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At 5°C, in nonbuffered purified water (reasonably analogous to ROWPU-treated water) at pH 9.0, 1 mg/L of free chlorine (FC) inactivated 99.99% of poliovirus 1 in 110 minutes and 5 mg/L of FC inactivated 99.99% of the virus in about 27 minutes. If tailing occurs (and it usually does) a margin of safety must be added to a treatment standard for this alone. And since there are undoubtedly enteric viruses more FC-resistant than the poliovirus used in these studies, the military treatment standard of 5 mg/L for 30 minutes at any temperature and any pH may be inadequate for ROWPU-treated water.

At 5°C, 526 mg/L of KCl (250 mg/L Cl<sup>-</sup>, a level far below the military's 600 mg/L permissible maximum for drinking water) potentiated the inactivation by FC at pH 9.0 of poliovirus 1 in nonbuffered purified water. In nonbuffered purified water, 526 mg/L of KCl increased the polioicidal effectiveness of FC by 700%. Under the same circumstances, 1,262 mg/L (600 mg/L Cl<sup>-</sup>, the maximum level acceptable by the military for drinking water)

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increased the poliocidal effectiveness of FC by 1400%.

At 5°C, poliovirus 1 was inactivated by FC at pH 9.0 more than 9 times more rapidly in nonbuffered tap water than in non-buffered purified water suggesting that KCl with or without other ions in tap water potentiated the virucidal effectiveness of FC.

Potassium chloride did not potentiate the poliocidal effectiveness of FC at pH 9.0 to nearly the degree in nonbuffered tap water that it did in non-buffered purified water suggesting that ions present in tap water potentiated the virucidal effectiveness of FC.

FC inactivated poliovirus 1 at pH 9.0 more than 3 times more rapidly in borate-buffered (0.05M) purified water than in nonbuffered purified water suggesting that the buffer ions potentiated the virucidal effectiveness of the FC. Clearly, disinfection studies should not be done in buffers without first determining the virucidal potentiating effect of the buffers.

Potassium chloride potentiated the virucidal effectiveness of FC in 0.05 M borate buffer to a lesser degree than in nonbuffered purified water further indicating that buffer ions potentiated the virucidal effectiveness of FC and that a maximum ion effect exists.

Potassium chloride, at a level 1, 262 mg/L, potentiated the virucidal effectiveness of FC at pH 4.5 in phthalate-buffered purified water, but to a lesser degree than it potentiated the virucidal effectiveness of FC at pH 9.0 in borate-buffered purified water.

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**EFFECT OF POTASSIUM CHLORIDE ON THE VIRUCIDAL EFFECTIVENESS  
OF CHLORINE DISINFECTION FOR MILITARY NEEDS**

**FINAL PHASE I REPORT**

**Gerald Berg, Ph.D.**

**10 November 1987**

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**EFFECT OF POTASSIUM CHLORIDE ON THE VIRUCIDAL EFFECTIVENESS  
OF CHLORINE DISINFECTION FOR MILITARY NEEDS**

**SUMMARY**

1. At 5°C, in nonbuffered purified (carbon-filtered, deionized) water (reasonably analogous to ROWPU-treated water) at pH 9.0, 1 mg/L of free chlorine (FC) inactivated 99.99% of poliovirus 1 in 110 minutes, and 5 mg/L of FC inactivated 99.99% of the virus in about 27 minutes. If tailing occurs (and it usually does) a margin of safety must be added to a treatment standard for this alone. And since there are undoubtedly enteric viruses more FC-resistant than the poliovirus used in these studies, the military treatment standard of 5 mg/L for 30 minutes at any temperature and any pH may be inadequate for ROWPU-treated water.
2. At 5°C, 526 mg/L of KCl ( $K^+$ -276 mg/L,  $Cl^-$ -250 mg/L) (a level of  $Cl^-$  far below the military's 600 mg/L permissible maximum for drinking water) potentiated the inactivation by FC at pH 9.0 of poliovirus 1 in nonbuffered purified water. In nonbuffered purified water, 526 mg/L of KCl increased the poliocidal effectiveness of FC by 700%. Under the same circumstances, 1,262 mg/L of KCl ( $K^+$ -662 mg/L,  $Cl^-$ -600 mg/L) (the maximum level of  $Cl^-$  acceptable by the military for drinking water) increased the poliocidal effectiveness of FC by 1,400%.
3. At 5°C, poliovirus 1 was inactivated by FC at pH 9.0 more than 9 times more rapidly in nonbuffered tap water than in nonbuffered purified water suggesting that salts or their ions in tap water potentiated the virucidal effectiveness of the FC.
4. Potassium chloride did not potentiate the poliocidal effectiveness of FC at pH 9.0 to nearly the degree in nonbuffered tap water that it did in nonbuffered purified water suggesting that salts or ions present in tap water potentiated the virucidal effectiveness of FC.
5. FC inactivated poliovirus 1 at pH 9.0 more than 3 times more rapidly in borate (boric acid [ $H_3BO_3$ ] [0.05M]-NaOH) buffered purified water than in nonbuffered purified water suggesting that the buffer ions potentiated the virucidal effectiveness of the FC. Clearly, disinfection studies should not be done in buffers without first determining the virucide potentiating effect of the buffers.
6. Potassium chloride potentiated the virucidal effectiveness of FC in  $H_3BO_3$  (0.05 M)-NaOH buffer to a lesser degree than in nonbuffered purified water indicating that the buffer or its ions potentiated the virucidal effectiveness of FC and that a maximum ion effect exists.
7. Potassium chloride, at a level of 1,262 mg/L ( $K^+$ -662 mg/L,  $Cl^-$ -600 mg/L), potentiated the virucidal effectiveness of FC at pH 4.5 in phthalate-buffered purified water but to a lesser degree than it potentiated the virucidal effectiveness of FC at pH 9.0 in  $H_3BO_3$  (0.005 M)-NaOH buffered purified water.

