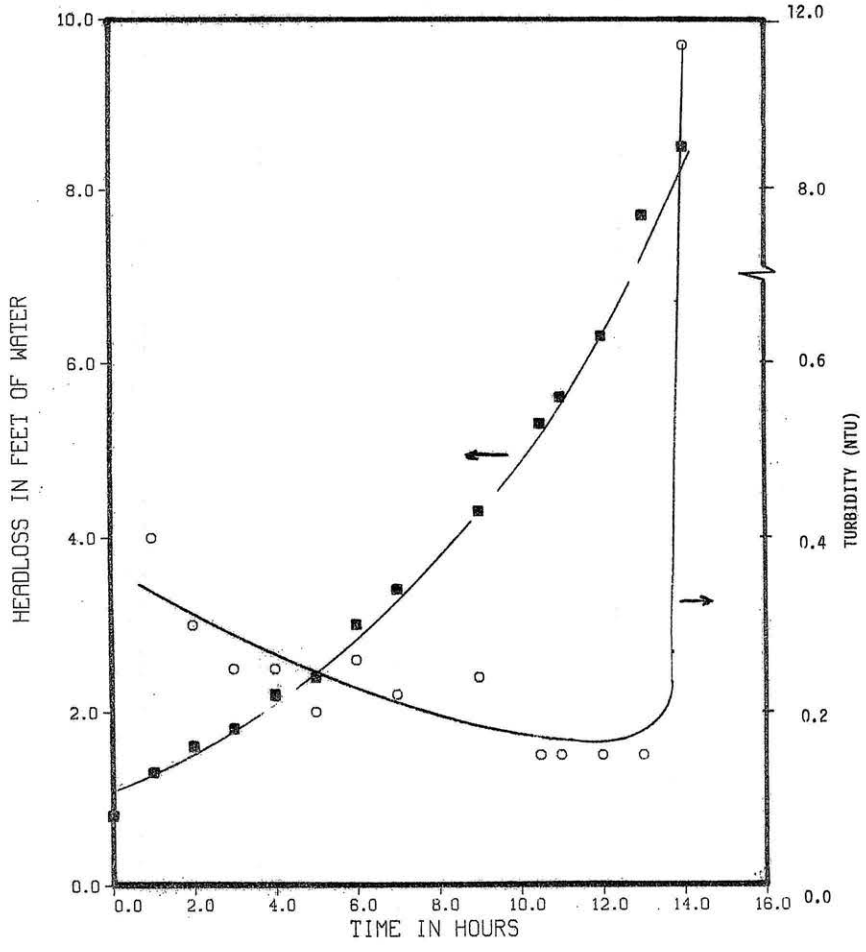


Figure H-21. Headloss and effluent turbidity vs time for Run 78. Raw water was 7.0 NTU, and 6 °C. Nalco 8102 dose used was 3.5 mg/L.

Spring Runoff Testing

The spring runoff testing was conducted from 4/23/83 to 6/1/83 at the Fort Collins Water Treatment Plant No. 1. The water used for testing was from the Cache La Poudre River during spring runoff. The intent was to begin testing in early March to experiment with low turbidity, i.e. less than 1 NTU, raw water. Disruption of electric power, however, required replacement of a buried cable. This delayed the start up of the WATER BOY and the testing did not begin until after the start of the spring runoff in late April. So, when the spring runoff began, it was decided to go ahead and treat the high turbidity water. Table H-2 summarizes results, and Figures H-22 to H-27 provide results in graphical form.

During spring runoff, the raw water turbidities of the Cache La Poudre River ranged between 12 and 44 NTU, and raw water temperatures ranged between 7 and 10°C. The river's properties were continually changing, raw water turbidity fluctuations were as much as 10 NTU per hour.

Horsetooth Testing

The Horsetooth Reservoir testing was conducted from 7/3/83 to 8/31/83 at the Engineering Research Center at Colorado State University using Horsetooth Reservoir water as a raw water source. During the first part of this phase, the contaminant injection and sampling system was incorporated into the flow scheme of the WATER BOY, and tests were run to establish procedures for tests to determine removals of Giardia cysts and coliform bacteria. Once the protocol for Giardia cysts and coliform bacteria removals vs coagulant dose tests was established, 22 of these tests were conducted with Horsetooth water.

Although the focus of this research was to determine removals using raw waters with turbidities lss than 1 NTU, the Horsetooth Reservoir testing validated testing procedures, and provided results to compare those obtained from low turbidity testing. Horsetooth Reservoir provided a raw water source with predictable and relatively constant properties. The turbidity and temperature ranges for Horsetooth Reservoir water are 3 to 12 NTU and 2 to 15°C, respectively, over the annual cycle.

Horsetooth Reservoir water has low turbidity when compared to water sources which have turbidities as high as 100 to 200 NTU. But if compared to the water of the Cache La Poudre River during late fall and winter, Horsetooth Reservoir water is within a "medium" range of turbidity.

The traditional filtration practice is comprised of: i) particle destabilization by coagulation, ii) floc production, and iii) floc penetration into the filter. This practice is applicable to Horsetooth Reservoir water. But when the raw water turbidity is below 1 NTU, this traditional practice of filtration does not seem to work. So, since Horsetooth Reservoir water could be properly coagulated and filtered in accordance with traditional practice, the use of this water source enabled

Table H-2. Spring Runoff Results[1/3/]

Run No.	Coagulant Dosage Category[2/]	Dose of Magnifloc 572-C mg/L	Turbidity (NTU)		
			Influent	Effluent[4/]	Percent Removal
34	none	0	23	10	57
36	none	0	35	11	69
25	optimum	8.5	27	1.0	96
27b	optimum	9.0	30	1.5	95
28a	optimum	17.0	30	1.5	95
29	optimum	10.5	44	2.0	95
30	optimum	8.0	44	1.5	97
33	optimum	6.5	23	1.0	96
35	optimum	9.0	34	1.5	96
37	optimum	12.5	35	1.3	96
38	optimum	7.0	16	1.1	93
23	nonoptimum	1.5	27	15	44
24	nonoptimum	4.0	27	8.5	69
26	nonoptimum	30	27	3.0	89
27a	nonoptimum	4.0	27	3.0	89
28b	nonoptimum	35	30	6.5	78
31	nonoptimum	5.0	44	2.5	94
32	nonoptimum	21	35	3.0	91

1/ Abstracted from Table B-1

2/ Based upon results in Figure H-22

3/ No Giardia cysts or coliform bacteria were injected during the spring runoff testing phase

4/ Effluent turbidity after 60 minutes of operation

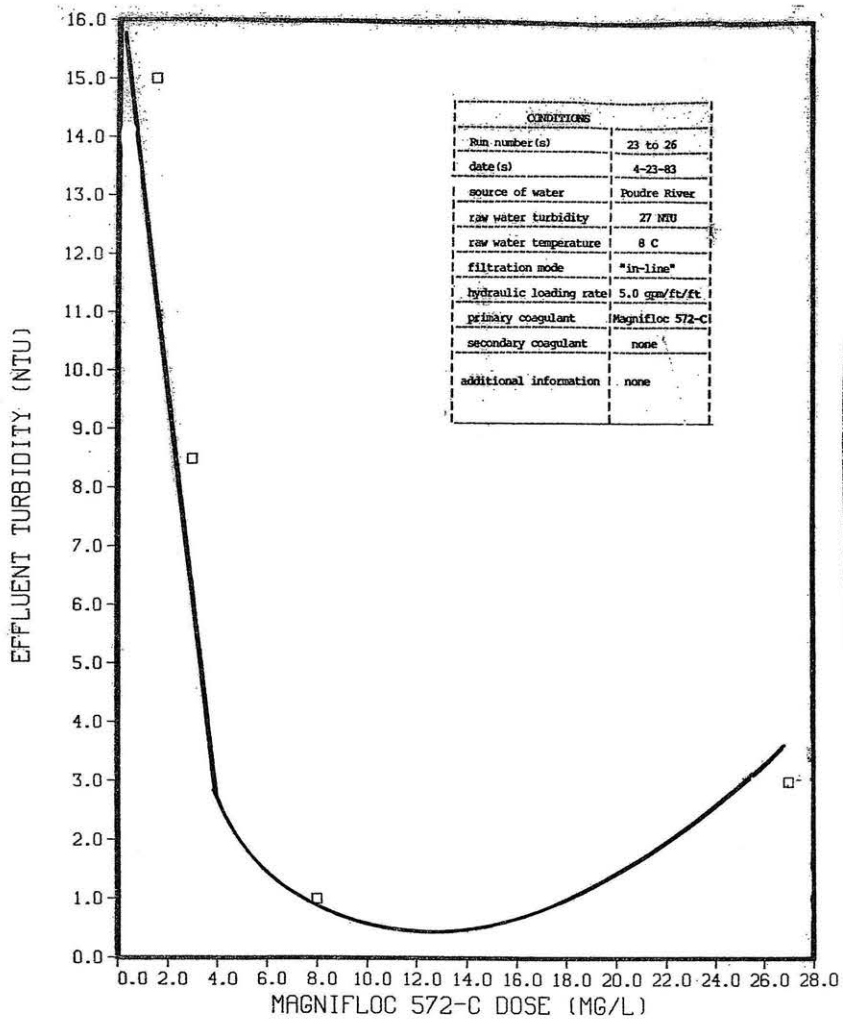


Figure H-22. Effluent turbidity vs Magnifloc 572-C dose. Horsetooth water.

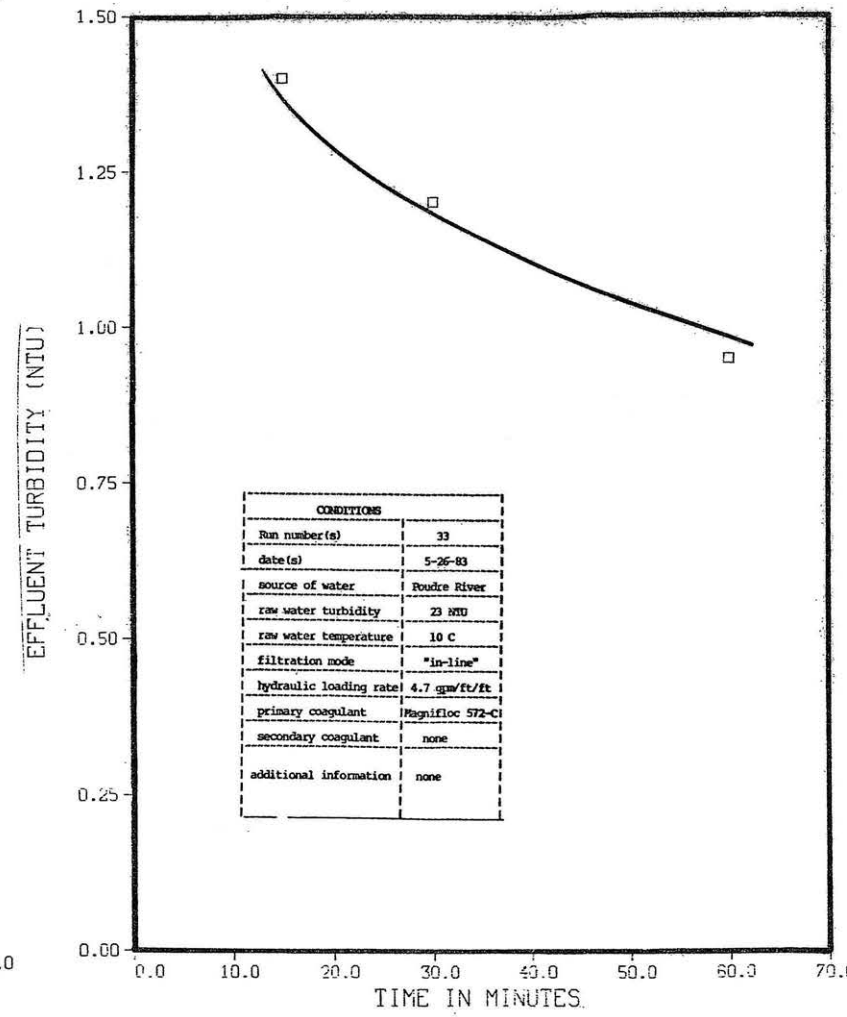


Figure H-23. Effluent turbidity vs time for spring runoff water using 6.5 mg/L of Magnifloc 572-C.

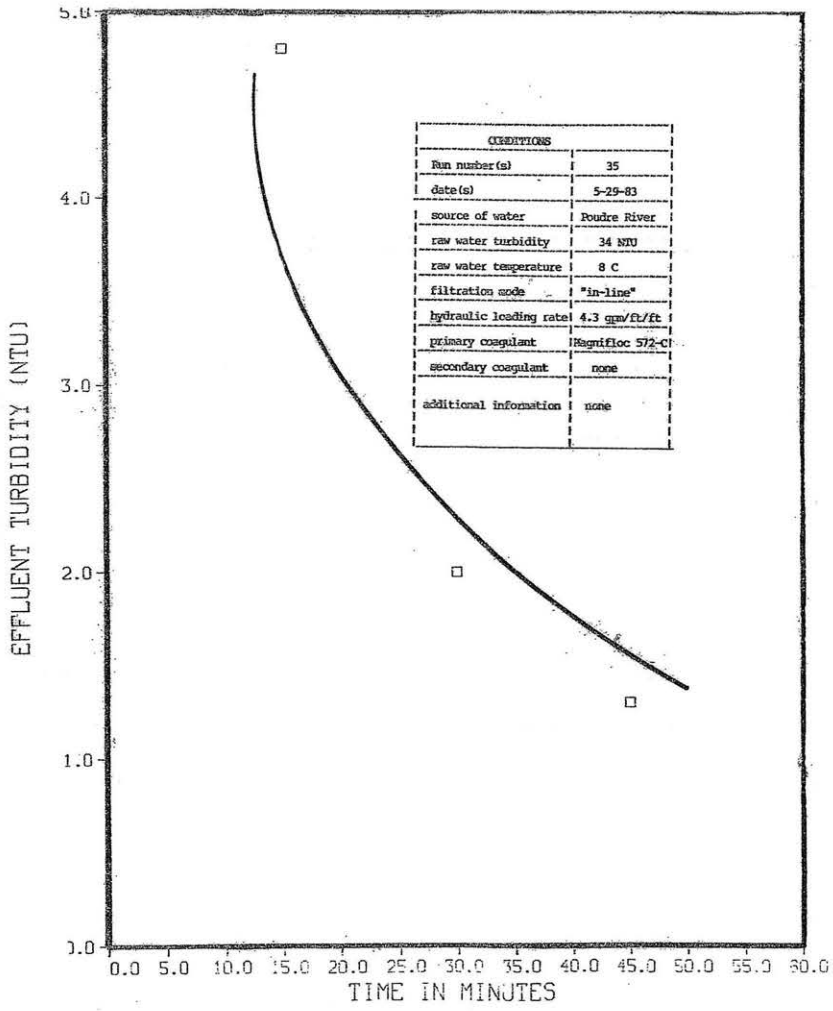


Figure H-24. Effluent turbidity vs time for spring runoff water using 9.0 mg/L of Magnifloc 572-C.

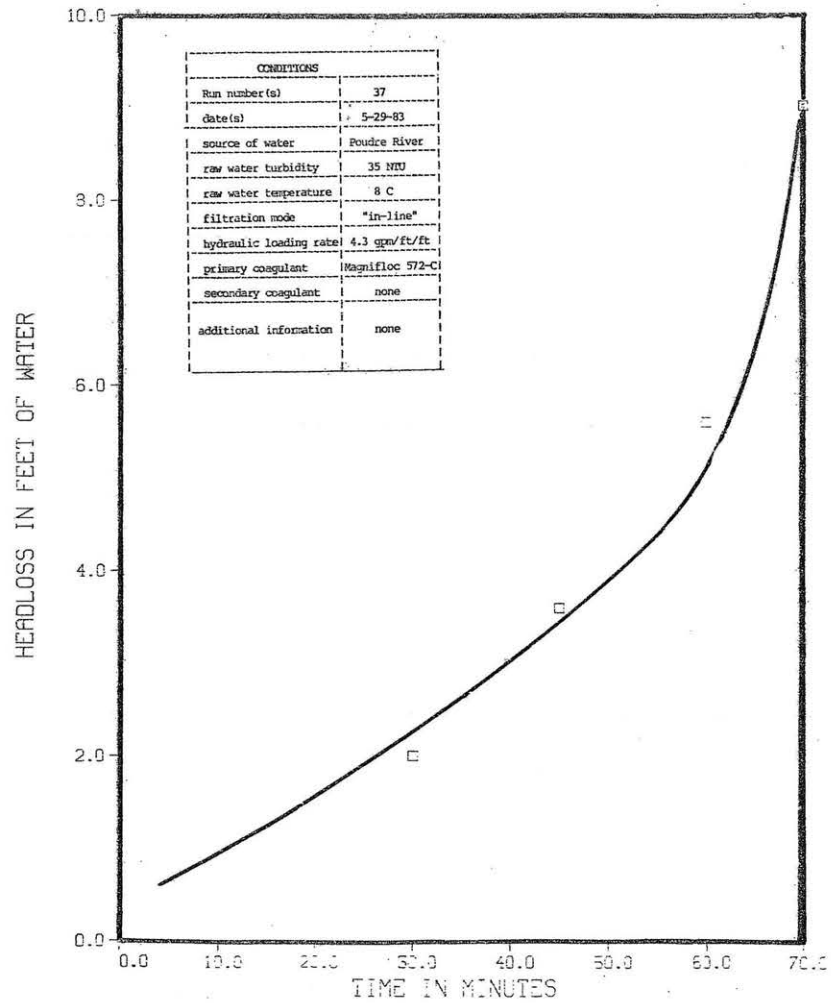


Figure H-25. Headloss vs time without coagulation. Spring runoff water; raw water was 35 NTU.

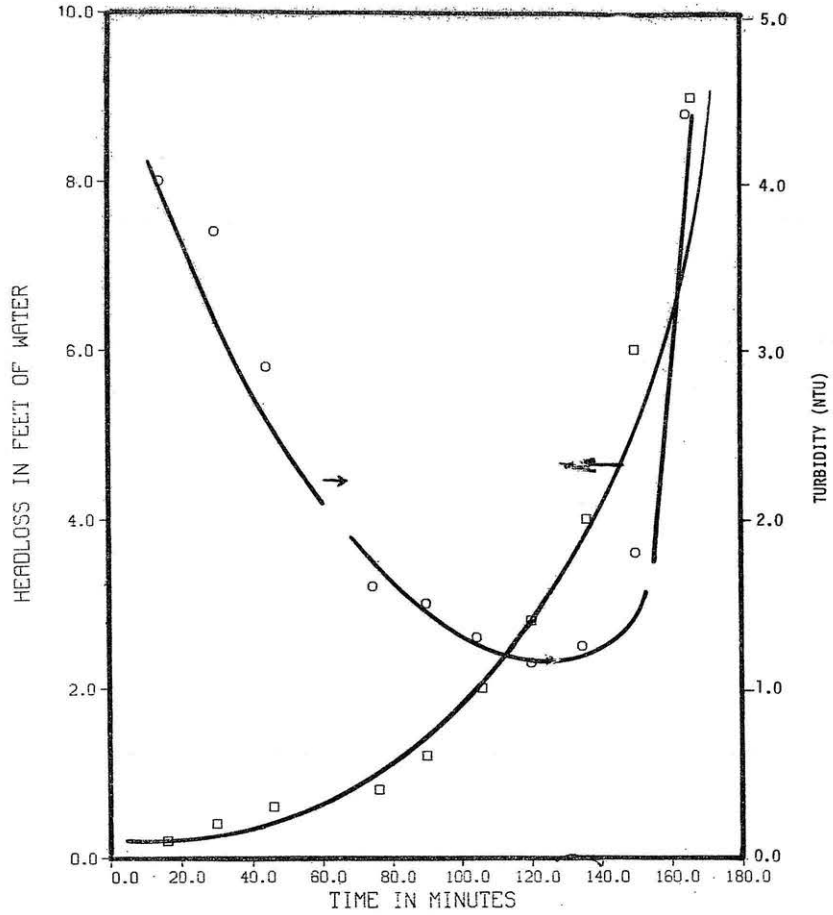


Figure H-26. Headloss and effluent turbidity vs time. Spring runoff water using 7 mg/L of Magnifloc 572-C; raw water was 16 NTU.

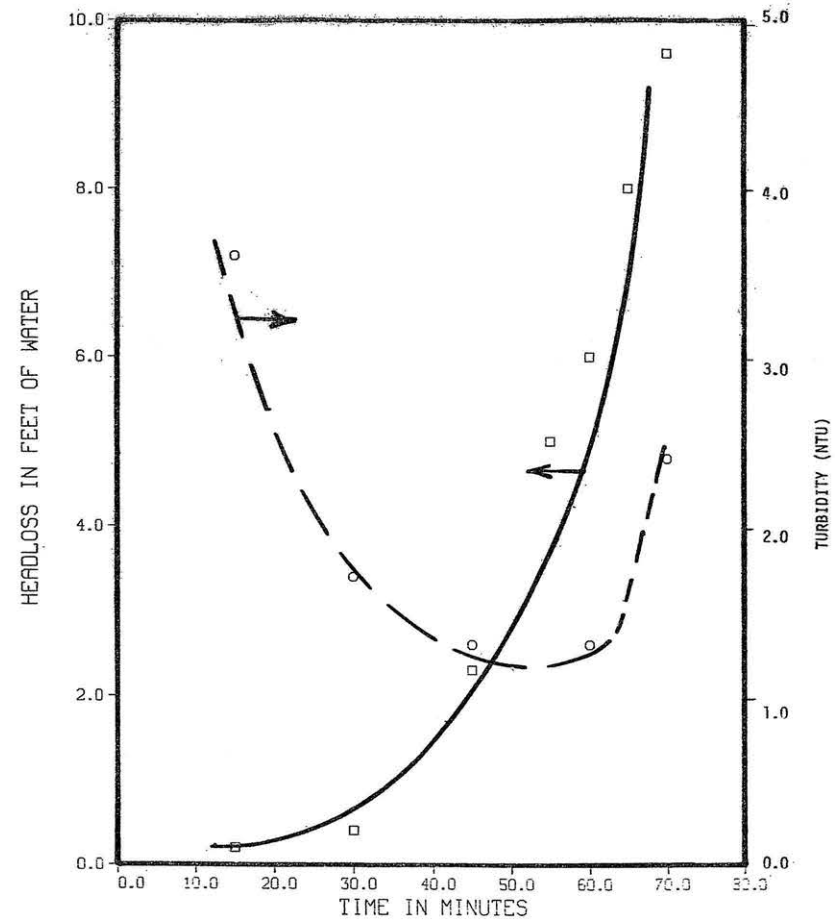


Figure H-27. Headloss and effluent turbidity vs time during spring runoff using 12.5 mg/L of Magnifloc 572-C; raw water was 35 NTU.

development of confidence in the WATER BOY's operation. This confidence was extended to the coliform and Giardia cyst sampling protocols, respectively.

In using Horsetooth Reservoir water the WATER BOY could be setup inside the Engineering Research Center at Colorado State University. This facilitated development of procedures since there was no need to protect against freezing. Also the Engineering Research Center shop and supply room were nearby. For these reasons, Horsetooth water was used to develop the Giardia cyst and coliform bacteria injection and sampling procedures.

HORSETOOTH TESTING

Coagulant Dosage Determination

Four chemical coagulants were used in testing with Horsetooth Reservoir water to obtain curves of effluent turbidity vs dose of coagulant. Table H-1 summarizes the overall results. Figures H-13 to H-16 show the four curves for Magnifloc 573-C, Nalco 8102, Magnifloc 572-C, and alum, respectively. The four curves all have the classical U-shape, permitting determination of "optimum" coagulant dosages. The "optimum" dosages so determined were then used during test runs in which coliform bacteria and Giardia cysts were injected into the influent piping of the WATER BOY. These curves also permitted selection of the "nonoptimum" coagulant dosages, which comprised part of the testing program in which Giardia cyst and coliform bacteria removals were evaluated.

Each of the three polymers used had the capability to reduce turbidity levels from 7.0 NTU, in the raw water, to 0.5 NTU for the product water, after one-hour of operation. After several hours of operation the turbidity levels were reduced to 0.15 NTU. This indicates that the polymers were highly effective in turbidity removal, using raw water from Horsetooth Reservoir. Similar results were obtained in the lab-scale pilot work. Therefore these polymers were expected to be effective in removal of bacteria and Giardia cysts. Alum was tested also, as seen in Figure H-16, producing finished water turbidity of 1.2 NTU, after one hour of operation.

Effect of Coagulant Dose on Filtration

After determination of "optimum" dosages of coagulants, based upon turbidity removal, and after selection of "nonoptimum" dosages to be used in testing, removals of coliform bacteria and Giardia cysts were evaluated using the WATER BOY. This evaluation was performed using coagulant dosages categorized as "none", "optimum", and "nonoptimum". Table H-1 summarizes the turbidity, coliform bacteria, and Giardia cyst removal data from 21 tests classified according to the type of coagulant dosage used.

Zero Coagulant Dose Tests—

The first seven runs of Table H-1, were tests in which "zero" coagulant dose was used, designated as "none". These tests were performed to establish a reference for evaluating Giardia cyst and coliform bacteria removals when coagulants were used.

Results showed that large numbers of cysts passed through the WATER BOY filter when chemicals were not used. Removals of Giardia cysts were less than one percent in three of the seven tests, while two others gave removals of 41 and 84 percent. Results for one of the seven tests showed a removal of >99 percent, which cannot be explained at this time. The results were supported by removals of turbidity and coliform bacteria. Turbidity removals ranged from 3 to 14 percent while coliform bacteria removals were about 30 percent for two tests. Only two coliform removal results are reported in the first seven tests due to difficulties in establishing proper dilutions in the analyses. These results further establish the contention that rapid rate filtration must be preceded by chemical coagulation to ensure effective treatment for production of drinking water.

Optimum Coagulant Dosage Tests--

Ten tests in Table H-1 used "optimum" dosages of coagulant. These ten runs are the second group of tests shown. The first eight tests used only a primary coagulant chemical, and the last two used alum as a primary coagulant and a polymer as a secondary coagulant. The tests using "optimum" coagulant dosages were performed with expectation that high removals of coliform bacteria and Giardia cysts would occur. For these "optimum" dosage tests, Table H-1 shows that removals of turbidity, coliform bacteria, and Giardia cysts all exceeded 90 percent, with only four exceptions, i.e. for turbidity when alum was used either alone or as the primary coagulant.

The data in Table H-1 show that removals of Giardia cysts exceeded 99 percent in five of the eight test runs having Giardia data. Cysts were not detected in the product water in these cases. Removals of less than 99 percent occurred only when alum was used; the reason is not evident. Removals of coliform bacteria exceeded 94 percent in all cases. Turbidity removals exceeded 90 percent with only four exceptions as noted above.

The cyst concentrations it may be noted were 10, 30, 45, 250, 800, 2000, 200, 75 cysts/liter for Runs 49, 52, 58, 90, 92, 98, 105, and 106, respectively. While alum could be less effective than the polymers, the cyst concentrations, which were not controlled easily, partially due to logistic problems, were quite high for the tests with alum. At the same time, however, the turbidity vs alum dose curve, shown in Figure H-16, indicates that alum is less effective as a coagulant than the polymers tested (see Figures H-13, H-14, H-15). These results indicate areas where further research effort is needed; i.e., to both better define the relationships and to explain the behaviors noted.

Nonoptimum Coagulant Dosage Tests--

The "nonoptimum" coagulant dosage tests, which used polymer only, are the last four tests shown in Table H-1. Cysts were not detected in the effluent for any of these "nonoptimum" Giardia test runs, while turbidity removals ranged between 76 and 86 percent, and removals of coliform bacteria ranged from 81 to >99 percent. Runs 51 and 59 used above-optimum dosages of Magnifloc 573-C and Nalco 8102, respectively. Runs 75 and 91 used below-optimum dosages of Magnifloc 572-C and Nalco 8102, respectively. Table H-1 shows the dosages used, which can be related to the turbidity-dose curves in

Figures H-13 to H-16 for the purpose of ascertaining the deviation from optimum. The "nonoptimum" dose tests were run to ascertain the sensitivity of removals of turbidity, bacteria, and Giardia cysts to deviations from optimum coagulant dosages, which could occur in practice. These results indicate that for Horsetooth Reservoir water, removals are not highly sensitive to coagulant dosage, but removals are not as high as when optimum dosages are used.

Associations Between Dependent Variables

Turbidity vs Giardia--

Table H-1 shows results for tests in which Giardia cyst removals were obtained. Figure H-17 shows these data for percent removals of Giardia cysts plotted against percent removal of turbidity. The plot shows that when removal of turbidity is high then removal of Giardia cysts is high also, e.g. exceeding 90 percent. The plot shows also that low removals of Giardia cysts occurred only when removals of turbidity were low also. The curve shown was drawn to indicate an association between the group of plotted points in the upper right portion of the plot and a fewer number of points in the lower left; it has no statistical significance. The curve shown has a rationale, however, based upon the work of Al-Ani and Hendricks (1983), which shows a similar association in a three-dimensional histogram plot. Their work shows that generally when turbidity removal exceeds 80 percent, Giardia cyst removal will exceed 98 percent. This work, at the field scale, supports their findings, except Figure H-17 indicates that Giardia removals exceed 90 percent (not 98 percent) when turbidity removal exceeds 80 percent. This supports generally their contention that turbidity removal can be used as a surrogate for Giardia cyst removal.

Coliform vs Giardia--

Table H-1 shows results for 10 tests in which coliform bacteria removals were obtained along with corresponding removals of Giardia cysts. Figure 3-6 is a plot showing percent removal of Giardia cysts vs percent removal of coliform bacteria. The plot shows that when removals of coliform bacteria are high then removals of Giardia cysts are high also. Only one point was obtained for low removals. The curve shown was drawn to indicate an association between the one point on the left side and the group of points on the right. These results are consistant also with those of the laboratory-scale testing and support the contention that removal of coliform bacteria can serve as an indicator of removal of Giardia cysts. The results indicate that when high removals of turbidity or coliform bacteria occur, then effective filtration is likely.

Turbidity vs Coliforms--

Figure H-19 shows plotted points, also obtained from Table -1, for percent removal of turbidity vs percent removal of coliform bacteria. The association between the two groups of plotted points is similar to the previous two plots, i.e. if 80 percent removal of turbidity occurs then removal of coliform bacteria can be expected to exceed 95 percent.

Coliforms vs Standard Plate Count--

Standard plate count measurements for the influent and effluent streams were made for only two runs, Runs 51 and 52. Removals were 99 percent and 98 percent, respectively with influent concentrations 29,000/mL and 15,000/mL. Removals of coliform bacteria were >99 percent and 95 percent for these two runs. Standard plate counts were not run routinely since coliform bacteria data were used and it was necessary to set priorities in order to control the work load.

Effect of Run Time on Filtration Effectiveness

Coliform bacteria concentrations in the product water were measured at intervals of time in Run 59. This was done during the initial 70 minutes of filter operation after backwash. Figure H-20 is a plot of the data obtained. The coagulant dosage was "nonoptimum", i.e. 11.0 mg/L of Nalco 8102. Figure H-20 shows that coliform counts in the effluent water dropped to 10/100 mL within 20 minutes of operation; this compares with 1200/100 mL in the influent water.

Figure H-21 shows how both headloss and effluent turbidity vary with time during Run 78 using optimum dosage of Nalco 8102 polymer. As shown in Figure H-21, 10-hours were required for the effluent turbidity to reach the lowest value of 0.15 NTU, while a turbidity level of 0.4 NTU was reached at 1-hour after backwashing. Breakthrough occurred about 14-hours after the start of the run, as indicated by the steep slope of the turbidity-time curve. Headloss increased continuously to about eight feet of water, which existed when breakthrough occurred.

After 12-hours of filtering during Run 78 Giardia cysts and coliform bacteria were fed into the system (at this point the run was designated as Run 79). Cysts were not detected in the effluent stream. Coliform counts were 8700/100mL in the influent, and 50/100mL in the effluent, giving >99 percent removal. It was not the purpose to have a prolonged run time, as is desired in practice. The purpose of Run 79 was to test the effectiveness of a "well-seated" filter in removing Giardia cysts and coliform bacteria.

RESULTS FROM SPRING RUNOFF TESTING AND FAMILIARIZATION TESTING - FIELD-SCALE

This appendix material summarizes results obtained in April and May 1983 during spring runoff of the Cache La Poudre River, when turbidity levels ranged from 12 to 44 NTU. The results are contained in this appendix to illustrate the contrast in treating high turbidity water compared with the low turbidity water, the focus of the research. The results indicate that although "in-line" filtration can produce water of 1 NTU, run times were only about two hours. Since run times of only two hours are not economical, the "in-line" mode is not feasible for treating high turbidity waters. The question was explored because of its significance if found feasible for high turbidity waters as well as waters having low turbidity.