## TABLE OF CONTENTS

|                                      | Page |
|--------------------------------------|------|
| SUMMARY.....                         | 2    |
| INTRODUCTION.....                    | 9    |
| MATERIAL AND METHODS.....            | 9    |
| RESULTS.....                         | 12   |
| DISCUSSION.....                      | 14   |
| CONCLUSIONS AND RECOMMENDATIONS..... | 15   |
| REFERENCES.....                      | 17   |
| APPENDIX A.....                      | 19   |
| APPENDIX B.....                      | 43   |
| APPENDIX C.....                      | 61   |
| APPENDIX D.....                      | 77   |
| DISTRIBUTION LIST.....               | 88   |

## APPENDIX A

### (LIST OF TABLES [pH 4.5 and 9.0])

|                                                                                                                                     |    |
|-------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 1. Inactivation of Poliovirus 1 at 5°C in Phthalate-.....                                                                     | 20 |
| Buffered Purified Water at pH 4.5 by<br>Approximately 1 mg/L of Free Chlorine in the Presence<br>and Absence of KCl.                |    |
| Table 2. Inactivation of Poliovirus 1 at 5°C in Phthalate-.....                                                                     | 22 |
| Buffered Purified Water at pH 4.5 by<br>Approximately 5 mg/L of Free Chlorine in the Presence<br>and Absence of KCl.                |    |
| Table 3. Inactivation of Poliovirus 1 at 5°C in Borate-.....                                                                        | 24 |
| Buffered Purified Water at pH 9.0 by<br>Approximately 1 mg/L of Free Chlorine in the Presence<br>and Absence of KCl (Experiment 1). |    |
| Table 4. Inactivation of Poliovirus 1 at 5°C in Borate-.....                                                                        | 26 |
| Buffered Purified Water at pH 9.0 by<br>Approximately 1 mg/L of Free Chlorine in the Presence<br>and Absence of KCl (Experiment 2). |    |
| Table 5. Inactivation of Poliovirus 1 at 5°C in Borate-.....                                                                        | 28 |
| Buffered Purified Water at pH 9.0 by<br>Approximately 5 mg/L of Free Chlorine in the Presence<br>and Absence of KCl.                |    |
| Table 6. Inactivation of Poliovirus 1 at 5°C in Borate-.....                                                                        | 30 |
| Buffered Tap Water at pH 9.0 by Approximately 5 mg/L<br>of Free Chlorine in the Presence and Absence of KCl.                        |    |
| Table 7. Inactivation of Poliovirus 1 at 5°C in Nonbuffered.....                                                                    | 32 |
| Purified Water at pH 9.0 by Approximately 1 mg/L<br>of Free Chlorine in the Presence and Absence of KCl.                            |    |
| Table 8. Inactivation of Poliovirus 1 at 5°C in Nonbuffered.....                                                                    | 34 |
| Purified Water at pH 9.0 by Approximately 5 mg/L<br>of Free Chlorine in the Presence and Absence of KCl<br>(Experiment 1).          |    |
| Table 9. Inactivation of Poliovirus 1 at 5°C in Nonbuffered.....                                                                    | 36 |
| Purified Water at pH 9.0 by Approximately 5 mg/L<br>of Free Chlorine in the Presence and Absence of KCl<br>(Experiment 2).          |    |
| Table 10. Inactivation of Poliovirus 1 at 5°C in Nonbuffered.....                                                                   | 38 |
| Tap Water at pH 9.0 by Approximately 1 mg/L of Free Chlorine<br>in the Presence and Absence of KCl.                                 |    |
| Table 11. Inactivation of Poliovirus 1 at 5°C in Nonbuffered.....                                                                   | 40 |
| Tap Water at pH 9.0 by Approximately 5 mg/L of Free Chlorine<br>in the Presence and Absence of KCl.                                 |    |

## APPENDIX B

### (LIST OF FIGURES [pH 4.5 and 9.0])

- Figure 1. Inactivation of Poliovirus 1 at 5°C in Phthalate-..... 44  
Buffered Purified Water at pH 4.5 by  
Approximately 1 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure 2. Inactivation of Poliovirus 1 at 5°C in Phthalate-..... 45  
Buffered Purified Water at pH 4.5 by  
Approximately 5 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure 3. Inactivation of Poliovirus 1 at 5°C in Borate-..... 46  
Buffered Purified Water at pH 9.0 by  
Approximately 1 mg/L of Free Chlorine in the Presence  
and Absence of KCl (Experiment 1).
- Figure 4. Inactivation of Poliovirus 1 at 5°C in Borate-..... 47  
Buffered Purified Water at pH 9.0 by  
Approximately 1 mg/L of Free Chlorine in the Presence  
and Absence of KCl (Experiment 2).
- Figure 5. Inactivation of Poliovirus 1 at 5°C in Borate-..... 48  
Buffered Purified Water at pH 9.0 by Approximately  
5 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure 6. Inactivation of Poliovirus 1 at 5°C in Borate-..... 49  
Buffered Tap Water at pH 9.0 by Approximately 5 mg/L of  
Free Chlorine in the Presence and Absence of KCl.
- Figure 7. Inactivation of Poliovirus 1 at 5°C in Nonbuffered..... 50  
Purified Water at pH 9.0 by Approximately  
1 mg/L of Free Chlorine in the Presence and Absence  
of KCl.
- Figure 8. Inactivation of Poliovirus 1 at 5°C in Nonbuffered..... 51  
Purified Water at pH 9.0 by Approximately  
5 mg/L of Free Chlorine in the Presence and Absence of KCl.
- Figure 9. Inactivation of Poliovirus 1 at 5°C in Nonbuffered..... 52  
Purified Water at pH 9.0 by Approximate.y  
5 mg/L of Free Chlorine in the Presence and Absence of KCl.
- Figure 10. Inactivation of Poliovirus 1 at 5°C in Nonbuffered..... 53  
Tap Water at pH 9.0 by Approximately 1 mg/L of Free Chlorine  
in the Presence and Absence of KCl.
- Figure 11. Inactivation of Poliovirus 1 at 5°C in Nonbuffered..... 54  
Tap Water at pH 9.0 by Approximately 5 mg/L of Free Chlorine  
in the Presence and Absence of KCl.