SPRING RUNOFF RESULTS

During spring runoff, turbidity levels of the Cache La Poudre River ranged between 12 and 44 NTU, changing as much as 10 NTU in one hour, and water temperatures ranged between 7 and 10°C. Since the WATER BOY was set up on the river when spring runoff began in late April 1983, it was decided to take advantage of these different conditions and conduct turbidity testing. The idea was to ascertain whether the "in-line" mode of filtration was effective for high turbidity waters.

There were 22 effluent turbidity vs chemical dose test runs using Cache La Poudre River water during spring runoff. Table H-2 summarizes the spring runoff results according to coagulant dose category. Testing was not done using Giardia cysts and coliform bacteria because of the limited scope of this phase. Eighteen test runs were conducted using Magnifloc 572-C polymer as sole coagulant, and four tests used alum as the sole coagulant. The alum test runs, Runs 39 to 42, were deemed invalid due to excessive dilution of the alum feed solution. The 18 effluent turbidity vs chemical dose tests, using Magnifloc 572-C as the sole coagulant involved two comparisons between the bench scale, laboratory scale, and field scale pilot plants (reported by McElroy and Hendricks, 1984). Three headloss and effluent turbidity vs time curves were developed.

Effect of Coagulant Dose on Filtration

Figure H-22 shows the effluent turbidity vs chemical dose curve developed using Magnifloc 572-C and water from the Cache La Poudre River during spring runoff. From this curve, the optimum dosage range for Magnifloc 572-C was estimated to be between 6 and 20 mg/L. This optimum dosage range is defined by turbidity removal, as shown in Figure H-22. The curve shown in Figure H-22 is unique for the particular ambient water conditions tested. Results, shown in Table H-2, are summarized in the following sections.

Zero Coagulant Dose Tests--

Runs 34 and 36 had zero coagulant dose. Raw water turbidities were 23 NTU and 35 NTU, respectively. These are the first two runs listed in Table H-2. After one-hour of operation, the effluent turbidity for these no chemical runs were 10 NTU, for Run 34; and 11 NTU for Run 36.

Optimum Coagulant Dose Test--

The optimum dose range was determined to be 6 to 20 mg/L of Magnifloc 572-C for 27 NTU spring runoff water. Runs 25, 27b, 28a, 29, 30, 33, 35, 37, and 38, had coagulation dosages within this 6 to 20 mg/L optimum dose range. Turbidity removals for these nine optimum dose runs ranged between 93 and 97 percent.

Nonoptimum Coagulant Dose Tests--

Runs 23, 24, 26, 27a, 28b, 31, and 32, all had nonoptimum coagulant dosages, as determined with 27 NTU raw water. None of these nonoptimum dose

runs had effluent turbidity below 3 NTU. Turbidity removals ranged between 44 and 94 percent.

Effect of Time on Filtration

Five effluent turbidity and headloss vs time runs were conducted using spring runoff water from the Cache La Poudre River. These were Runs 33, 35, 36, 37, and 38.

The results of Run 33 is shown in Figure H-23, which shows how effluent turbidity varies with time immediately following backwashing for raw water having 23 NTU. Effluent turbidity readings declined steadily to 1.4 NTU, 1.2 NTU, and 0.9 NTU, at 10 minutes, 30 minutes, and 60 minutes, respectively. Figure H-24 shows the results of Run 35, which was similar to Run 33, but with 34 NTU water. Both Figures H-23 and H-24 illustrate the need for "filtering to waste". A period of about 30 minutes duration appears adequate based upon the curves shown.

Figure H-25 shows how headloss varies with time without chemicals for the "in-line" mode of filtration, and using raw water having turbidity of 35 NTU. Figure H-26 and H-27 show headloss and effluent turbidity vs time relations using an optimum dose of Magnifloc 572-C, "in-line" filtration, and raw water turbidity of 16 and 35 NTU respectively.

Figures H-25, H-26, and H-27 are the results of Runs 36, 38, and 37, respectively. The length of run for these three runs, before backwashing was required, were approximately 60 min, 160 min, 60 min, respectively. These short runs show that "in-line" filtration is not a suitable treatment mode during spring runoff. Conventional treatment would allow flocculation which would permit sedimentation, thus reducing the turbidity load on the filters.

APPENDIX I

CHEMICALS

Coagulant chemicals are described in this appendix, which includes manufacturer's literature as available. Also, because it is often not clear how alum is expressed, e.g. as mg/L AL^{+++} or mg/L $Al_2(SO_4)_3$, etc., a chart of equivalents is shown.

The alum and the polymers used are indicated below by manufacturer and brand name.

Manufacturer	Brand Name	Literature Included
Allied	Liquid alum	yes
Nalco	650	yes
	8100	no
	8102	yes
	8152	yes
	8170	no
	8181	no
Dow	Separan NP10	yes
American Cynaid	Magnifloc 572C	yes
	Magnifloc 573C	yes

Allied Chemical

CUSTOMER ENGINEERING
TECHNICAL SERVICE
P.O. BOX 6, SOLVAY, N.Y. 13209
INDUSTRIAL CHEMICALS DIVISION

September 1, 1972

DATA SHEET LA-1 - EQUIPMENT AND PIPING FOR LIQUID ALUM AT AMBIENT TEMPERATURES

A. PHYSICAL PROPERTIES

1. Description

Liquid alum, an aqueous solution of aluminum sulfate, $Al_2(SO_4)_3$ plus H_2O, is a very pale green liquid. The commercial strength, 36.5° Baume', has 8.3% available Al_2O_3.

2. Physical Constants

Density (gm/cc) at 60°F	1.34
Density (lb/gal) at 60°F	11.2
Gallons/ton at 60°F	180
Viscosity (cp) at 32°F	52
Viscosity (cp) at 70°F	21
Boiling point (°F)	214
Freezing point (°F)	5

3. Conversion from Dry Alum

There are 5.4 pounds of dry alum (17% Al_2O_3) per gallon of liquid alum. Convert dry alum to liquid alum as follows:

$$\frac{\text{pounds dry basis}}{\text{5.4 pounds per gallon}} = \text{gallons liquid basis}$$

CALCULATIONS SHOWING EQUIVALENT CHEMICAL EXPRESSIONS FOR ALUM CONCENTRATION

According to the Allied Data Sheet I-1, Appendix I, the density of the liquid alum is 1.34 gm/mL at 60°F. The data sheet states that the density of dry alum is 5.4 lb dry alum (17 percent Al_2O_3) per gallon of liquid alum. The converson of these data to concentrations of Al_2SO_4 or Al_2O_3 are not apparent. The conversions are explained as follows:

1. Determine percentage of dry alum in liquid alum solution:

$$\frac{5.4 \text{ lb dry alum}}{\text{gal liquid alum}} \times \frac{\text{gal liquid alum}}{8.34 \text{ lb water} \cdot 1.34 \frac{\text{lb liquid alum}}{\text{lb water}}}$$

$$= \frac{4.5 \text{ lb dry alum}}{11.17 \text{ lb liquid alum}} = \frac{0.48 \text{ gm dry alum}}{\text{gm liquid alum}}$$

2. Deduce form of "dry alum"

The MW of $Al_2(SO_4)_3 \cdot 14H_2O$ is 592

The MW of Al_2O_3 is 102

The Al_2O_3 equivalent weight in $Al_2(SO_4)_3 \cdot 14H_2O$ is $= \frac{102}{592}$

$$= 0.172 \frac{\text{gm } Al_2O_3}{\text{gm} Al_2(SO_4)_3 \cdot 14H_2O}$$

This means that the "dry alum" has the form $Al_2(SO_4)_3 \cdot 14H_2O$ per gm liquid alum, or

$$\frac{0.48 \text{ gm } Al_2(SO_4)_3 \cdot 14H_2O}{\text{gm liquid alum}} \times \frac{1.34 \text{ gm liquid alum}}{\text{mL liquid alum}}$$

$$= \frac{0.643 \text{ gm alum as } Al_2(SO_4)_3 \cdot 14H_2O}{\text{mL liquid alum}}$$

3. Equivalent expressions are:

$$[Al^{+++}] = \frac{0.643 \text{ gm } Al_2(SO_4)_3 \cdot 14H_2O}{\text{mL liquid alum}} \times \frac{54 \text{ gm } Al^{+++}}{592 \text{ gm} Al_2(SO_4)_3 \cdot 14H_2O}$$

$$= \frac{0.058 \text{ gm } Al^{+++}}{\text{mL liquid alum}} \times \frac{\text{mL liquid alum}}{1.34 \text{ gm liquid alum}}$$

$$Al_2(SO_4)_3 = \frac{0.64 \text{ gm } Al_2(SO_4)_3 \cdot 14H_2O}{\text{mL liquid alum}} \times \frac{396 \text{ gm } Al_2(SO_4)_3}{592 \text{ gm } Al_2(SO_4)_3 \cdot 14H_2O}$$

$$= \frac{0.428 \text{ gm } Al_2(SO_4)_3}{\text{mL liquid alum}}$$

$$Al_2O_3 = \frac{0.64 \text{ gm } Al_2(SO_4)_3 \cdot 14H_2O}{\text{mL liquid alum}} \times \frac{102 \text{ gm } Al_2O_3}{592 \text{ gm } Al_2(SO_4)_3 \cdot 14H_2O}$$

$$= \frac{0.11 \text{ gm } Al_2O_3}{\text{mL liquid alum}}$$

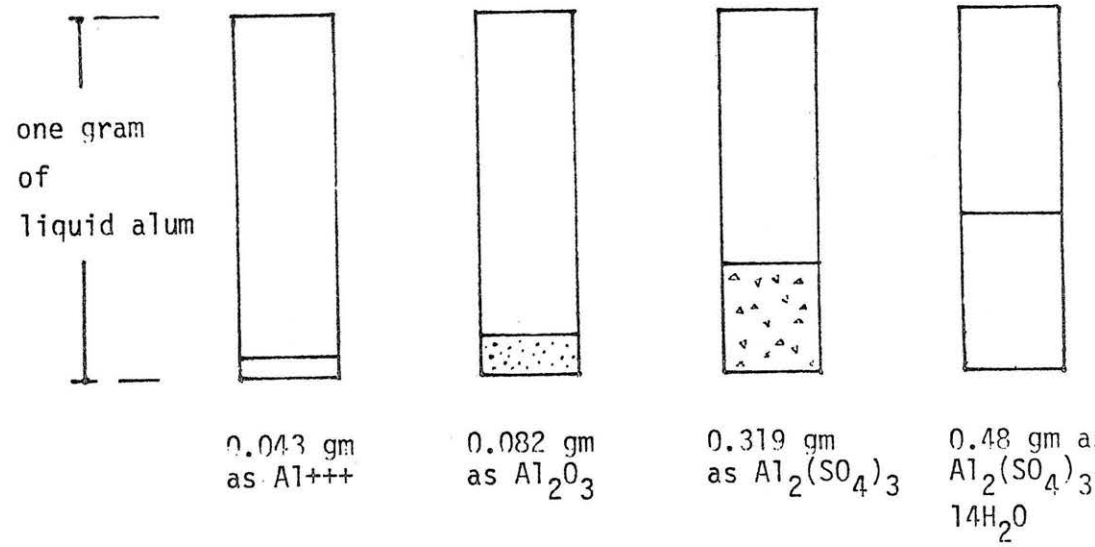
It is possible that operators may express concentrations in terms of mg/L of liquid alum. To do this, the actual weight of liquid alum is used as the basis and is added to water to make one liter of solution. For example, to make 10 mg liquid alum/L solution we find the volume of liquid alum solution that has 10 mg. This is calculated as follows:

$$\frac{X \text{ mL liquid alum}}{10 \text{ mg liquid alum} \times \frac{\text{gm}}{10^3 \text{ mg}}} = \frac{1 \text{ mL liquid alum}}{1.34 \text{ gm liquid alum}}$$

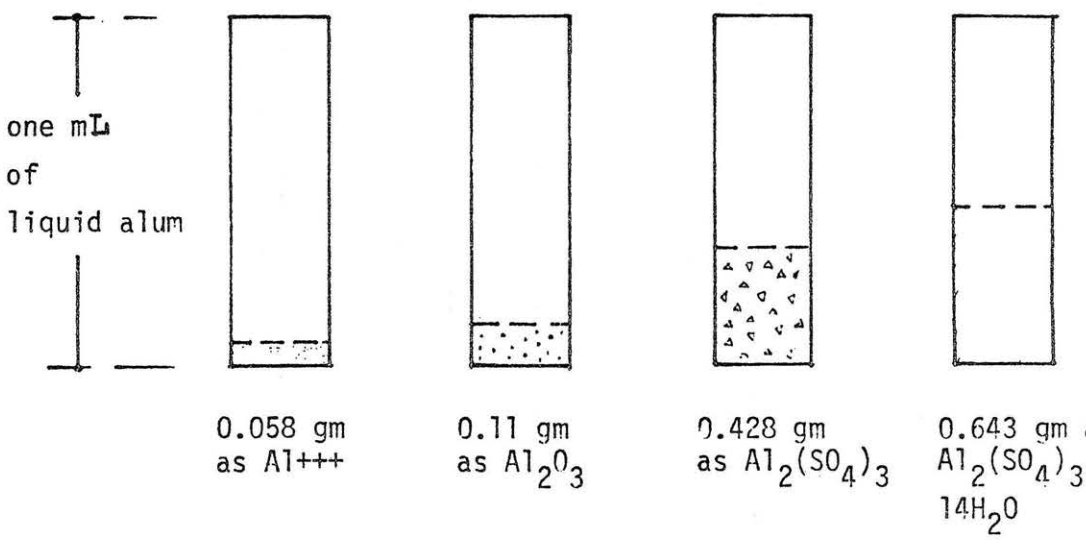
$$X = 0.00746 \text{ mL}$$

Of course we can not measure volumes this small so if the liquid alum solution is diluted to say 1.34 gm/1000 mL the volume would be 7.46 mL instead.

The results of all of the above arithmetic are summarized in Figure I-3. Thus, using Figure I-1 (a), it is easy to convert from "grains of liquid alum" to any form of expression desired, e.g., grains as Al^{+++}, $Al_2(SO_4)_3$, etc. For example, if an operator says he uses 10 mg/L of alum, and it is determined that he or she means liquid alum, one merely multiplies this figure by 0.48 to get the dosage in terms of $Al_2(SO_4)_3 \cdot 14H_2O$. Similarly, if we wish to meter alum in terms of say, $Al_2(SO_4)_3 \cdot 14H_2O$, we can consult Figure I-3 (b) and note that each milliliter of liquid alum contain 0.643 gm of $Al_2(SO_4)_3 \cdot 14H_2O$. If the specifications for the liquid alum are different than stated in this Appendix, the above calculations can be used as a model. For example, if the density of liquid alum happens to be say 1.45 (an arbitrarily chosen number) instead of 1.34, all preceding calculations and Figures I-1 (a) and I-1 (b) would be changed accordingly.



(a) One gram of liquid alum



(b) One milliliter of liquid alum

Figure I-1. Equivalent expressions of alum in Allied Chemical Commercial liquid alum based upon molecular weight conversions.

MANUFACTURER'S LITERATURE ON POLYMERS USED

(1) Nalco 650 (p. 1 of 1)

NALCO 650 functions as an effective aid for adsorbing oils, clay, organics (including color) and microorganisms. It is particularly effective when used in combination with cationic polyelectrolyes, such as NALCO® 600, NALCOLYTE® 603, 605, or 607, and with inorganic coagulants, such as sodium aluminate. NALCO 650 rapidly forms a fast-settling floc, and should be considered whenever increased throughput is desired. It neither increases the dissolved solids content nor alters the pH of the water being treated.

PRINCIPAL USES

NALCO 650 is a highly surface-active colloid used principally as a complementary coagulant aid and weighting agent in water clarification and softening. NALCO 650 is used extensively where natural turbidity is insufficient to form heavy, low water content floc. The floc thus formed is more readily dewatered or dried than that formed by metallic salts such as alum or iron salts. As a consequence, a decrease in alum or other salt dosage may result, with a lower resulting cost

GENERAL DESCRIPTION

A processed montmorillonite. Forms a uniform, insoluble suspension when dispersed in water.

Form	Pulverized
Color	Light buff
Odor	None
Density	61 lb/cu ft

DOSAGE

Varies between 5 and 50 ppm, depending upon application.

FEEDING

For batch preparation, make up to a 6% slurry in water, by adding NALCO 650 through a 1" screen. To facilitate wetting, the addition of 0.01 lb of NALCO® 680 sodium aluminate per gallon of water, before addition of NALCO 650, is recommended. Feed into the vortex of a Lightnin' or equal mixer. Mix vigorously, initially, then continue slow mixing to maintain a uniform suspension.

In dry chemical feeders where solids are fed continuously. NALCO 650 flows freely in standard proportioning equipment. For optimum results, introduce NALCO 650 into the slurry vat via a Nalco Type III Coagulant Disperser. Make certain that the disperser effluent stream is fed into the vortex of the Lightnin' mixer in the slurry vat. The Lightnin' mixer should have two standard propellers to insure adequate agitation.

Introduce the NALCO 650 slurry into the rapid agitation section of the clarifier or to the suction side of the raw water supply pumps.

HANDLING AND SHIPPING

No special precautions are required, but avoid prolonged contact. Does not deteriorate with age. Store in a dry place. NALCO 650 is shipped in bulk hopper bottom cars, hopper trucks, or in 50 lb multiwall bags.

NALCO CHEMICAL COMPANY
WATER TREATMENT CHEMICALS
2901 BUTTERFIELD ROAD □ OAK BROOK, ILLINOIS 60521

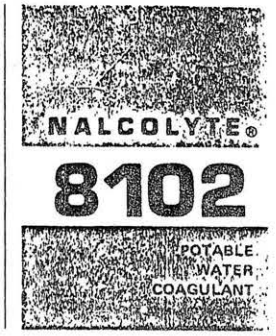
SUBSIDIARIES IN AUSTRIA BRAZIL CHILE COLOMBIA FRANCE HOLLAND ITALY MEXICO PHILIPPINES SAUDI ARABIA SPAIN SWEDEN VENEZUELA AND WEST GERMANY □ AFFILIATES IN AUSTRALIA CANADA JAPAN SOUTH AFRICA TAIWAN AND UNITED KINGDOM

(2) Nalco 8102 (p. 1 of 2)

NALCOLYTE 8102 is a moderate molecular weight, cationic polyelectrolyte developed for both potable water clarification and waste treatment systems.

NALCOLYTE 8102 aids in:

- *Improving effluent quality*
- *Reducing or eliminating the need for metal salts*
- *Producing a dense, rapid-forming, easily settled floc*
- *Forming a compact, easily dewatered sludge*
- *Producing quality water in prechlorinated systems*
- *Reducing or eliminating the need for pH adjustment*



PRINCIPAL USES

NALCOLYTE 8102 is a moderate molecular weight, polycationic polymer recommended for use as a primary coagulant in raw water clarification and lime softening. NALCOLYTE 8102 is approved by the USEPA for treatment of potable water at an application rate up to 50 ppm.

Application Programs	Use
• Conventional Clarification or Lime Softening	Primary coagulant to partially or completely replace inorganic salts
• Direct Filtration	Primary coagulant for low turbidity and colored water
• Filter Aid	Secondary coagulant to improve filter effluent quality
• Clay/Polymer	Primary coagulant in a total alum or iron replacement

Your local Nalco representative can help determine the best clarification program for your needs.

GENERAL DESCRIPTION

Form	Liquid
Charge in Solution	Cationic
Density (Typical)	9.09 lb/gal
Color	Pale amber
Appearance	Slight haze
Odor	Slight
pH Neat (Typical)	4.5
Viscosity	See Figure 1
Freeze Point (Neat)	14°F
Freeze-Thaw Recovery	Complete

DOSAGE

The specific dosage of NALCOLYTE 8102 will depend on raw water characteristics, the type of application, equipment operation, and the results required. Your Nalco representative will recommend the dosage ranges expected for your system.

FEEDING

Conventional Clarification, Clay/Polymer, Direct Filtration

NALCOLYTE 8102 can be fed neat or as a diluted stock solution. See Figure 2. Use a positive displacement pump, BIF 1711 Series or equivalent, to meter the product or solution into a water line where it can be continuously diluted to 0.5% or less before application. NALCOLYTE 8102 should be applied prior to the rapid mix zone to ensure efficient distribution into the water.

When preparing a stock solution in cold water (less than 60°F) additional mixing may be required. NALCOLYTE 8102 solutions greater than 10% concentrations are stable for one week.

Filter Aid Application

NALCOLYTE 8102 should be fed as a 10–30% solution to the distribution header prior to the filters. In some cases, better performance can be obtained by feeding each filter individually at the inlet flume. The feed point should be selected to ensure distribution of the polymer within the water prior to filter contact.

MATERIALS OF CONSTRUCTION

PVC feed lines should be used when handling concentrated NALCOLYTE 8102. Stainless steel pump heads can be used. Store NALCOLYTE 8102 in fiberglass (DK-411 or equivalent), polyethylene, or rubber-lined tanks. If existing mild steel tanks are used, line with Plasite 4005 or equivalent. Consult your local representative for recommended tank designs.

SHIPPING AND STORAGE

NALCOLYTE 8102 is available in bulk or 55-gallon, non-returnable lined drums. Drums can be stored for one year in unopened containers. Although freezing is harmless, it should be avoided, since lower temperatures increase product viscosity.

HANDLING

NALCOLYTE 8102 should be handled as a mildly acidic product. Avoid contact with skin, eyes, and clothing. Use in a well ventilated area. Avoid prolonged or repeated breathing of vapors and do not take internally. In case of contact with skin, wash with water. For eyes, wash with water for 15 minutes and call a physician.

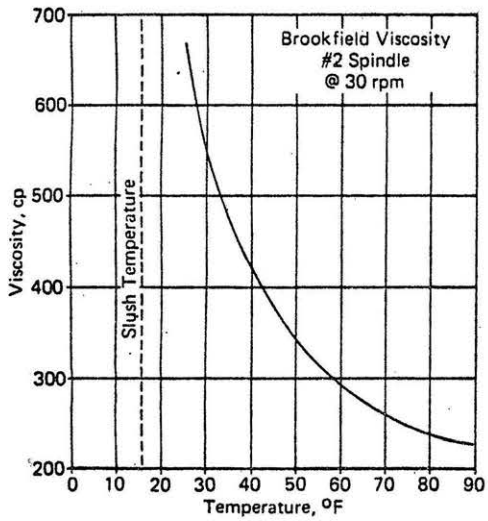


FIGURE 1 — *Typical viscosity as function of temperature NALCOLYTE 8102*

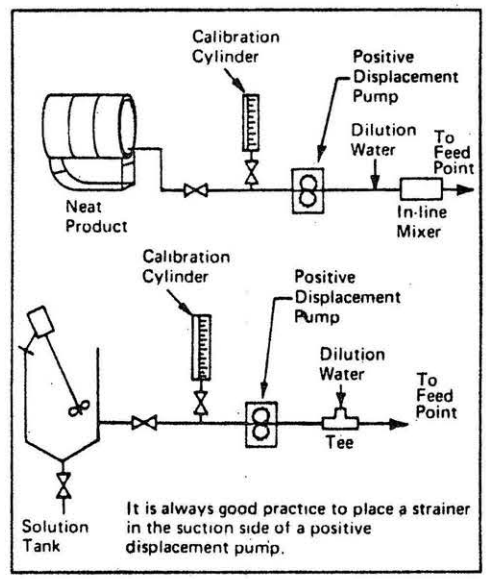


FIGURE 2 — *Recommended coagulant feed systems*

(3) Nalco 8152 (p. 1 of 1)

NALCO 8152 is a highly active colloid that provides excellent performance in water systems where the floc is not dense enough to settle. NALCO 8152 is recommended in raw water clarification of low turbidity systems or in waste water clarification.

PRINCIPAL USES

NALCO 8152 is recommended as a complementary coagulant aid and weighting agent in water clarification where the natural turbidity is insufficient to form a dense floc. NALCO 8152 is also used in primary and secondary waste clarification. NALCO 8152 is particularly effective when used in combination with Nalco cationic polyelectrolytes.

GENERAL DESCRIPTION

Forms a uniform, insoluble suspension when dispersed in water.

Form	Pulverized
Color	Grayish tan
Odor	None
Bulk Density:	
Loose	27 lb/cu ft
Packed	57 lb/cu ft

DOSAGE

Dosage varies between 2 and 30 ppm depending on application.

FEEDING

For batch preparation, make up a 1—5% slurry in water by the slow addition of NALCO 8152 into the vortex of a high speed mixer. Once the clay is in suspension the agitation can be reduced to maintain uniformity. For continuous feeding, meter the clay into the vortex of a high speed meter (Double prop, 1725 rpm) which thoroughly wets and slurries the clay. The recommended retention time is 40—60 minutes before the slurry is educted or pumped to the application point.

HANDLING AND SHIPPING

Avoid prolonged or repeated breathing of dust. If dusting is a problem, wear an approved dust mask or respirator for protection against pneumocomosis. NALCO 8152 does not deteriorate with age. Store in a dry place.

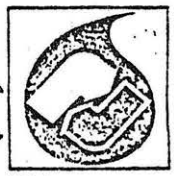
NALCO 8152 is shipped in 50 pound multi-wall bags and in bulk.

NALCO CHEMICAL COMPANY
INDUSTRIAL DIVISION :: WATER TREATMENT CHEMICALS
2901 BUTTERFIELD ROAD II OAK BROOK, ILLINOIS 60521

SUBSIDIARIES IN AUSTRIA, BRAZIL, COLOMBIA, FRANCE, ITALY, MEXICO, PHILIPPINES, SPAIN, SWEDEN, UNITED STATES, VENEZUELA, AND WEST GERMANY. AFFILIATES IN AUSTRALIA, CANADA, HOLLAND, JAPAN, SOUTH AFRICA, TAIWAN, UNITED KINGDOM AND UNITED STATES.

(4) Magnifloc 572C (p. 1 of 4)

MAGNIFLOC® 572C flocculant



Water Clarification Treatment

MAGNIFLOC® 572C flocculant is a low molecular weight liquid cationic flocculant which is effective as a primary coagulant in raw water clarification and is especially effective on low-turbidity/highly colored waters.

Advantages

- Easy to apply, pourable liquid which simplifies dilution, feed and handling operations
- Economical to use – effectiveness at low dosage levels results in reduced handling and storage costs. Added savings can be realized in bulk deliveries
- Works over a wide pH range and eliminates the need for post-treatment pH adjustment.
- Resistant to chlorine
- Eliminates alum or ferric muds when used as a primary coagulant
- Forms a low volume, compact, high-solids sludge which is both biodegradable and burnable
- Less blow-down of sludge is required resulting in more useable water and increased effective throughput

Application

MAGNIFLOC 572C flocculant should be metered to the system by use of a corrosion-resistant, positive displacement pump and diluted 100:1 with clean water prior to being fed to the process stream. Best results are obtained by dispersing feed stream and promoting high turbulence for rapid mixing beyond the addition point.

Principal Uses

MAGNIFLOC 572C flocculant is a highly effective cationic polyelectrolyte which may be used as a primary flocculant or as a coagulant aid in clarifiers and other types of gravity settling operations and in air flotation units.

MAGNIFLOC 572C flocculant is recommended for use in:

- Raw water clarification and especially for low turbidity, highly-colored waters, as a total or partial replacement for inorganic coagulants.
- Treatment of oily waste waters and emulsions as a demulsifier.
- Clarification of latex wastes.

Treatment Level

Raw water clarification	1.0 - 10 ppm
Waste water treatment	1.0 - 20 ppm
Demulsification	10 - 250 ppm

Typical Properties

Appearance	Straw-colored liquid
Specific Gravity (25°C)	1.14 - 1.18
Viscosity (25°C, cps)	100 - 125
pH	5.7
Solubility	Infinite in water
Chemical Reactivity	Non-reactive
Shelf-Life	12-24 months
Freezing Point (°F)	0

Environmental Properties

BOD (mg/L)	100,000
COD (mg/L)	200,000

CYANAMID · AMERICAN CYANAMID COMPANY • Water Treating Chemicals • Wayne, N.J. 07470 • (201) 831-1234

Handling

Animal tests have shown that MAGNIFLOC 572C flocculant has a low order of toxicity, and is not irritating to skin or eyes. Consequently no more than ordinary measures of personal hygiene and plant housekeeping are needed in handling this product. Spilled polymer on floors, etc. is very slippery and difficult to "hose down" spills should be scooped and/or wiped up prior to flushing with water. Storage in glass, stainless steel, plastic or epoxy-lined vessels is recommended. Do not use aluminum or iron in feed or storage systems. If MAGNIFLOC 572C flocculant freezes during shipment or storage, it may be thawed and used after agitation to insure homogeniety.

Shipping

MAGNIFLOC 572C flocculant is shipped in 55 gallon non-returnable steel drums, F.O.B. Avondale, La. and Kalamazoo, Mich. For information on bulk delivery, contact your Cyanamid Representative or Sales Office.

Technical Service

Good water management for you and for our environment depends on using the best products and the latest technology in a totally balanced program. At Cyanamid, we offer a full system of water treatment products designed to meet your needs. Your Cyanamid Sales Representative stands ready to offer information and assistance in using this product and helping you achieve our common goal — clean water.

EPA

MAGNIFLOC 572C flocculant is approved by the Environmental Protection Agency for use in the clarification of potable water at dosages of up to 10 ppm.

Important Caution

The information and statements herein are believed to be reliable but are not to be construed as a warranty or representation for which we assume legal responsibility. Users should undertake sufficient verification and testing to determine the suitability for their own particular purpose of any information or products referred to herein. NO WARRANTY OF FITNESS FOR A PARTICULAR PURPOSE IS MADE.

Nothing herein is to be taken as permission, inducement or recommendation to practice any patented invention without a license.

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5-1384 7M 10/75

CYANAMID

MSDS NO. 1869-01
CAS NO.
DATE: 08/03/82

MATERIAL SAFETY DATA

PRODUCT IDENTIFICATION	TRADEMARK:	**MAGNIFLOC® 572C Flocculant**
	SYNONYMS:	Polyquaternary amine
	CHEMICAL FAMILY:	Cationic polyamine
	MOLECULAR FORMULA:	Mixture
	MOLECULAR WGT.:	Mixture

WARNING SPILLS OF THIS PRODUCT ARE VERY SLIPPERY

HAZARDOUS INGREDIENTS

COMPONENT	CAS. NO.	%	TWA/CEILING	REFERENCE
No Permissible Exposure Limits (PEL), have been established by OSHA				

NFPA HAZARD RATING Not Established

HEALTH HAZARD INFORMATION

EFFECTS OF OVEREXPOSURE:	Acute oral (rat) and acute dermal (rabbit) LD50 values are 5.36 ml/kg and > 16.0 ml/kg, respectively. No eye irritation and no significant skin irritation were produced during primary irritation studies with rabbits.
FIRST AID:	No specific first aid procedures are necessary for accidental exposure to this product.

EMERGENCY PHONE: 201/835-3100

AMERICAN CYANAMID COMPANY, WAYNE, NEW JERSEY 07470

MSDS NO. 1869-01
MAGNIFLOC® 572C Flocculant

FIRE AND EXPLOSION HAZARD INFORMATION	FLASH POINT: METHOD:	>200 F (>93.3 C) Closed Cup
	FLAMMABLE LIMITS (% BY VOL):	Not Available
	AUTOIGNITION TEMP:	Not Available
	DECOMPOSITION TEMP:	Not Available
	FIRE FIGHTING:	Use water, carbon dioxide or dry chemical to extinguish fires. Wear self-contained, positive pressure breathing apparatus and full firefighting protective clothing.
REACTIVITY DATA	STABILITY: CONDITIONS TO AVOID:	Stable None known
	POLYMERIZATION: CONDITIONS TO AVOID:	Will Not Occur None known
	INCOMPATIBLE MATERIALS:	Strong oxidizing agents. This material reacts slowly with iron, copper and aluminum, resulting in corrosion and product degradation.
	HAZARDOUS DECOMPOSITION PRODUCTS:	Thermal decomposition or combustion may produce carbon monoxide, carbon dioxide, ammonia, oxides of nitrogen and/or hydrogen chloride gas.
PHYSICAL PROPERTIES	APPEARANCE AND ODOR:	Straw colored liquid; slight amine odor
	BOILING POINT:	Not Available
	MELTING POINT:	0 F (−18 C)
	VAPOR PRESSURE:	Not Available
	SPECIFIC GRAVITY:	1.14-1.18
	VAPOR DENSITY:	Not Available
	% VOLATILE (BY VOL):	∾50%
	OCTANOL/H_2O PARTITION COEF.:	Not Available
	pH:	5-7 as is
	SATURATION IN AIR (BY VOL):	Not Available
	EVAPORATION RATE:	Not Available
	SOLUBILITY IN WATER:	Complete
SPILL OR LEAK PROCEDURES	STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED:	Spills of this material are very slippery. Cover spills with some inert absorbent material and scoop into a container. Wash area thoroughly with water. Repeat if slipperiness remains.
WASTE DISPOSAL	Disposal must be made in accordance with applicable governmental regulations.	
SPECIAL PRECAUTIONS	HANDLING AND STORAGE/OTHER:	None

Marvin A. Friedman Marvin A. Friedman, Ph.D., Director of Toxicology and Product Safety

(5) Magnifloc 573C (p. 1 of 4)

MAGNIFLOC®
573-C COAGULANT

POTABLE GRADE

PRODUCT BULLETIN WTC 115

MAGNIFLOC 573-C is a liquid cationic flocculant which works effectively as a primary coagulant in raw water clarification or as a powerful coagulant aid in the clarification of water such as surface waters common to Texas and Louisiana.