|                                                                                     |    |
|-------------------------------------------------------------------------------------|----|
| Figure 12. Effect of KCl on the Inactivation of Poliovirus 1.....                   | 55 |
| (99.99%) at 5°C by Free Chlorine at pH 4.5 in Phthalate-Buffered Purified Water.    |    |
| Figure 13. Effect of KCl on the Inactivation of Poliovirus 1.....                   | 56 |
| (99.99%) at 5°C by Free Chlorine at pH 9.0 in Borate-Buffered Purified Water.       |    |
| Figure 14. Effect of KCl on the Inactivation of Poliovirus 1.....                   | 57 |
| (99.99%) at 5°C by Free Chlorine at pH 9.0 in Nonbuffered Purified Water.           |    |
| Figure 15. Effect of 0.05 M Borate Buffer on the Inactivation.....                  | 58 |
| of Poliovirus 1 (99.99%) at 5°C by Free Chlorine at pH 9.0.                         |    |
| Figure 16. Effect of KCl on the Inactivation of Poliovirus 1.....                   | 59 |
| (99.99%) at 5°C by Free Chlorine at pH 9.0 in Nonbuffered Tap Water.                |    |
| Figure 17. Inactivation of Poliovirus 1 (99.99%) at 5°C by.....                     | 60 |
| Free Chlorine at pH 9.0 in Nonbuffered Purified Water and in Nonbuffered Tap Water. |    |

## APPENDIX C

### (LIST OF TABLES [pH 10.0])

|                                                                |    |
|----------------------------------------------------------------|----|
| Table A1. Inactivation of Poliovirus 1 at 5°C in Borate-.....  | 63 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 0.63-0.65 mg/L of Free Chlorine in the Presence                |    |
| and Absence of KCl.                                            |    |
| Table A2. Inactivation of Poliovirus 1 at 5°C in Borate-.....  | 64 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 0.63 mg/L of Free Chlorine in the Presence and Absence         |    |
| of KCl.                                                        |    |
| Table A3. Inactivation of Poliovirus 1 at 5°C in Borate-.....  | 65 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 1.3 mg/L of Free Chlorine in the Presence and Absence          |    |
| of KCl.                                                        |    |
| Table A4. Inactivation of Poliovirus 1 at 5°C in Borate-.....  | 66 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 5.63-5.69 mg/L of Free Chlorine in the Presence                |    |
| and Absence of KCl.                                            |    |
| Table A5A. Inactivation of Poliovirus 1 at 5°C in Borate-..... | 68 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 0.81-0.83 mg/L of Free Chlorine in the Presence                |    |
| and Absence of KCl.                                            |    |
| Table A5B. Inactivation of Poliovirus 1 at 5°C in Borate-..... | 69 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 0.61-0.77 mg/L of Free Chlorine in the Presence                |    |
| and Absence of KCl.                                            |    |
| Table A6. Inactivation of Poliovirus 1 at 5°C in Borate-.....  | 70 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 0.60-1.30 mg/L of Free Chlorine in the Presence                |    |
| and Absence of KCl.                                            |    |
| Table A7. Inactivation of Poliovirus 1 at 5°C in Borate-.....  | 72 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 0.65-4.21 mg/L of Free Chlorine in the Presence                |    |
| and Absence of KCl.                                            |    |
| Table A8. Inactivation of Poliovirus 1 at 5°C in Borate-.....  | 74 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 0.95-1.15 mg/L of Free Chlorine in the Presence                |    |
| and Absence of KCl.                                            |    |
| Table A9. Inactivation of Poliovirus 1 at 5°C in Borate-.....  | 76 |
| Buffered Purified Water at pH 10.0 by Approximately            |    |
| 4.63-4.76 mg/L of Free Chlorine in the Presence                |    |
| and Absence of KCl.                                            |    |

## APPENDIX D

### (LIST OF FIGURES [pH 10.0])

- Figure A1. Inactivation of Poliovirus 1 at 5°C in Borate-.....78  
Buffered Purified Water at pH 10.0 by Approximately  
0.63-0.65 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure A2. Inactivation of Poliovirus 1 at 5°C in Borate-.....79  
Buffered Purified Water at pH 10.0 by Approximately  
0.63 mg/L of Free Chlorine in the Presence and Absence  
of KCl.
- Figure A3. Inactivation of Poliovirus 1 at 5°C in Borate-.....80  
Buffered Purified Water at pH 10.0 by Approximately  
1.3 mg/L of Free Chlorine in the Presence and Absence  
of KCl.
- Figure A4. Inactivation of Poliovirus 1 at 5°C in Borate-.....81  
Buffered Purified Water at pH 10.0 by Approximately  
5.63-5.69 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure A5A. Inactivation of Poliovirus 1 at 5°C in Borate-.....82  
Buffered Purified Water at pH 10.0 by Approximately  
0.81-0.83 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure A5B. Inactivation of Poliovirus 1 at 5°C in Borate-.....33  
Buffered Purified Water at pH 10.0 by Approximately  
0.61-0.77 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure A6. Inactivation of Poliovirus 1 at 5°C in Borate-.....84  
Buffered Purified Water at pH 10.0 by Approximately  
0.60-1.30 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure A7. Inactivation of Poliovirus 1 at 5°C in Borate-.....85  
Buffered Purified Water at pH 10.0 by Approximately  
0.65-4.21 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure A8. Inactivation of Poliovirus 1 at 5°C in Borate-.....86  
Buffered Purified Water at pH 10.0 by Approximately  
0.95-1.15 mg/L of Free Chlorine in the Presence  
and Absence of KCl.
- Figure A9. Inactivation of Poliovirus 1 at 5°C in Borate-.....87  
Buffered Purified Water at pH 10.0 by Approximately  
4.63-4.76 mg/L of Free Chlorine in the Presence  
and Absence of KCl.

## EFFECT OF POTASSIUM CHLORIDE ON THE VIRUCIDAL EFFECTIVENESS OF CHLORINE DISINFECTION FOR MILITARY NEEDS

### INTRODUCTION

The military looks to disinfection with free chlorine (FC) as the final barrier to prevent viruses and other pathogenic microorganisms from producing disabling enteric diseases in military personnel who must drink field-produced drinking water. The disinfection barrier is paramount in all of the variety of conditions under which the military must produce drinking water in the field.

It has been demonstrated repeatedly that in the presence of 0.05 to 0.1M (3,728 to 7,456 mg/L) KCl (because KCl is completely ionized in water, KCl exists as  $K^+$  and  $Cl^-$ ) hypochlorite ion ( $OCl^-$ ), the otherwise slow virucidal form of FC, is 10 to 20 times more virucidal than hypochlorous acid ( $HOCl$ ), the rapid virucidal form of FC (1-6). The virucidal activity of  $HOCl$  also is potentiated by KCl. It is not clear whether  $K^+$  or  $Cl^-$  or both is responsible for the potentiation. (It is now clear from the research reported herein that many ions in drinking water potentiate the virucidal effectiveness of FC.) The occurrence of  $K^+$  and/or  $Cl^-$  in a drinking water, if present in sufficient concentration, may potentiate the virucidal effectiveness of the free chlorines. Conversely, removal of these entities from a drinking water may significantly reduce the virucidal effectiveness of the free chlorines. The range of  $Cl^-$  concentrations in natural waters may span from only 10 or 20 mg/L (fresh water treated with Reverse Osmosis Water Purification Units [ROWPU]) to 600 mg/L (field water quality standard maximum). Potassium ions also occur in natural waters in low concentrations, and there is no field water quality maximum standard for these ions. Reverse osmosis removes most  $K^+$ ,  $Cl^-$ , and other ions from water. Thus, ROWPU may reduce the virucidal effectiveness of the FC applied to such treated waters to prevent post ROWPU treatment microbiological contamination. In this situation, it might be necessary for the military to increase the concentration of FC in the field-disinfected waters to meet the military's required disinfection standards. It was therefore of considerable importance to the military to determine the effect of small concentrations of ions on the virucidal capability of FC ( $OCl^-$  and  $HOCl$ ).

This study was divided into two phases, PHASE 1 was to determine whether KCl ( $Cl^-$  in the range of 10 to 600 mg/L) has a significant effect (2 to 3-fold difference) on disinfection by the free chlorines. In the event that a significant KCl effect was demonstrated, PHASE 2 was to be initiated to provide comprehensive data on the adjustment of FC that will be necessary to meet the military's required disinfection capability or to develop cautionary guidance for field disinfection at low  $K^+$  and/or  $Cl^-$  levels (for example, ROWPU operations). *Keywords: Reverse Osmosis Water Purification Unit (ROWPU); Drinking water; (17)*

### MATERIAL AND METHODS

#### 1. Water.

All of the disinfection tests were done in chlorine demand-free purified (carbon-filtered, deionized) water, in tap water obtained in the City of Cincinnati, or in buffers prepared in those waters. Purified water was obtained from a Millipore Super Q System.