ADVANTAGES

- Easy to apply, pourable liquid which simplifies dilution, feed and handling operations.
- Economical to use – effectiveness at low dosage levels results in reduced handling and storage costs. Added savings can be realized in bulk deliveries.
- Works over a wide pH range and eliminates the need for post treatment pH adjustment.
- Resistant to chlorine.
- Eliminates alum or ferric muds when used as a primary coagulant.
- Forms a low volume, compact, high-solids sludge which is both biodegradable and burnable.
- Less blow-down of sludge is required resulting in more useable water and increased effective throughput.

PRINCIPAL USES

MAGNIFLOC 573-C is a highly effective cationic poly-electrolyte which may be used as a primary flocculant or as a coagulant aid.

MAGNIFLOC 573-C is recommended for use in:

- Gravity Settling Operations – improves floc formation yielding larger floc size and faster settling rates.
- Clarifiers of all types.
- Air Flotation – Results in clearer underflows and greater through-put.
- Solids-liquid separation systems containing residual chlorine up to several ppm.
- Settling basins.

APPLICATION

MAGNIFLOC 573-C should be metered to the system by use of a corrosion resistant, positive displacement pump and diluted 100:1 with clean water prior to being fed to the process stream. Best results are obtained by dispersing feed stream and promoting high turbulence for rapid mixing beyond the addition point.

TREATMENT LEVEL

When used as the primary coagulant, treatment ranges from 1.0–10 ppm. Normally, MAGNIFLOC 573-C is more effective if added prior to the lime slurry.

CYANAMID WATER TREATING CHEMICALS, WAYNE, N. J. 07470

TYPICAL PROPERTIES	
Appearance	Straw, liquid
Specific Gravity (25°C)	1.14 – 1.18
Viscosity (25°C, cps)	200 – 400
pH	6 – 8
Solubility	Water –
Chemical Reactivity	Non-reactive
Shelf Life (50° – 100°F)	6 Months

PRELIMINARY ENVIRONMENTAL PROPERTIES	
BOD (mg/L)	100,000
COD (mg/L)	200,000
Odor Threshold No.	115
Taste Threshold No.	8,500

HANDLING

Animal tests have shown that MAGNIFLOC 573-C has a low order of toxicity, and is not irritating to skin or eyes. Consequently, no more than ordinary measures of personal hygiene and plant housekeeping are needed in handling this product. Spilled polymer on floors, etc. is very slippery and difficult to "hose down" – spills should be scooped and/or wiped up prior to flushing with water.

SHIPPING

MAGNIFLOC 573-C coagulant is shipped in 55 gallon non-returnable steel drums. F.O.B. Avondale, La. For information on bulk delivery, contact your Cyanamid Representative or Sales Office.

TECHNICAL SERVICE

Tech[illegible] service and information for making the best use of this product are available through your Cyanamid P[illegible]tative.

SALES OFFICES

P.O. Box 868
Mobile, Ala. 36601
(205) 342-4622

P.O. Box 3381
Portland, Ore. 97208
(503) 228-6281

P.O. Box 31
Linden, N.J. 07037
(201) 486-6000

P.O. Box 8889
Chicago, Ill. 60666
(312) 827-8871

1550 Tennessee Street
San Francisco, Cal. 94107
(415) 826-8150

4337 N. American Street
Philadelphia, Penn. 19140
(215) 455-0700

CYANAMID INDUSTRIAL CHEMICALS AND PLASTICS DIVISION
WAYNE, NEW JERSEY 07470

1-1354 3M 5/71

CYANAMID

MATERIAL SAFETY DATA

MSDS NO. 1868-01
CAS NO. ----------
DATE: 10/13/82

PRODUCT IDENTIFICATION

TRADEMARK:	**MAGNIFLOC® 573C Flocculant**
SYNONYMS:	Polyquaternary amine
CHEMICAL FAMILY:	Cationic polyamine
MOLECULAR FORMULA:	Mixture
MOLECULAR WGT.:	Mixture

WARNING

SPILLS OF THIS PRODUCT ARE VERY SLIPPERY

HAZARDOUS INGREDIENTS

COMPONENT	CAS. NO.	%	TWA/CEILING	REFERENCE
No Permissible Exposure Limits (PEL), have been established by OSHA				

NFPA HAZARD RATING

Not Established

HEALTH HAZARD INFORMATION

EFFECTS OF OVEREXPOSURE:	Acute oral (rat) LD50 value is 4.67 ml/kg. Acute dermal (rabbit) LD50 value for a similar product is > 10.0 ml/kg. No signs of irritation of sensitization were produced by MAGNIFLOC 573C treated paper during a repeat insult patch test in humans. No eye irritation was produced during primary irritation studies with rabbits.
FIRST AID:	No specific first aid procedures are necessary for accidental exposure to this product.

EMERGENCY PHONE: 201/835-3100

AMERICAN CYANAMID COMPANY, WAYNE, NEW JERSEY 07470

MSDS NO. 1868-01
MAGNIFLOC® 573C Flocculant

FIRE AND EXPLOSION HAZARD INFORMATION	FLASH POINT: METHOD:	> 200 F > 93.3 C Closed Cup
	FLAMMABLE LIMITS (% BY VOL):	Not Available
	AUTOIGNITION TEMP:	Not Available
	DECOMPOSITION TEMP:	Not Available
	FIRE FIGHTING:	Use carbon dioxide, dry chemical, or water spray to extinguish fires. Wear self-contained, positive pressure breathing apparatus and full firefighting protective clothing.
REACTIVITY DATA	STABILITY: CONDITIONS TO AVOID:	Stable None known
	POLYMERIZATION: CONDITIONS TO AVOID:	Will Not Occur None known
	INCOMPATIBLE MATERIALS:	Strong oxidizing agents. This material reacts slowly with iron, cooper and aluminum, resulting in corrosion and product degradation.
	HAZARDOUS DECOMPOSITION PRODUCTS:	Thermal decomposition or combustion may produce carbon monoxide, carbon dioxide, ammonia, oxides of nitrogen and/or hydrogen chlorde gas.
PHYSICAL PROPERTIES	APPEARANCE AND ODOR:	Amber liquid; slight amine odor
	BOILING POINT:	Similar to water
	MELTING POINT:	0 F (−18 C)
	VAPOR PRESSURE:	Similar to water
	SPECIFIC GRAVITY:	1.08-1.18
	VAPOR DENSITY:	Similar to water
	% VOLATILE (BY VOL):	∞50%
	OCTANOL/H_2O PARTITION COEF.:	Not Available
	pH:	5 - 7
	SATURATION IN AIR (BY VOL):	Not Available
	EVAPORATION RATE:	Similar to water
	SOLUBILITY IN WATER:	Complete
SPILL OR LEAK PROCEDURES	STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED:	Wear impervious boots. Spills of this material are very slippery. Spilled material should be absorbed onto an inert material and scooped up. The area should be thoroughly flushed with water and scrubbed to remove residue.
WASTE DISPOSAL	Disposal must be made in accordance with applicable governmental regulations.	
SPECIAL PRECAUTIONS	HANDLING AND STORAGE/OTHER:	None

Marvin A. Friedman — Marvin A. Friedman, Ph.D., Director of Toxicology and Product Safety

(6) Separan NP-10 (p. 1 of 3)

M A T E R I A L S A F E T Y D A T A S H E E T PAGE: 1
DOW CHEMICAL U.S.A. MIDLAND MICHIGAN 48640 EMERGENCY PHONE: 517-636-4400

EFFECTIVE DATE: 09 OCT 79 PRODUCT CODE: 77669

PRODUCT NAME: SEPARAN (R) NP10 SYNTHETIC POLYMER MSD: 0290

INGREDIENTS (TYPICAL VALUES-NOT SPECIFICATIONS)	: % :
SLIGHTLY HYDROLYZED POLYACRYLAMIDE	: :
SOLIDS	: 95 :
MOISTURE	: 5 :

SECTION 1 PHYSICAL DATA

BOILING POINT: DECOMPOSES : SOL. IN WATER: VERY SOLUBLE
VAP PRESS: (MMHG @ 20C) NEGLIGIBLE: SP. GRAVITY: ----
VAP DENSITY (AIR=1): NOT APPLIC. : % VOLATILE BY VOL: NOT APPLICABLE
APPEARANCE AND ODOR: WHITE, FREE FLOWING AMORPHOUS SOLID, LITTLE ODOR.

SECTION 2 FIRE AND EXPLOSION HAZARD DATA

FLASH POINT: NONE (TESTED TO 400F): FLAMMABLE LIMITS (STP IN AIR)
METHOD USED: CLEVELAND OPEN CUP. : LEL: NONE UEL: NONE
EXTINGUISHING MEDIA: FOAM, CO2, DRY CHEMICAL, WATER FOG.
SPECIAL FIRE FIGHTING EQUIPMENT AND HAZARDS: NONE.

SECTION 3 REACTIVITY DATA

STABILITY: SOLIDS SOFTEN AT 220-230C. DECOMPOSITION EVIDENT AT 270C. PRODUCTS FORMED ON COMPLETE COMBUSTION: CARBON DIOXIDE, WATER, AND NITROGEN DIOXIDE.
INCOMPATIBILITY: OXIDIZING MATERIAL.
HAZARDOUS DECOMPOSITION PRODUCTS: NITROGEN OXIDES.
HAZARDOUS POLYMERIZATION: WILL NOT OCCUR.

SECTION 4 SPILL, LEAK, AND DISPOSAL PROCEDURES

ACTION TO TAKE FOR SPILLS (USE APPROPRIATE SAFETY EQUIPMENT): SWEEP UP AND SALVAGE AS MUCH AS POSSIBLE.
DISPOSAL METHOD: BURN OR BURY IN ACCORDANCE WITH LOCAL, STATE, AND FEDERAL REGULATIONS.

SECTION 5 HEALTH HAZARD DATA

INGESTION: LOW ACUTE ORAL TOXICITY; LD50 (RATS) GREATER THAN 2000 MG/KG.

(CONTINUED ON PAGE 2)
(R) INDICATES A TRADEMARK OF THE DOW CHEMICAL COMPANY

Separan NP-10 (p. 2 of 3)

DOW CHEMICAL U.S.A. MIDLAND MICHIGAN 48640 EMERGENCY PHONE: 517-636-4400

EFFECTIVE DATE: 05 OCT 79 PRODUCT CODE: 77659
PRODUCT (CONT'D): SEPARAN (R) NP10 SYNTHETIC POLYMER MSD: 0290

SECTION 5 HEALTH HAZARD DATA (CONTINUED)

EYE CONTACT: SLIGHT TRANSIENT IRRITATION ONLY.
SKIN CONTACT: ESSENTIALLY NON-IRRITATING.
SKIN ABSORPTION: NOT LIKELY TO BE ABSORBED IN TOXIC AMOUNTS.
INHALATION: DOW GUIDE 10 MG/M3. POSSIBLE NUISANCE DUST.
EFFECTS OF OVEREXPOSURE: POSSIBLE THROAT DRYNESS.

SECTION 6 FIRST AID--NOTE TO PHYSICIAN

FIRST AID PROCEDURES:

EYES: IRRIGATION OF THE EYE IMMEDIATELY WITH WATER FOR FIVE MINUTES IS GOOD SAFETY PRACTICE.
SKIN: WASH OFF IN FLOWING WATER.
INHALATION: REMOVE TO FRESH AIR IF EFFECTS OCCUR. CONSULT MEDICAL PERSONNEL.
INGESTION: LOW IN TOXICITY. INDUCE VOMITING IF LARGE AMOUNTS ARE INGESTED.

NOTE TO PHYSICIAN:
EYES: MAY CAUSE MILD IRRITATION. STAIN FOR EVIDENCE OF CORNEAL INJURY.
SKIN: NO EFFECT EXPECTED.
RESPIRATORY: INJURY IS UNLIKELY.
ORAL: LOW IN TOXICITY.
GENERAL: CONSULT STANDARD LITERATURE. NO SPECIFIC ANTIDOTE. TREATMENT BASED ON THE SOUND JUDGMENT OF THE PHYSICIAN AND THE INDIVIDUAL REACTIONS OF THE PATIENT.

SECTION 7 SPECIAL HANDLING INFORMATION

VENTILATION: RECOMMEND CONTROL OF DUST TO SUGGESTED GUIDE.
RESPIRATORY PROTECTION: IF REQUIRED, USE AN APPROVED DUST RESPIRATOR.
PROTECTIVE CLOTHING: NONE REQUIRED.
EYE PROTECTION: NOT NORMALLY NECESSARY. SAFETY GLASSES WITHOUT SIDE SHIELDS RECOMMENDED.

SECTION 8 SPECIAL PRECAUTIONS AND ADDITIONAL INFORMATION

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE: PRACTICE REASONABLE CAUTION AND PERSON CLEANLINESS. PRODUCT BECOMES SLIPPERY WHEN WET. PRACTICE GOOD HOUSEKEEPING AT ALL TIMES TO PREVENT SLIPPERY FLOORS.

(CONTINUED ON PAGE 3)

M A T E R I A L S A F E T Y D A T A S H E E T PAGE: 3
DOW CHEMICAL U.S.A. MIDLAND MICHIGAN 48640 EMERGENCY PHONE: 517-636-4400

EFFECTIVE DATE: 09 OCT 79 PRODUCT CODE: 77669
PRODUCT (CONT'D): SEPARAN (R) NP10 SYNTHETIC POLYMER MSD: 0290

SECTION 8 SPECIAL PRECAUTIONS AND ADDITIONAL INFORMATION (CONTINUED

ADDITIONAL INFORMATION: REVISIONS 10/09/79 --- FLASH POINT, SKIN CONTACT, SKIN ABSORPTION, INHALATION, VENTILATION, PROTECTIVE CLOTHING.

LAST PAGE

(R) INDICATES A TRADEMARK OF THE DOW CHEMICAL COMPANY

CONSULT THE DOW CHEMICAL COMPANY FOR FURTHER INFORMATION.

THE INFORMATION HEREIN IS GIVEN IN GOOD FAITH, BUT NO WARRANTY, EXPRESSED OR IMPLIED, IS MADE.

APPENDIX J

GIARDIA PROCUREMENT, ANALYSIS AND DETECTION LIMITS

APPENDIX J

GIARDIA CYST PROCUREMENT, ANALYSIS AND DETECTION LIMITS

This appendix contains information on procurement of Giardia cysts, including processing of the fecal samples obtained, sampling efficiency by membrane filtration, techniques for processing samples washed from membrane filters for microscopic counting, detection limits, and an overall discussion of sample processing and cyst counting. The material was based upon the work of Dr. Charles Hibler and was written mostly by Dr. Hibler, with portions written by Dr. W. D. Bellamy (e.g. the portion on detection limits, with editing by the authors.

PROCUREMENT OF GIARDIA CYSTS

Securing Giardia Cysts

Giardia cysts were obtaned from fecal samples of infected dogs. Positive Giardia samples commonly appear as soft to watery stools but normal, firm stools should not be excluded as possibilities. Puppies about six weeks old are the best source but older dogs, bitches, and kennel dogs break frequently.

Fecal samples were collected in baggies and securely closed with twist-tie type closures. Samples were labeled with the pen number, dog number, etc. for future reference and notifying appropriate personnel of the results. The samples were placed in a cooler with ice and transported to the laboratory.