2. Phosphate buffer.  
A 0.2 M phosphate buffer solution was prepared by adding 23.7 g of  $\text{KH}_2\text{PO}_4$  (Fisher P-382) and 4.2 g of  $\text{K}_2\text{HPO}_4$  (Fisher P-288) to 1 L of purified water. This solution was filtered through a 0.45  $\mu\text{m}$  Millipore membrane. A 0.05 M phosphate buffer solution was prepared by adding 250 mL of 0.2 M phosphate buffer to 750 mL of purified water.
3. Sodium thiosulfate neutralizer solution.  
A 100 mg/mL solution of  $\text{Na}_2\text{S}_2\text{O}_3$  was prepared by adding 12.5 g of  $\text{Na}_2\text{S}_2\text{O}_3$  (Fisher S-446) to 125 mL of purified water. One mL of this solution was added to 1 L of 0.05 M phosphate buffer to yield a 100 mg/L solution of  $\text{Na}_2\text{S}_2\text{O}_3$  in phosphate buffer.
4. Beef extract (1%).  
A 1% solution of beef extract was prepared by adding 10 g of beef extract (Gibco M00040) to 1 L of purified water. This solution was used for preparing dilutions of virus.
5. Chlorine demand-free waters.  
Chlorine demand-free waters were prepared according to Standard Methods (7). Waters, purified and tap, were made demand-free by chlorinating them to a free residual of 5 mg/L and storing them for three days. The waters were then dechlorinated by exposure to ultraviolet light for several days. To make certain that no chlorine remained in the waters, amperometric titrations were performed on them when chlorine was no longer demonstrable by DPD test. The demand-free dechlorinated waters were stored until needed.
6. Chlorine demand-free buffers.
  - a. Phthalate buffer. Phthalate buffer was prepared by adding 40.846 g of potassium hydrogen phthalate to 1 L of purified water. The molarity was 0.2 M. Chlorine was added to the buffer to a final concentration of 5 mg/L. The chlorinated buffer was stored in the laboratory for three days to satisfy the chlorine demand, and the buffer was dechlorinated by exposing it to ultraviolet light. To make certain that no chlorine remained, amperometric titrations were performed on the buffer when chlorine was no longer demonstrable by the DPD test. For tests, the 0.2 M buffer was diluted in chlorine demand-free purified water to 0.005 M and its pH was adjusted to 4.5 with chlorine demand-free 2 M  $\text{H}_2\text{SO}_4$  or NaOH.
  - b. Borate buffer. Borate buffer was prepared by adding 12.366 g of boric acid to 1 L of purified water. The molarity was 0.2. The buffer was made chlorine demand-free and its pH was adjusted in the same way that this was done with the phthalate buffer.
7. Chlorine demand-free NaOH and chlorine demand-free  $\text{H}_2\text{SO}_4$ .  
Appropriate strength solutions of NaOH and  $\text{H}_2\text{SO}_4$  were prepared and made chlorine demand-free in the same way that the buffers were made demand-free.
8. Chlorine demand-free KCl solution.  
KCl (Matheson, Coleman, and Bell PX1405) solutions were prepared in three concentrations as follows: A solution containing 21 mg of KCl/10 mL was prepared by dissolving 0.21 g of KCl in 100 mL of purified water. A solution containing 526 mg of KCl/10 mL was prepared by dissolving 5.26 g of KCl in 100 mL of purified water. A solution

containing 1,262 mg of KCl/10 mL was prepared by dissolving 12.62 g of KCl in 100 mL of purified water. All of the solutions were prepared in volumetric flasks and all of the solutions were brought to final volume with a few drops of chlorine solution of sufficient strength to yield about 5 mg/L of chlorine. The solutions were made chlorine demand-free in the same way the buffers were made demand-free. In the test procedures, 4 mL of KCl solution added to 396 mL of test solution yielded the required concentration of  $\text{Cl}^-$ .

9. Chlorine demand-free glassware.

Chlorine demand-free glassware was prepared by the procedure described in Standard Methods (7). Chlorine demand-free beakers and cylinders used in the test procedures were rinsed three times in the test waters just before the tests were done.

10. Amperometric titrations for FC.

Amperometric titration with phenylarsene oxide for FC were done by the procedures described in Standard Methods (7). The titrations were done on a total volume of 300 mL of each test fluid.

11. Virus.

A chlorine demand-free preparation of poliovirus 1 (Mahoney LP, RKP42 of 10/7/69) was used in all of the experiments described herein.

12. Cell cultures. All poliovirus assays were done in BCM cells (8) by the plaque technique (9). Plaques were generally read 3, 4, and 7 days after cultures were inoculated.

13. Test procedure.

One-and-a-half to two-liter quantities of chlorine demand-free water, sometimes buffered, sometimes not, sometimes purified water, sometimes tap water, were chlorinated to either 1 or 5 mg/L residuals, and 396 mL quantities were distributed into 600 mL beakers. Four mL of an appropriate concentration of KCl solution was added to each of three beakers to yield concurrently  $\text{K}^+$  plus  $\text{Cl}^-$  concentrations of 11 and 10, 276 and 250, and 662 and 600 mg/L. In many of the tests, phthalate buffer was used to maintain a pH of 4.5 and borate buffer was used to maintain a pH of 9.0. When buffers were not used, the pH of each test water was adjusted with 2 M and 0.25 M  $\text{H}_2\text{SO}_4$  or NaOH, respectively, and rechecked and readjusted if necessary at the beginning of each test. Two control beakers were set up with each test. In most experiments, one contained 396 mL of the test water to which had been added 4 mL of KCl solution of sufficient strength to yield a final concentration of 662 mg/L of  $\text{K}^+$  and 600 mg/L of  $\text{Cl}^-$  in the control beaker. The second beaker contained 198 mL of the test water, 198 mL of  $\text{Na}_2\text{S}_2\text{O}_3$  neutralizer solution and 4 mL of KCl solution of sufficient strength to yield a final concentration of 662 mg/L of  $\text{K}^+$  and 600 mg/L of  $\text{Cl}^-$  in the control beaker. Test and control waters were brought to  $50^\circ\text{C}$  ( $\pm 1^\circ\text{C}$ ) in a water bath, and under continuous stirring on a magnetic stirrer, 1 mL of a chlorine demand-free poliovirus 1 suspension was added to each beaker. Five mL samples were removed from each test beaker at appropriate time intervals and mixed rapidly with 5 mL volumes of a solution that contained 100 mg/L of  $\text{Na}_2\text{S}_2\text{O}_3$ . A similar sampling procedure was used for the control beakers, but the first control beaker was sampled only after one minute, and the second control beaker was sampled at the longest time interval used in any of

the tests. In tests where nonbuffered systems were used, pH was redetermined in each test beaker at the conclusion of each test. Amperometric titrations for FC were also done on all test fluids at the conclusion of each test. All test samples were diluted in 1% beef extract and inoculated onto cell cultures. Some test samples were stored at -70°C for several days before inoculation onto cell cultures. Control tests showed that freezing test samples at -70°C before inoculation onto cell cultures did not alter results.

## RESULTS

The first year of this study was devoted to determining whether KCl, at levels of up to 1,262 mg/L ( $K^+$ -662 mg/L;  $Cl^-$ -600 mg/L [the maximum  $Cl^-$  level permissible for drinking water under Military standards]) significantly increases the rate of inactivation of poliovirus by FC at pH 4.5 and at pH 9.0. Early experiments were undertaken at pH 10.0 to maximize the  $OCl^-$  levels present, and many of the preliminary studies were done at this pH level. At the request of the Project Officer, the pH of the alkaline level tests was reduced to 9.0, because this pH level is more in line with the higher extremes usually experienced under field conditions. The data obtained at pH 10.0 are presented in Appendices C and D.

In early experiments in 0.05 M borate buffer, more than 7,456 mg/L of KCl (0.1 M) did not affect the rate of inactivation of poliovirus 1 by FC at pH 10.0. Several lots of sodium and potassium chloride from different manufacturers yielded results similar to those in these earlier studies, but lots of boric acid (a buffer component) from manufacturers other than the one used in the first studies allowed a salt effect to manifest itself.

In borate buffer that allowed manifestation of a salt effect, 526 and 1,262 mg/L quantities of KCl ( $K^+$ -276 and 662 mg/L, respectively;  $Cl^-$ -250 and 600 mg/L, respectively) increased the rate of poliovirus 1 inactivation by FC at pH 9.0.

### Inactivation of Poliovirus 1 in Phthalate-Buffered Purified Water, pH 4.5

In phthalate buffered purified water at pH 4.5, where most of the FC exists as  $HOCl$ , 1.17 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in just over 4.0 minutes (Table 1, Figure 1). In the presence of 21 mg/L of KCl ( $K^+$ -11 mg/L;  $Cl^-$ -10 mg/L), approximately the same quantity of FC inactivated poliovirus 1 at about the same rate, and in the presence of 526 mg/L of KCl, a little bit more rapidly. In the presence of 1,262 mg/L of KCl, however, 0.97 mg/L of FC inactivated 99.99% of poliovirus 1 in about 2.8 minutes.

In phthalate-buffered purified water at pH 4.5, 5.27 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in about 1.2 minutes. The same quantity of FC in the presence of 21 mg/L of KCl inactivated poliovirus 1 at about the same rate. In the presence of 526 mg/L of KCl, 5.20 mg/L of FC inactivated poliovirus 1 a little more rapidly, and in the presence of 1,262 mg/L of KCl, 5.15 mg/L of FC inactivated poliovirus 1 twice as rapidly as the same quantity of FC did in the absence of KCl (Table 2, Figure 2).

### Inactivation of Poliovirus 1 in Borate-Buffered Purified Water, pH 9.0

In borate-buffered purified water at pH 9.0, 1.22 mg/L of FC (primarily  $OCl^-$ ), in the absence of KCl, inactivated 99.99% of poliovirus 1 in about 22 minutes. In the presence of 21 mg/L of KCl, the rate of inactivation of poliovirus 1 by 1.16 mg/L of FC was about the same. In the presence of 526

mg/L of KCl, 99.99% of the virus was inactivated by 1.14 mg/L of FC in about 16 minutes, and in the presence of 1,262 mg/L of KCl, 1.20 mg/L of FC inactivated 99.99% of the virus in about 10 minutes (Table 3, Figure 3).

In another experiment in the same buffer, in the absence of KCl, 1.08 mg/L of FC inactivated 99.99% of poliovirus 1 in 22 minutes. In the presence of 21 mg/L of KCl, 1.05 mg/L of FC inactivated the virus at about the same rate. In the presence of 526 mg/L of KCl, 1.04 mg/L of FC inactivated 99.99% of the virus in about 18 minutes. In the presence of 1,262 mg/L of KCl, however, 1.01 mg/L of FC inactivated 99.99% of the virus in about 12 minutes (Table 4, Figure 4).

In the same buffer, 5.05 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in about 9.5 minutes. In the presence of 21 mg/L of KCl, 5.00 mg/L of FC inactivated the poliovirus at about the same rate. In the presence of 526 mg/L of KCl, 5.05 mg/L of FC inactivated 99.99% of the virus in about 3.5 minutes, and in the presence of 1,262 mg/L of KCl, 5.00 mg/L of FC inactivated 99.99% of the virus in about 2.2 minutes (Table 5, Figure 5).

#### Inactivation of Poliovirus 1 in Borate-Buffered Tap Water, pH 9.0

In borate-buffered tap water at pH 9.0, 5.20 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in about 3.6 minutes. In the presence of 21 mg/L of KCl, 5.17 mg/L of FC inactivated the poliovirus at about the same rate. In the presence of 526 mg/L of KCl, 5.10 mg/L of FC inactivated 99.99% of the virus in about 2.7 minutes, and in the presence of 1,262 mg/L of KCl, 5.00 mg/L of FC inactivated 99.99% of the virus in about 2.3 minutes (Table 6, Figure 6).

#### Inactivation of Poliovirus 1 in Nonbuffered Purified Water, pH 9.0

In nonbuffered purified water at pH 9.0, 0.95 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in a little more than 100 minutes. In the presence of 21 mg/L of KCl, 0.93 mg/L of FC inactivated 99.99% of the virus in about 63 minutes. In the presence of 526 mg/L of KCl, 0.90 mg/L of FC inactivated 99.99% of the poliovirus in about 18 minutes, and in the presence of 1,262 mg/L of KCl, 0.85 mg/L of FC inactivated 99.99% of the virus in about 5 minutes (Table 7, Figure 7).

In the same nonbuffered system, 5.00 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in 23 to 24 minutes. In the presence of 21 mg/L of KCl, 5.00 mg/L of FC inactivated the virus at about the same rate. In the presence of 526 mg/L of KCl, 4.88 mg/L of FC inactivated 99.99% of the poliovirus in about 4.5 minutes, and in the presence of 1,262 mg/L of KCl, 4.83 mg/L of FC inactivated 99.99% of the poliovirus in about 2.5 minutes (Table 8, Figure 8).