The sources of fecal samples were:

(1) CHRL - Collaborative Radiological Health Laboratory

Foothills Campus - Beagle Colony
Call Esther 491-8522 ext 29 for clothes in women locker
Jim Winic 49208522 for information on puppy liters
(age, births, breeding, etc.)

(2) Humane Society for Larimer County

6317 Kyle Ave., Fort Collins 226-3647
Collect at 7:30 am (before cage cleaning)
1:00 pm (after feeding)
Call before collecting to alert staff

(3) Vet. Teaching Hospital

Parasitology Lab 491-7101 ext 233
Glenda Taton (Parasite Lab Tech) will collect heavy infected samples

(4) Oncology - Vet. Teaching Hospital

Oncology 491-7101
Call Dee or Sharon or Dr. Gillette
They use beagles from CHRL which break with *Giardia* when moved to the Vet. Hospital

Preparing Cysts for Experimentation

In the laboratory, zinc flotations were performed on each fecal sample to check for the presence of *Giardia* cysts. This procedure is described below. If cysts were presented, the sample(s) were weighed and added to an equal amount of cool distilled water. The sample was then mixed thoroughly to break apart any aggregates.

If the sample appears extremely dirty it may be filtered through cheese cloth or gauze or the solution may be mixed thoroughly, quickly allowed to settle, poured into another container and the sediment discarded. Each of these procedures will, however, result in the loss of some cysts. Cyst samples and suspensions were refrigerated at all times.

Cyst Identification--

The zinc flotation procedure was used to ascertain the presence of cysts in fecal samples. The procedure is described following. A fecal sample about the size of a pea is placed in a centrigue tube, 5 to 6 drops of Lugol's Iodine is added to the sample and is mixed well. Fill the tube half way with zinc sulfate solution (spgr 1.18 or 1.20) and mix well. Fill the tube with more solution until the meniscus buldges and affix a coverslip. Place the tube in the centrifuge and tap the coverslip with a pencil end to form a secure bond. *If the coverslip is not firmly in place it will come off during centrifugation and the procedure will have to be repeated. *The coverslip must always be handled by its edge as body oils will prevent attachment of the cysts to its surface. Centrifuge the samples at 1500 rpm for 5 minutes. Remove the coverslip and place on a glass slide. Examine the coverslip for *Giardia* at 100x magnification.

Labelling and Storing Cyst Suspensions

Jars containing suspensions of concentrated cysts were labelled with the date and the number of cysts per mL. The sample should be counted at least every 3rd day and before used in experiments to ensure accurate counts and cyst condition.

Cyst Counting Techniques Obtained from Membrane Filter Sampling

There are two techniques used to process a sample obtained from membrane filtering to concentrate the cysts for counting. These are: 1) Stoll dilution, and 2) micropipette. For a sample with a large number of cysts, i.e. a fecal suspension, the Stoll technique is usually used. For a sample with a low cyst population, the micropipette technique usually is used. The zinc flotation technique was used for the first six slow sand filter test runs (see report by Bellamy, et al., 1984) and for identifying cysts in fecal samples.

Stoll Dilution Technique--

The procedure for the Stoll dilution technique is described as follows. Add 3 mL Lugol's Iodine to a Stoll flask and fill the flask to the 56 mL mark with cool distilled water. Mix the fecal suspension well and remove 4 mL liquid. Add the 4 mL to the flask and shake thoroughly. A 0.075 mL aliquot is removed via micropipette and is placed in a vaseline well. A coverslip is affixed and the number of cysts counted at 400x. The total number seen on one slip is multiplied by 200 to give the total number per mL sample. A minimum of 2 coverslips should be read and averaged.

Micropipette Technique for Samples from Experimentation--

The procedure for the micropipette technique is described as follows. Samples in mason jars under ice will arrive at the laboratory and must sit overnight to allow settling of the cysts and debris. The following day the samples are pipetted down to approximately 200 mL liquid without disturbing the sediment. After the excess water is removed, mix the sample well and pour in a 50 mL conical centrifuge tube. Centrifuge for 5 minutes at 1500 rpm. Pipette off the supernant to about 5 mLs and repeat the procedure until all the sample has been concentrated to 1 mL and the sample jar rinsed well with distilled water. The final volume of the concentrate will depend on the amount of debris present in the sample.

To a 1 mL concentrate add 5 to 6 mLs Lugol's Iodine and to a 5 mL sample add 10 to 15 mls Iodine. Mix the sample thoroughly and remove a 0.050 mL aliquot via micropipette. Place in a vaseline well, affix coverslip, and scan entire slip at 400x. Note the characteristics of the debris present (protozoa, amorphous, fungal bodies, etc.) and count the number of cysts if any. If cysts are seen a minimum of two aliquots are counted and averaged.

To calculate the number of cysts present in the entire sample the number is multiplied by its corresponding dilution factor, i.e.

a 1 mL concentrate is multiplied by 20

a 5 mL concentrate is multiplied by 100

and a 10 mL concentrate is multiplied by 200

All results are recorded and reported on the standard forms, e.g. Figure J-1. Information which must be included is: date, information included on the sample label, initials of the analyst, counts of duplicate sample readings, final cyst number reported and the observations of the debris appearance.

Reagents and Supplies

Lugol Iodine

1000 mL warm distilled water
100 gm Potassium Iodine
50 gm Iodine

Mix till Iodine crystals are in solution. Store in dark bottle light will deactivate the solution.

$ZnSO_4$ Solution

2-3 gallons distilled water
3 kg or 1-6.6 lb jar of $ZnSO_4$ crystals
Mix till crystals are in solution, place hydrometer into solution to read specific gravity. Keep adding $ZnSO_4$ till a specific gravity of 1.18 or 1.20 is reached. Store in one gallon glass jars.

Coverslips

VWR Micro cover glasses 1 ounce
Cat No. 48366-227
22 x 22 mm No. 1 1/2

Slides

Scientific Products Micro Slides
Plain Pre-cleaned
1.2 mm thick Size 3 x 1 inch
Cat No. M6130

Micro-pipette Tips

Micro-selectapette pipette tips
Siliconized - For - micro - pipetting
50-75-100 µL 250 pipettes
Clay Adams Re-order No. 4711
Cat No. 53517-423 VWR

GIARDIA QUANTITATION

Filtration System_____

Run Information (sample label)	Analysis Date	Am't. Conc. in Sample	Analysis by	Counts of Replicates	Cyst # Reported	Observations and Comments

Figure J-1. Giardia analysis record form.

MEMBRANE RECOVERY EFFICIENCY

Giardia cysts sampling of filter influent and of filter effluent streams were obtained by the use of 5 micrometer pore size Nucleopore R polycarbonate membrane filters. The filters used with the laboratory-scale pilot plant were 142 mm diameter, while 293 mm diameter filters were used wth the field-scale pilot plant. Questions about the recovery efficiency of this technique was addressed in brief experiments and is described here.

Recovery Efficiency of 5 µm Pore Size, 142 mm Polycarbonate Membrane Filters

Several tests were conducted by Dr. Hibler to determine the Giardia cyst recovery efficiency of the membrane filters. Table J-1 summarizes the test results.

The tests were conducted to determine if there was a difference in recovery resulting from different cyst source or resulting from different sampling techniques. Tests 1 and 2 in Table J-1 compared different cyst sources and Tests 2 and 3 compared differences in sampling techniques, i.e., pumping the sample through the membrane filter or sucking the sample through.

These results demonstrate that the variation in recovery of Giardia cysts is a function of the cysts and not the sampling techniques. Test 1 results range from 36 to 54 percent and average 44 percent. Test 2 results (using a different source of cysts) produced recovery results ranging from 74 to 89 percent and averaged 79 percent. This demonstrated the marked effect the cyst source has on recovery. Comparing Test 2 at 79 percent recovery to Test 3 at 81 percent recovery demonstrates the minor variation caused by different sampling techniques.

Tests which complement these results are those which are performed routinely on the filter feed tank during Giardia cyst test runs. Table J-2 summarizes the recovery information developed during the slow sand filter tests (see Bellamy, et al. 1984). Each of these tests represents a different cyst source. Again large variations in recovery, i.e., 18 to 80 percent result when different cysts sources were tested, thus confirming the dependence of recovery on the "state" of the cyst.

The "state" of the cyst and its resultant behavior during the sampling procedure is probably dependent on a number of factors. But, based on our observations and Dr. Hibler's experience, the two most apparent factors are: 1) the source of the cyst, and 2) the age of the cyst.

Based on these results it became apparent that the membrane recovery factor should be determined for each test run and that an average recovery for all test runs should not be used. When a membrane recovery factor for a particular run cannot be calculated, e.g., no influent sample was taken, an average from similar tests has been used.

Table J-1. Cyst concentration by membrane filter sampling compared cyst concentration in source tank as determined by grab sample. Analysis by micropipette technique for both sampling methods. Tests conducted in laboratory of Dr. Charles Hibler, July 1982.

	Test Condition and Technique	Run Number	Cyst Concentration Based Upon Grab Sample of Tank (cysts/liter)	Cyst Concentration Resulting from Given Test Condition in the First Column (cysts/liter)	Recovery (%)
1.	Cysts concentrated with a pump and membrane filter (cyst batch 1)	1	3,333	1,200	36
		2	3,333	1,800	54
		3	3,333	1,500	45
		4	3,333	1,300	39
		5	3,333	1,550	46
		Average	3,333	1,470	4
2.	Cysts concentrated with a pump and membrane filter (cyst batch 2)	1	35,00	25,000	71
		2	35,000	30,000	86
		3	35,000	27,000	77
		4	35,000	26,000	74
		5	35,000	31,000	89
		Average	35,000	27,800	79
3.	Cysts concentrated with a membrane filter and vacuum, i.e., no pump (cyst batch 2)	1	35,000	26,000	74
		2	35,000	31,000	89
		3	35,000	28,000	80
		Average	35,000	28,300	81

Table J-2. Comparison of cyst recovered by sampling milk cooler feed tank water using membrane filters with cyst concentrations in tank as computed after adding cyst concentrate suspension. Analyses performed during slow sand filter experiments.

Slow Sand Filter Run Number	Cyst Concentration[1] in Filter Feed Tank	Cyst Concentration Determined by Subsampling the Filter Feed Tank with a Membrane Filter[2]	Membrane Filter Percent Recovery
48	420	196.8	46.8
60	500	399.3	79.9
66	50	35.8	71.7
69	50	31.3	62.6
75	50	15.9	31.8
81	50	32.2	64.3
87	1,000	183.8	18.4
90	1,000	221.0	22.1

[1]Each of these results are the average of 3 to 6 analyses performed on a cyst concentrate of liquefied dog feces which is added to the filter feed tank on a batch basis. Cyst concentraton equals a number of cysts in concentrate suspension divided by volume of water in tank.

[2]Each of these results are the average of 4 to 11 analyses. The samples are concentrated with a membrane filter.

The mathematical determination of the membrane recovery factor is:

100 x (detected cyst conc.)/(known ("added") cyst conc.)

The known ("added") cyst concentration is determined by analyzing a cyst concentration, i.e., liquified dog feces, numerous times, then adding the concentrate to the batch filter feed tank. The concentration in the tank is then calculated by the appropriate dilution factor. The "detected" cyst concentration is then determined by analyzing a sample from the filter feed tank. This sample is concentrated with a membrane filter thus allowing for the membrane recovery calculation. The membrane filter recovery efficiencies given in Table J-2 were determined this way.

Passage of Cyst Through Membrane Filter

A 5-liter glass container was filled with 4 liters of water. The water was cooled to 5°C and dog feces containing Giardia cysts were added to the container. The feces added contained a sufficient number of cysts to bring the cyst concentration up to 2500 c/L. One liter of the mixture was then filtered through a 5 µm pore size polycarbonate membrane filter. The filtrate was collected in two 500 mL flasks.

There were no Giardia cysts found in either of the filtrate flasks. The entire sample volume was concentrated and analyzed by zinc flotation.

Retention of Cysts on Surface of Membrane Filter After Washing

A portion of the membrane filter used in the above experiment, approximately the area of one cover slip, was examined microscopically after it had been washed; no cysts were seen on the membrane filter. A similar but more in-depth examination was performed by Luchtel et al. (1980). This analysis also showed that very few cysts were retained by the membrane filter after washing.

DETECTION LIMIT DETERMINATIONS

There are two detection limit calculations used for this experimentation: 1) for each individual micropipette analyses, and 2) for an average detection limit when numerous samples are being considered. Each of these methods are discussed below.

Micropipette Detection Limit

The micropipette method of analysis begins by concentrating a sample to one milliliter. In other words all the cysts present in the sample are concentrated to the 1 mL volume. A 0.05 mL (1/20 mL) aliquot is then taken and microscopically examined. This means that if there is only one cyst present in the 1 mL sample concentrate, there is 1/20 chance that it will be withdrawn in the 0.05 mL aliquot. This accounts for the first detection limit factor of: (20)/(Number of aliquots examined). In other words, 20 cysts must be present in the 1 mL, and uniformly distributed, to be sure that

one cyst will be withdrawn by one aliquot. If the sample filtered is 100 liters, the detection limit is 20/100, or 0.20 cysts/liter. The total detection limit for a sample on a per liter basis is then calculated by:

[(20)/Number of aliquots]/[(Fractional membrane filter recovery efficiency) (liters of sample concentrated)]

This equation accounts for the analysis dilution, the membrane filter recovery, and the size of sample. For example:

Sample size = 100 liters
Membrane recovery efficiency = 45%
One aliquot analyzed
Detection limit = [(20/1)]/[(0.45) (100)] = 0.44 cyst/liter

Zinc Sulfate Detection Limit

The zinc float method of analysis is characterized by microscopically examining the entire sample for Giardia cysts. There is no dilution factor associated with this analysis technique. It did become apparent when comparing this technique to the micropipette technique that it resulted in cyst counts of approximately 20 percent less than the micropipette method. Consequently, the detection limit for zinc float analyses on a per liter basis is calculated by:

(1)/[(0.80) (Fractional Membrane Recovery) (liters of sample conc.)]

This calculation accounts for the 80 percent recovery by zinc flotation, the membrane filter recovery and the size of sample. For example

Sample size = 50 liters
Membrane recovery = 35%
Detection limit = 1/[(0.80) (0.35) (50)] = 0.07 cysts/liter

Average Detection Limit

The average detection limit is used when more than one analysis has been performed for a test run. Rather than physically combining all of the samples into one container and performing one analysis, each sample was analyzed separately and then the results were mathematically combined. This leads to slightly different results but both results are valid. The mathematical approach requires an averaging of detection limits since individual detection limits are not suitable for multiple analyses of the same source. For example, a single source of water is analyzed 100 times for coliforms and none are found in any of the 100 mL samples. The true test accuracy is not demonstrated by reporting the individual test detection limits, i.e. that the source has less than one coliform per 100 mL, when in fact 10 liters of sample were analyzed and no coliforms were found.

The individual detection limit for Giardia analyses is based on the probability of finding one cyst. This can be understood by envisioning the analysis of a thousand 1 mL samples, each having one cyst in them. If one 0.05 mL aliquot is taken from each sample and examined it will be determined, after completing all of the analyses, that there is a one in twenty chance of finding a cyst. The detection limit for each analyses was 20/1 mL or the inverse of the probability of finding one cyst, i.e. 1/20. This factor of 20 is the multiplication factor already discussed.

Since the detection limits are the inverse of the probabilities of finding a cyst it is then appropriate to apply probability calculations to multiply analyses when determining the combined detection limit. The following calculations describe the analysis:

P = Probability of finding one cyst

N = Number of tests

(1-P) = Probability of not finding a cyst

$(1-P)^N$ = Probability of not finding a cyst in N samples

$1-(1-P)^N$ = Probability of finding a cyst in N samples

$1/[1-(1-P)^N]$ = Detection limit for N tests, i.e. inverse of probability of finding a cyst

For example, assume 5 samples were collected with an average membrane recovery factor of 50 percent and that each sample was concentrated from 10 liters.

Individual detection limits = (20 cysts/1 aliquot)/(0.5, membrane recovery factor) = 40 cysts
(only one aliquot was analyzed)

Individual probability of detecting one cysts = 1/40

Average detection limit for the 5 tests = $1/[1-(1-1/40)^5]$
= 8.41 cysts

Average detection limit per liter = 8.41/10 = 0.841 cysts/liter

As an alternative the 5 samples in the above example could have been physically combined and the detection limit would have been:

Individual detection limit = (20/1)/0.5 = 40 cysts
(only one aliquot was analyzed)

Individual detection limit per liter = 40 cysts/50 liter
= 0.8 cysts/liter

This result, as expected, is somewhat lower than the mathematical combination, but for each technique the detection limit is valid.