In another series of tests, in the same nonbuffered system, 5.00 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in about 31 minutes. In the presence of 21 mg/L of KCl, 5.05 mg/L of FC inactivated the poliovirus at about the same rate. In the presence of 526 mg/L of KCl, 4.95 mg/L of FC inactivated 99.99% of the virus in about 3 minutes, and in the presence of 1,262 mg/L of KCl, 5.00 mg/L of FC inactivated 99.99% of the virus in about 2 minutes (Table 9, Figure 9).

#### Inactivation of Poliovirus 1 in Nonbuffered Tap Water, pH 9.0

In nonbuffered tap water at pH 9.0, 0.95 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in about 13 minutes. In the presence of 21 mg/L of KCl, 1.02 mg/L of FC inactivated 99.99% of the virus in about 13 minutes. In the presence of 526 mg/L of KCl, 1.00 mg/L of FC inactivated 99.99% of the poliovirus in about 9 minutes, and in the

presence of 1,262 mg/L of KCl, 1.02 mg/L of FC inactivated 99.99% of the virus in 4 to 5 minutes (Table 10, Figure 10).

In the same nonbuffered tap water system, 5.00 mg/L of FC, in the absence of KCl, inactivated 99.99% of poliovirus 1 in about 3 minutes. In the presence of 526 mg/L of  $\text{Cl}^-$ , 4.85 mg/L of FC inactivated 99.99% of the poliovirus in 2 to 2.5 minutes, and in the presence of 1.62 mg/L of KCl, 4.80 mg/L of FC inactivated 99.99% of the virus in just under 2 minutes (Table 11, Figure 11).

#### DISCUSSION

At pH 4.5 in phthalate buffer, FC inactivated poliovirus 1 about 80% more rapidly in the presence of 1,262 mg/L of KCl than in the absence of KCl (Figures 1, 2, and 12). Potassium chloride, at a level of 526 mg/L, also appeared to potentiate the poliocidal effectiveness of FC at pH 4.5, but to a lesser degree. At a level of 21 mg/L, KCl did not appear to significantly potentiate FC at pH 4.5 in phthalate buffer or under any of the other conditions subsequently tested. The potentiation of FC by KCl in buffer at pH 4.5 is clearly worthwhile, but  $\text{HOCl}$ , the predominant form of FC that occurs at pH 4.5, is a rapid virucide even in the absence of KCl. The military's greatest need is on the alkaline side of the pH spectrum because  $\text{OCl}^-$ , which forms at alkaline pH levels as the result of the ionization of  $\text{HOCl}$ , is a relatively slow virucide and because most natural waters are alkaline. At pH 9.0 in borate buffer, FC inactivated poliovirus 1 almost three times more rapidly in the presence of 1,262 mg/L of KCl than in the absence of KCl (Figures 3-5, and 13). Potassium chloride, at a level of 526 mg/L, potentiated the poliocidal effectiveness of FC by almost 2-fold.

At pH 9.0, in the presence of 1,262 mg/L of KCl, FC inactivated poliovirus 1 as rapidly in tap water as in borate-buffered purified water (compare Figures 6 and 13). In the absence of KCl, however, FC inactivated the poliovirus 1 considerably more rapidly in tap water than in borate-buffered purified water. This finding, along with the earlier finding that different commercial preparations of  $\text{H}_3\text{BO}_3$  (the major component of borate buffer), differently affected the poliocidal effectiveness of FC, strongly suggested a need for testing the virucidal effectiveness of FC (with and without KCl) in nonbuffered purified water. Buffers have always been used to maintain pH at required levels. Preliminary studies, however, showed that pH levels in purified water, adjusted with  $\text{H}_2\text{SO}_4$  or  $\text{NaOH}$ , remained stable for long periods within 0.1 (occasionally 0.2) pH units. Subsequent studies in nonbuffered waters produced remarkable results.

In studies with nonbuffered purified water at pH 9.0, FC inactivated poliovirus 1 about 15 times more rapidly in the presence of 1,262 mg/L of KCl than in the absence of KCl. In the presence of 526 mg/L of KCl, FC inactivated the virus about 7 times more rapidly than in the absence of KCl (Figures 7-9, 14). In the presence of 526 and 1,262 mg/L of KCl, FC inactivated the poliovirus about as rapidly in nonbuffered purified water as in borate-buffered purified water (Figures 13 and 14). But in the absence of KCl, FC inactivated the virus more than 3 times faster in the borate-buffered purified water than in the nonbuffered purified water (Figure 15 - from Figures 13 and 14). The 0.05 M borate buffer had a powerful virucide potentiating effect on FC. It is clear that disinfection studies should not be done in buffers without first determining the virucide potentiating effect of the buffers. The borate buffer did not add to potentiation in the presence of either 526 or 1,262 mg/L of KCl.

The experiments in Figures 10 and 11 are exactly the same as those in Figures 7-9 and 14 except that in Figures 10 and 11 the experiments were done in nonbuffered tap water and those in Figures 7-9 and 14 were done in nonbuffered purified water. When 1,262 mg/L of KCl was present, the virucidal effectiveness of FC at pH 9.0 was not much different in nonbuffered purified water than in nonbuffered tap water. When 526 mg/L of KCl was present in both waters, the inactivation rate was faster in tap water than in purified water, and when no KCl was present, the inactivation rate dropped off sharply in purified water. In the absence of KCl, poliovirus 1 was inactivated by FC about 10 times more rapidly in nonbuffered tap water than in nonbuffered purified water (Figure 17). It would appear that ions in the tap water markedly enhanced the poliocidal effectiveness of  $\text{OCl}^-$ .