Detection limits in this report can be for individual analyses or an average for a test run; each is applied to its specific case. An average detection limit is not applied to an individual analysis.

Conclusions

The counting and sampling experiments conducted in July, August and September of 1982 by Dr. C. Hibler, established that the micropipette technique is the most suitable technique for this work. Different samples from the same suspension, different replicates of the sample, and three persons counting resulted in a maximum difference between any two counts of only about fifteen percent. Although there is no suspension of known cyst concentration to use for a standard, it is believed that the counts by this technique represent the Giardia cyst population in the sample counted.

On sampling efficiency, the use of the 5 µm pore size, 142 mm polycarbonate membrane filter represents the best state-of-the-art on sampling at this time. Sampling efficiency of the pump membrane filter system was determined to be primarily dependent on the source and age of cysts being used for a particular experiment. This discovery resulted in the determination of a cyst recovery factor from the membrane filters on a test run by test basis.

GIARDIA QUANTIFICATION TRIALS

This section describes the preliminary Giardia analytical evaluations performed by Charles P. Hibler, Consetta M. Helmick and Donna G. Howell. The purpose was to develop an accurate and reliable means for quantifying cysts of Giardia.

Introduction

Accurate quantification for eggs, larvae and cysts of parasitic organisms is extremely difficult because parasites do not produce eggs and larvae continuously, nor do the cyst-producing forms of protozoan parasites encyst (or produce cysts, depending on the species) on a continuous basis. For example, examination of numerous dogs clinically infected with giardiasis has revealed that cyst production (cysts in feces) may vary from extremely low numbers in a fecal sample taken in the morning, to extremely high numbers in a sample taken at noon: results are inconsistent and vary from hour to hour and day to day. Diarrhea causes dilution, resulting in inaccuracy by some techniques, and compaction also results in inaccuracy. Moreover, cyst numbers in one portion of the fecal mass often are much higher (or lower) than those in another portion. Thus, unlike bacteria which frequently continue to multiply as they pass the digestive system, and can be cultured to obtain accurate counts, uniform mixing of eggs, larvae or cysts does not

occur; nor does multiplication occur in the intestinal contents. Quantification of eggs, larvae and cysts necessitates visualization of the organism. Needless to say, experience is a factor, fatigue is a factor, and technique is a factor. If thorough mixing does not occur, or if sampling techniques are poor, inconsistent, or sloppy, the end result is highly variable data.

Parasitologists use two types of techniques for reporting presence or absence of parasites in blood, urine or stool specimens: (1) qualitative, and (2) quantitative. The reason for both qualitative and quantitative techniques is that the presence of parasites in pets and/or humans indicates treatment is necessary; however, since most domestic ruminants and horses, as well as wild species, harbor a few parasites, economics enter into the decision. Irrespective of the technique used, the factors given in the preceding paragraph must be taken into consideration. Moreover, qualifications and experience of the parasitologist in diagnosing parasitism and recognition of a given technique's limitations are additional factors to be considered. Although the author has had 20 years experience diagnosing all of the known types of parasites of man and animals, I will confine my remarks to techniques employed for Giardia.

Qualitative techniques (hopefully) reveal to the parasitologist the presence or absence of parasitic infection. There are a number of qualitative techniques purported to be effective in diagnosing Giardia; however, the actual number of techniqes suitable for this purpose are limited. The direct smear, the Willis technique (and a myriad of modifications on the market, most of which are made to sell rather than diagnose), the formalin-ether sedimentation technique (formalin-ethyl acetate), and the $ZnSO_4$ centrifugal-flotation techniques are those generally employed.

The direct smear, although with obvious limitations, does have a place and can be effectively used by experienced parasitologists, especially when seeking cysts or trophozoites from clinical cases of parasitism if the sample is extremely fresh. Moreover, since some parasitic organisms do not encyst and are too fragile for any flotation technique, (Trichomonas), it is the only means available. The direct smear should not, when negative, be used as the only diagnostic criterion when seeking cysts, eggs, or larvae.

The Willis technique and its many modifications (Fecalyzer, Ovassay, etc.) employ $MgSO_4$, NaCl, $NaNO_3$, or sucrose. These generally are concentrated to a specific gravity of 1.20 to 1.30 (depending upon the chemical) to "float" eggs, larvae and cysts to the surface of a vial, centrifuge tube, etc. They often are allowed to attach to a microscope slide, a coverslip or, alternatively a bacterial loop is used to sample for the presence of organisms in the meniscus. Unfortunately, the chemicals generally used will destroy the fragile cysts of the species of Giardia found in most of the animals of interest (dog, cat, man, beaver, muskrat, etc.). Even if the specific gravity is reduced to 1.17 or 1.18, the great majority of cysts are destroyed. However, if the "overlay" technique is used with sucrose at a specific gravity of 1.13 then cyst destruction in minimal.

The formalin-ethyl acetate sedimentation technique is widely employed by medical technologists, using the excuse that since schistosome eggs are too heavy to float, a sedimentation technique must be employed in the interest of accuracy. The author is not aware of any cases of schistosomiasis occurring among native Americans who have not left the United States; the parasite does not occur in the contiguous 48 states. An advantage of formalin-ethyl acetate is that preservation maintains cysts, eggs, larvae and trophozoites of parasites for extended periods of time, facilitating shipment to a laboratory for diagnosis. Two disadvantages are inherent: (1) The technique does not selectively concentrate anything, for 90 percent of the material placed in the centrifuge tube is present in the centrifugate; and (2) Cysts of Giardia will not maintain for more than two weeks in formalin. Thereafter they disappear (cysts probably rupture, although reason says they should not do so in this preservative). Therefore, it must be considered that if only a few cysts are present they would be difficult to find in the centrifugate, due either to the lack of selective concentration or because they possibly will have ruptured before examination was initiated. Nevertheless, in the hands of an experienced parasitologist this is a valuable tool; time consuming, but valuable.

Experienced parasitologists working with Giardia have stated, on numerous occasions and in a considerable number of publications, that the $ZnSO_4$ centrifugal-flotation technique is the only reliable concentration technique available for qualitative examination of stool samples. $ZnSO_4$ is used as a specific gravity of 1.18 when examining fresh (unpreserved) samples and at a specific gravity of 1.20 when examining formalin-fixed specimens. The advantages of $ZnSO_4$ are that you obtain a selective concentration of cysts at the meniscus (or on a coverslip). A disadvantage is that some cysts are trapped in the fecal mass, or do not attach to the coverslip. This is not a severe disadvantage and does not effect reliability of the method. Another disadvantage is that formalin-fixed cysts (if examined before they rupture) are heavier (more dense?) than fresh cysts and do not float as well. Recently, we have discovered a third disadvantage: when cysts are maintained in water for extended periods of time (several weeks) (is this a mature cyst?), while viable and infective, either rupture in $ZnSO_4$, or (as with formalin) become heavier and fail to float well. Possibly the cyst becomes more fragile, even when maintained in water at $5^{\circ}C$, over an extended period of time. However, in the authors (limited) experience, they simply do not float, indicating that density has increased. Mixing fresh stool specimens with water (highly diluted specimen), centrifuging and removing the supernatant, followed by $ZnSO_4$ centrifugal flotation, gives a more accurate estimation of the number of cysts (in this case, per milliliter of suspension) than the examination of the formed stool specimen - providing the specimen is fresh and providing the coverslip is not "greasy." Coverslips marketed by some manufacturers are "greasy" and cysts, eggs, etc., do not attach well. Even though this technique (diluting the specimen with water) approaches quantitative procedures (essentially identical with the modified Stoll technique) it is at best 80 percent accurate in the hands of an experienced parasitologist and much less accurate when done by inexperienced parasitologists who are not aware of the limitations of the technique.

The $ZnSO_4$ centrifugal-flotation technique, although admittedly qualitative, and not accurate, provides the only means of selective separation of cysts from the dirt, debris, plant material, algae, diatoms, pollen, nematode eggs and larvae, crustaceans and their eggs, arthropod parts, and the myriads of protozoa, etc., found in surface waters that are sources of domestic supply. Currently this is the only means to determine if Giardia cysts are present, and in what relative numbers, in raw water or finished water. When the concentration of cysts is very low (often 1-5 cysts/gallon) any other analytical system currently available would be like looking for the proverbial needle in a haystack! Indeed, in the examination of raw or finished water obtained during epidemics of giardiasis, any procedure other than the selective concentration technique would be an exercise in futility because when you have 8, 16, or 32 water filters to be examined and the Department of Health begging for results, there are not enough experienced people in the United States to provide the answer. Therefore, the state-of-the-art in the real world is that in surface water there are myriads of organisms, together with Giardia and selective concentration is the only reliable means of separation of the cysts from other organisms. In fact, this investigator could care less whether Giardia is or is not present: if organisms the size of (or larger than) Giardia are present in the raw water, and these same organisms (in about the same quantity) are present in the finished water, then the system is at risk. The $ZnSO_4$ centrifugal-flotation technique is an ideal and very quick method to tell the Department of Health and/or water treatment operator that their filtration and/or treatment system is not removing particulate matter the size of or larger than Giardia. Therefore, the system is at risk if Giardia is introduced into the water supply.

Parasitologists have used, from time to time, a number of quantitative techniques: (1) McMaster Counting Chamber; (2) Whitlock Paracytometer; (3) Stoll Dilution Technique; and (4) Modified Stoll Dilution Technique for the recovery of eggs, larvae, and cysts. Recently investigators working with Giardia have initiated the use of the hemacytometer as well as the direct counting procedure (use of a calibrated micropipette). All of these procedures are simply modifications of similar techniques. All have advantages (depending on the parasite and the host) and all have disadvantages: inaccurate mixing, inaccurate sampling, inexperienced parasitologists, and inexperience with the parasite the individual is counting can result in highly variable data. An excellent example is Giardia. In any given sample some of the cysts are fresh and "plump," the morphology is excellent; some of the cysts are not fresh, and are not "plump," rather they are distorted (dying or dead). Over time (a short period of time!) a dead or dying cyst may not be recognized by the parasitologist. When using a quantitative (dilution) technique, irrespective of what has been diluted (erythrocytes, leukocytes, or Giardia cysts), missing a few can result in highly variable data. Moreover, since Giardia cysts are not readily visualized without some form of stain (e.g., Lugol's Iodine), then overstaining, understaining, or the lack of experience necessary to realize that live, dying, and dead cysts all stain differently will result in variable data.

The technique developed by Stoll requires the use of a special (Erlenmeyer) flask marked at 56 milliliters and at 60 milliliters. Fluid (generally water) is added to the 56 milliliter mark and sufficient fecal matter added (determined by Stoll to be 2 grams) to bring the material to the 60 milliliter mark. This is thoroughly shaken and a 0.075 or a 0.10 milliliter sample removed, the eggs, larvae or cysts counted and multiplied by 200 or 100, respectively, to obtain organisms/gram. Since the Stoll technique is essentially a 1:15 dilution, addition of 4 milliliters of suspenson provides the parasitologist with the number of organisms/milliliter. A modification of this technique is to mix the sample in the flask with a solution of high specific gravity ($MgSO_4$, NaCl, etc.), mix, remove a specific amount, add to a centrifuge tube, affix a coverslip and centrifuge. The coverslip is removed and the organisms counted. The problems inherent in the modification of the Stoll technique are the same as the $ZnSO_4$ centrigual flotation technique.

The McMaster Counting Chamber, and the Whitlock Paracytometer are specially manufactured slides containing coverslips (calibrated) permanently affixed. The Chamber (or well) for these techniques (similar to the hemocytometer) are constructed to hold a specific volume of fluid (such as the chemicals of high specific gravity previously mentioned). The specimen is mixed with the fluid, pipetted into the Chamber, and the organisms allowed to float. The disadvantages are that they might not float or, as is the case with Giardia, flotation occurs very slowly. Those that float immediately (being closer to the surface) distort and become unrecognizable, only to sink before the remainder have floated. Therefore, the disadvantages of these two techniques make them essentially of no value for counting Giardia and will not be considered further.

While the hemacytometer appears to be a likely candidate for counting Giardia cysts, and will be evaluated, there are several inherent disadvantages that perhaps bear discussion at this point. The hemacytometer was designed for counting blood cells; consequently, the volume of liquid held in the chamber is extremely small. If the number of cysts in the sample are not in sufficient concentration (such as might be anticipated with erythrocytes and/or leukocytes) to provide accurate results, the end result is highly variable data. Moreover, in the authors experience, most parasitologists do not realize that the cysts of Giardia are quite heavy. The simple act of mixing a diluted sample, pipetting this sample, and then transferring a small portion to the hemacytometer chamber generally results in inordinately high counts because the cysts settle just enough to affect the results. Moreover, considering once again the volume of the chamber, extremely low cyst numbers result in inordinately low counts.

Since quantitative procedures have not been developed or evaluated for Giardia, and because evaluation of experimental filtration and/or treatment systems for removal of Giardia necessitate reliable and accurate counting procedures, the purpose of this experiment is to develop accurate procedures and evaluate their reliability when performed by experienced parasitologists. Since all quantitative procedures offering any hope of accuracy and

reliability are dilution procedures, the techniques to be evaluated are: (1) Stoll Technique; (2) Micropipette Technique; and (3) Hemacytometer Technique.

Materials and Methods

Source of Giardia--

A large amount of feces was collected from four dogs, each with clinical giardiasis, and the fecal matter mixed with distilled water. The water level was adjusted to 2 liters of suspension. This was then refrigerated at 5°C.

Holding Vat--

A 40 liter tank was filled to the 38 liter level with tap water, the chlorine allowed to evaporate for 1 day, and the vat then refrigerated until the water temperature became 5°C.

Techniques--

As indicated in the introductory remarks, all of the techniques to be evaluated are essentially dilution techniques, irrespective of their name. Since Giardia cysts must be stained to facilitate visualization, Lugol's Iodine was used for this purpose. Needless to say, this necessitated a considerable amount of emperical experimentation to determine the best procedure for staining cysts without interfering with accuracy, reliability, or causing undue distortion, overstaining, or understaining that would likewise affect accuracy and reliablity. Ultimately it was determined that a concentrated solution of Lugol's Iodine (parasitologists use many different modifications of this stain) must be added prior to any attempt at counting.

For any given suspension of material another factor needed to be considered: the amount of organic material present which could interfer with accuracy. If too much organic debris is present, and too many cysts per field, microscope fatigue quickly intervenes resulting in inaccuracies affecting the reliability.

Micropipette--

A calibrated micropipette manufactured by Clay Adams, with calibrations of 0.05, 0.075 ad 0.10 milliliters (Silicone coated glass pipettes #4711) here used throughout for both micropipette (MP) and Stoll dilution technique (SD).

With the MP technique, one milliliter samples were obtained after very thorough mixing, concentrated iodine added, and a 0.05 mL sample withdrawn. This sample was introduced into a vaseline well, a coverslip affixed, and the counts performed. The number of cysts counted, for the 1 mL sample, was multiplied by 20 to obtain cysts/mL.

If the amount of organic debris, and the number of cysts, was too concentrated for accuracy, an additional 4 mL of water was added, making a 1:5 dilution. A 0.05 mL sample removed (as before) and the number of cyst multiplied by 100 to obtain cysts/mL. If the material was still too dense, the sample was diluted to 1:10, a 0.05 mL sample removed and the number of cysts multiplied by 200 to obtain cysts/mL. If further dilution was

necessary, either the SD technique (see below) was employed or the original suspension was diluted.

Stoll Dilution Technique--

Iodine was added to a Stoll flask (in about the same proportion as with the MP technique) and the flask filled to the 56 mL mark with distilled water. To this was added enough suspension (4 mL) to bring the volume to 60 mL. This was thoroughly shaken, and a 0.075 mL sample removed, introduced into a vaseline well, a coverslip affixed and the number of cysts counted multiplied by 200 to obtain cysts/mL.

Initial Counting--

The 2 liter suspension of fecal material was counted by the MP and SD techniques before addition to the 38 liters of water. The results were (expressed is cysts/mL):

	MP (1:10 dilution)	SD (1:15 dilution)
	24,600	24,400
	25,000	25,200
	25,400	25,400
Average	25,000	25,000

The 2 liters of suspension, when added to the 38 liters of water (1:20 dilution) should provide 50,000,000 cysts in 40,000 mL of water or 1,250 cysts/mL. After addition of the 2 liters of suspension, the vat was maintained at 5°C. Before removing samples for analysis the vat was thoroughly stirred with a large, flat paddle (12 inches wide) for 1.5-2.0 minutes. A 1 mL sample was obtained for use with the MP technique, and a 4 mL sample for the SD technique. Counting of cysts were performed at 450x for evaluation.

Results

Micropipette--

Counting by the MP technique was performed at 1:1 and 1:5. All counting was performed at 450x. Two (replicates) readings were performed on each sample. The results with 1:1 dilution are tabulated.

Comments--

Direct MP counting at 1:1 is an extremely effective, but very time consuming procedure, primarily because of the amount of organic material present. With some water, which contains a considerable amount of algae, diatoms, pollen, protozoa, etc., it is even more time consuming and requires about 1 hour to do a thorough count. An individual can count about 3 samples before the fatigue factor causes data to become variable.

Table J-3. Giardia cysts counted by three individuals using the micropipette technique at 1:1 dilution.

	Individual #1	Individual #2	Individual #3
Sample 1	1180	1140	640
Rep	1180	1160	1680
Sample 2	1240	1120	1100
Rep	1240	1160	1080
Sample 3	1300	1160	1000
Rep	1160	1100	940
Sample 4	1340	1160	1000
Rep	1160	1120	1140
Sample 5	1320	Average 1140	Average 1073
Rep	1180		
Average	1230		

Table J-4. Giardia cysts counted by three individuals using the micropipette technique at 1:5 dilution.

	Individual #1	Individual #2	Individual #3
Sample #1			
Replicate	1300	1000	1100
Replicate	1100	1200	900
Replicate	1200	1400	1100
Replicate	1400	1100	1000
Average	1250	1175	1025
Sample #2			
Replicate	1200	1000	1400
Replicate	1400	1100	1200
Replicate	1100	1200	1200
Replicate	1100	1400	1300
Average	1200	1200	1250
Sample #3			
Replicate	1500	1200	1400
Replicate	1100	1100	1100
Replicate	1100	1300	1400
Replicate	1200	1400	1400
Average	1225	1250	1325
Sample #4			
Replicate	1200	1200	
Replicate	1200	1100	
Replicate	1400	1300	
Replicate	1100	1100	
Average	1225	1175	

Dilution of the sample to 1:5 resulted in less debris, required less time and the fatigue factor was lower.

Individuals #1 and #2 are more experienced than individuals #3; moreover, #3 had another assignment and often several days would pass between the examination of samples. She commented that becoming accustomed to looking for cysts after a 2-3 days break from the routine was difficult.

The results, with 1:1 or 1:5, are extremely consistent. As might be expected, there appears to be as much variation between individuals as within individuals.

When very little organic debris was present, counting at 100x was possible, however, the individuals discovered that cysts were often missed causing the data to be variable. A recount at 450x in variable resuled in more accuracy.

Stoll Dilution--

This is essentially the same techniques as the MP, but the dilution factor is 1:15, a rather dilute solution of material.

Comments--

Counting cysts by the SD technique is not as fatiguing, nor is it as time consuming as the MP technique, primarily because of the dilution factor of 1:15; however, accuracy was dependent upon a sufficient number of replicates to obtain a good average. As to be expected, considerable variation occurred within and between individuals. Subsequent trials by the author, reducing the number of cysts (further dilution of the vat sample to an estimated 625 cysts/mL) resulted in even more variation; however, concentration of the vat sample to 2500 cysts/mL indicated that variation in counts was reduced. Tests (replicates) similar to those above were not performed, but it stands to reason that increasing the number of cysts (up to a point!) would increase sampling accuracy, while further dilution would decrease the accuracy.

Considerable time (2 weeks) had elapsed before individual #3 completed her sample #3.

Table J-5. *Giardia* cysts counted by three individuals using the Stoll technique.

Individual #1	Individual #2	Individual #3	
Sample #1			
Replicate	1200	1400	1800
Replicate		1800	1200
Replicate	800	1400	1600
Replicate	2200	1800	
Replicate	1000	1400	1600
Average	1300	1560	1550
Sample #2			
Replicate	1000	1400	1800
Replicate	1600	1200	1200
Replicate	1400	1400	1200
Replicate	1400	1400	1200
Replicate	0800	1400	1000
Average	1240	1360	1280
Sample #3			
Replicate	1400	1600	1000
Replicate	2200	1800	1000
Replicate	800	1400	1000
Replicate	1000	1200	1000
Replicate	1000	1200	1200
Average	1280	1440	1040

*Cysts were old and had begun to rupture or die.

Hemacytometer—

Initially a 1:5 dilution of the vat sample (250 cysts/ml) was examined with the hemacytometer; however, after repeated samples, no cysts were obtained; therefore, a direct examination of the vat sample (1250 cysts/ml) was attemped, using both stained and unstained cysts. The results were: 0, 0, 0, 0, 1 cysts, 0, 0, 2 cysts, 0, 1 cyst, 0, 0, 0, 0, 1 cysts, 0.

Comments—

The hemacytometer holds a very small volume of liquid. Dilution of a sample to 1000-2000 cysts/ml is far too dilute for any semblance of accuracy. However, if the sample is more concentrated (to what extent I do not know but I suspect in the neighborhood of 10,000-20,000 cysts/ml) no doubt accuracy will increase. Nevertheless, I question the accuracy of the hemacytometer under any circumstances unless the investigator is using extremely clean and/or highly concentrated numbers of cysts. Moreover, it was observed that if cysts were withdrawn from the source, and not pipetted onto the chamber very quickly, the cysts tended to settle to the tip of the pipette. When the small volume is considered, together with the high multiplication factor, this would result in inordinately high numbers of cysts/ml.

Quantification After Passing the Push-Pull Pump and the Nucleopore Membrane--

Evaluation of data on filtration runs indicates a 60 percent loss of cysts, either in the counting accuracy, the vat, the pump, the membrane filter, or during the processing. Therefore, a vat, containing approximately 38 liters of water was placed into a refrigerator at 5°C. A sufficient quantity of cysts were added to obtain approximately 2 cysts/ml. Results (see below) indicated 2.0 cysts/ml in the vat. A series of pump-filter examinations were made on this material. A total of 2 liters of material was pumped through the filters, the pads washed, and then examined at a 1:5 dilution by the MP technique.

The procedure, during trial runs, is to pump the material through the filter pad then aspirate (with vacuum) the liquid remaining in the filter canister on through the pad. Therefore, to determine where losses were occurring, some runs were aspirated, some were not. For one run, material was not pumped through the filter, but directly into a flask.

Number of cysts/ml in the Vat--

To determine the number of cysts/ml in the vat 20 ml samples (1 percent of the proposed filtration samples-2 liters) were examined. The 20 ml samples were concentrated to 1 ml and examined by the MP technique.

The results are given below:

	Total Cysts Recovered	
	Sample #1	Sample #2
Replicate	3	3
Replicate	3	0
Replicate	2	1
Replicate	200	200
Average	2.5	1.5

For a 1:1 MP the correction factor is X20; therefore in Sample #1, the average is 50 cysts in 20 ml or 2.5 cysts/ml and in Sample #2 is 30 cysts in 20 ml or 1.5 cysts/ml. This indicates an average of 2.0 cysts/ml. However, the individual reading sample #2 (CPH) had many interruptions and he (CPH) questions the accuracy of his results. The results indicate 4000-5000 cysts should be present in 2 liters. Two to 2.5 cysts/ml, with 20 ml subsamples, is far too dilute to obtain greater accuracy.

Results Through the Pump and Filter--

A total of 2 liters was passed through the pump at each run. After each filter run, a 20 ml subsample was taken for examination. All counting for the filter-pump trials was done at 1:5 dilution by the MP technique; all counting for the 20 ml subsamples was done at 1:1 by the MP technique. The results are expressed as total cysts present in the sample. The average and percent recovery is also given. Percent recovery was determined, at the time of sampling, by the subsample count using the micropipette technique (Table J-6).

Table J-6. Giardia cysts recovered after passage through the push-pull pump and the filter.

Run #	Procedure	2 liter sample				20 ml subsample			
		#1 Repli-cate	#2 Repli-cate	Average	(%)	#1 Repli-cate	#2 Repli-cate	#3 Repli-cate	Ave
1	Aspirated	1200	1200	1200	36%	4000	4000	2000	3333
2	Aspirated	1800	1800	1800	54%	2000	4000	4000	3333
3	Not Aspirated	1600	1400	1500	45%	4000	2000	4000	3333
4	Not Aspirated	1200	1400	1300	39%	0	000	6000	3333
5	Aspirated	1700	1400	1550	46%	2000	4000	4000	3333
6	Not Filtered	1700	1500	1600	48%	0	6000	4000	3333

Comments--

These results indicate that 3,333 cysts should have been recovered from the 2 liter filter samples. Recovery percentages ranged from 36-54 percent (Ave: 45 percent). Losses no doubt occur in all steps of the operation as a result of the pump, filter and the subsequent centrifugation procedures to concentrate the filter washings.

A series of 50 ml subsamples were obtained subsequent to the filter runs and concentrated to 1:1 and read by the MP technique. The results were:

Sample Size	#1	#2	#3	#4	#5	Ave
50 ml	100	80	60	80	60	76

An average of 76 cysts in 50 ml = 152 cysts/100 ml or 1.52 cysts/ml, which extrapolates to 3040 cysts in 2 liters. The original subsamples, taken 2 days earlier, indicated there should be 3,333 cysts in 2 liters.

The cysts, when introduced into the vat had been maintained 5 weeks and were excellent, morphologically. However, the results from this sample of cysts indicated that losses due to death and dissolution of cysts was occurring very rapidly. For example, counts on the day the vat was charged indicated 2-2.5 cysts/ml. The next day results were 1.67 cysts/ml. Two days later the results indicated 1.52 cysts/ml. Microscopic examination indicated they were dying and/or dead: no doubt many ruptured before processing was initiated and, as might be anticipated, processing through the pump and filter, followed by centrifugation, destroyed many more. It is reasonable to assume that dying/dead cysts are more fragile than fresh cysts. Yet there is little way to predict when cysts are going to die, for this seems to vary between individual samples. We have observed (over the past 12 years) that some Giardia samples will keep for weeks and be in excellent shape; others are gone and/or unrecognizable in less than a week. Maintenance in water increases their life span: some cysts will look excellent (morphologically) for 2 months a 5°C while others are beginning to deteriorate at 2-3 weeks. Some cysts in all samples are no doubt more fragile than others and processing will result in a certain amount of dissolution.

EFFECT OF PUMPING ON CYST RECOVERY

Objective

To determine if a loss and/or destruction of Giardia cysts occurs in the process of pumping cysts through the push-pull pump or the nucleopore filter, or if the loss occurs as a result of counting error.

Procedure

As in the preceding trial, a 10-gallon tank was filled with tap water, the chlorine allowed to evaporate and the water refrigerated to 5°C. The tank was then charged with 30 ml of dog feces containing 31,000 Giardia cysts/ml or 930,000 cysts (estimated).

The trials were initiated on 9/16/82. A total of five runs were made with the push-pull pump and the nucleopore filter, and one run with the push-pull pump, but no nucleopore filter. Three runs were made with the vacuum pump connected to the nucleopore filter and one run with the vacuum pump, but no nucleopore filter. As in the preceding trial, the number of Giardia cysts/ml was determined prior to the trial, and subsamples from the vat were taken during each trial run.

Results

The results are presented in tabular form. Table G-7 lists the cysts/ml determined prior to the runs, Table G-8 lists the results of the push-pull pump through the nucleopore filter, and Table G-9 lists the results using the vacuum pump to pull material through the nucleopore filter. At the end of all runs, a 2 liter grab sample was counted. The 2 liter sample was counted by the direct micropipette technique, 1:15 dilution. A total of 78,200 cysts were calculated to be present in 2 liters or 39 cysts/ml.

Table J-7. Giardia cysts/ml determined to be present in the tank prior to trial. Direct micropipette counts, 1:1 dilution of 1 cc samples.

	Total (cysts/ml)	Average (cysts/ml)
Sample 1	40	
Replicate	40	40
Sample 2	40	
Replicate	20	30
Sample 3	40	
Replicate	20	30
Sample 4	20	
Replicate	60	40
	Average: 35 cysts/ml	

Discussion

The results of this trial indicate that when fresh Giardia cysts are used together with the push-pull pump or the vacuum pump through the nucleopore filter, there is very little cyst loss and/or destruction if the personnel are conscientious in preparation, washing of the filter disc, and performing sufficient replicate counts. A comparison of the push-pull pump with the 50 ml grab samples indicates that results are consistent: an average of the six samples revealed 26.2 cysts/ml by the push-pull pump and 26.5 cysts/ml by the grab sample. Although some problems developed with the vacuum pump, an average of four samples revealed 23.5 cysts/ml by vacuum pump and 22.7 cysts/ml grab sample.

Unfortunately, examination of the material on this tank trial took an inordinate amount of time to complete, possibly resulting in a loss of cysts. It took approximately 2 to 2.5 hours to read each sample (22 samples) because of the large number of cysts, debris, and microscope fatigue. From the time the trial was initiated, samples taken, and samples analyzed, there was a 1

Table J-8. *Giardia* cysts/mL obtained following the push-pull pump and filtering through the nucleopore filter. All counts performed by direct micropipette, 1:5 dilution.

Run #	H_2O Passed	Pressure	Counts	Cysts/mL	50mL Grab Sample	
					Counts	Cysts/mL
1	2000 mL	10 lbs	50,700	25	1600	32
Rep.			49,800	25	1640	33
Rep.			49,500	25	1680	34
Rep.			50,300	25		
Avg.			50,050	25	1640	33
2	1500 mL	15 lbs	45,400	30	1100	22
Rep.			44,700	30	1200	24
Rep.			43,600	29	1120	22
Rep.			43,700	29		
Avg.			44,350	30	1140	23
3	1500 mL	15 lbs	40,200	27	1220	24
Rep.			39,500	26	1100	22
Rep.			39,800	27	1200	24
Rep.			40,500	27		
Avg.			40,000	27	1173	24
4	1500 mL	15 lbs	39,200	26	1400	28
Rep.			39,800	27	1560	31
Rep.			40,300	27	1500	30
Rep.			39,600	26		
Avg.			39,725	26	1486	30
5	1500 mL	20 lbs	45,700	30	1240	25
Rep.			45,500	30	1160	23
Rep.			45,700	30	1300	26
Rep.			46,000	31		
Avg.			45,725	31	1233	25
6*	2000 mL	0 lbs	35,800	18	1140	23
Rep.			40,200	20	1180	24
Rep.			28,200	14	1200	24
Rep.			37,200	19		
Avg.			35,350	18	1173	24

*Used push-pull pump but not the nucleopore filter

Table J-9. Giardia cysts/mL obtained using the vacuum pump to pull sample through the nucleopore filter. All counts performed by direct micropipette, 1:5 dilution.

Run #	H_2O Passed	Counts	Cysts/mL	50 mL Grab Sample	
				Counts	Cysts/mL
1	1200 mL	30,300	25	1040	
Rep.		33,400	28	1020	20
Rep.		30,400	25	1100	22
Rep.		31,600	26		
Averages		31,425	26	1053	21
2	1500 ml	31,100	21	1100	22
Rep.		30,400	25	1180	24
Rep.		30,800	21	1120	22
Rep.		31,500	21		
Averages		31,200	21	1133	23
3	1300 mL	26,500	20	1180	24
Rep.		29,000	22	1200	24
Rep.		27,800	21	1140	23
Rep.		29,500	23		
Averages		28,200	22	1173	24
4*	2000 mL	48,800	24	1100	22
Rep.		52,600	26	1160	23
Rep.		46,200	23	1120	22
Rep.		49,600	25		
Averages		49,300	25	1126	23

Note: Only 4 runs were made because of pump problems.
*No nucleopore filter, only the vacuum pump.

week to 10-day span which could account for cyst loss via death of cysts. Also centrifugation of samples could apply enough pressure to fragile cysts to cause rupturing and cyst loss. The entire trial took 80 working hours to accomplish, from start to finish. The pretrial count indicated 30-40 (Average 35) cysts/ml, and the 2 liter grab sample, analyzed shortly after the trial, indicated 39 cysts/ml; yet all counts after a 2-day lag were within the 23-33 cyst/ml range. This indicated that, over time, cyst loss was occurring. Possibly, this was due to increased fragility because of age of cysts or that a certain percentage of cysts are fragile initially. Nevertheless, cyst loss was consistent irrespective of the procedure.