Since RO-treated waters contain few ions, the poliocidal effectiveness of  $\text{OCl}^-$  in such waters will be about that seen in the nonbuffered purified water study. This is to say that in RO-treated waters at pH 9.0, about 110 minutes will be required to inactivate 99.99% of poliovirus 1 at 50°C by 1 mg/L of FC (about the same as in nonbuffered purified water), and 27 minutes will be required to inactivate 99.99% of the virus by 5 mg/L of FC under the same conditions (Figure 17). If tailing occurs (and it usually does because naturally occurring viruses are usually clumped to some degree) as it frequently did in the experiments reported herein, a margin for safety must be added to a treatment standard for this alone. And, since there are undoubtedly enteric viruses more FC-resistant than the poliovirus used in these studies, the military treatment standard of 5 mg/L for 30 minutes at any temperature and any pH may be inadequate for ROWPU-treated waters and other drinking waters with low ionic content.

For waters that contain a sufficient quantity of ions, the treatment standard may be excessive. But, we must know more than we do now about the relationship of ion concentration and FC inactivation rates and more about the levels of salts present in such waters before we can say that with assurance.

Although, it seems clear now that many different ions may potentiate the poliocidal effectiveness of  $\text{HOCl}$  and  $\text{OCl}^-$ , we know little about which ions do so. Moreover, even though at least one theory suggests that cations rather than anions are responsible for the enhanced poliocidal effectiveness of  $\text{HOCl}$  and  $\text{OCl}^-$  (4,10,11), even this is not yet certain.

#### CONCLUSIONS AND RECOMMENDATIONS

In PHASE 1 of this study, we showed that an enhancement of poliocidal activity by FC occurs at the salt levels present in natural waters. This study also showed that tap water contains substances (probably ions) that enhance the virucidal activity of FC (both  $\text{HOCl}$  and  $\text{OCl}^-$ ). Potassium chloride levels of 526 mg/L and 1,262 mg/L potentiated the virucidal effectiveness of FC. In purified water, KCl potentiated the virucidal capability of  $\text{OCl}^-$  by 10-fold and sometimes more. In the absence of salts, 5 mg/L of FC at pH 9.0 inactivated poliovirus 1 so slowly in purified water, it may be necessary for the military to adjust the dosage of FC in order to meet its required disinfection capability in ROWPU-treated waters and in other waters of low salt content.

Thus, the military needs to pursue PHASE 2 of this study to determine the degree to which the ion effect occurs with viruses other than poliovirus 1 and to determine which ions potentiate the virucidal effectiveness of FC. With this information, the military will be better

able to decide whether it must adjust the dosage of FC in field-produced drinking waters such as ROWPU-treated waters and other waters of low ion content in order to meet the military's required disinfection capability.

Moreover, to the extent that FC concentrations can be reduced in waters that contain ions that potentiate the virucidal effectiveness of FC, the production of carcinogenic halomethanes will be reduced. Although the problems produced by such carcinogens may not be manifested for many years and are therefore of no battlefield significance, eventual consequences of the kind currently experienced with the agent orange situation, and the accompanying negative public relations, are always better avoided.

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APPENDIX A  
(LIST OF TABLES [pH 4.5 and 9.0])

TABLE 1. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN PHTHALATE-BUFFERED  
PURIFIED WATER AT PH 4.5 BY APPROXIMATELY 1 MG/L  
OF FREE CHLORINE IN THE PRESENCE & ABSENCE OF KCL

TEST #1 CHLORINE (MG/L): 1.17 KCL (MG/L): 0 CONTROL (PFU/ML): 250000  
INITIAL CHLORINE (MG/L): 1.22

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 48000                | 28000                | 38000               | 15.1999         | 84.8001             |
| 1              | 20400                | 15200                | 17800               | 7.1199          | 92.8801             |
| 1.5            | 10800                | 6400                 | 8600                | 3.44            | 96.56               |
| 2              | 5200                 | 4000                 | 4600                | 1.8399          | 98.1601             |
| 2.5            | 840                  | 840                  | 840                 | .336            | 99.664              |
| 3              | 320                  | 248                  | 284                 | .1136           | 99.8864             |
| 3.25           | 104                  | 136                  | 120                 | .0479           | 99.9521             |
| 3.5            | 132                  | 116                  | 124                 | .0495           | 99.9505             |
| 3.75           | 60                   | 28                   | 44                  | .0175           | 99.9825             |
| 4              | 4                    | 16                   | 10                  | 4E-03           | 99.996              |

TEST #2 CHLORINE (MG/L): 1.11 KCL (MG/L): 21 CONTROL (PFU/ML): 250000  
INITIAL CHLORINE (MG/L): 1.22

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 22400                | 24800                | 23600               | 9.44            | 90.56               |
| 1              | 12800                | 14800                | 13800               | 5.5199          | 94.4801             |
| 1.5            | 8000                 | 6800                 | 7400                | 2.9599          | 97.0401             |
| 2              | 8800                 | 8000                 | 8400                | 3.36            | 96.64               |
| 2.5            | 2080                 | 2280                 | 2180                | .8719           | 99.1281             |
| 3              | 680                  | 1040                 | 860                 | .344            | 99.656              |
| 3.25           | 360                  | 400                  | 380                 | .152            | 99.848              |
| 3.5            | 236                  | 252                  | 244                 | .0976           | 99.9024             |
| 3.75           | 140                  | 172                  | 156                 | .0623           | 99.9377             |
| 4              | 72                   | 68                   | 70                  | .0279           | 99.9721             |

TABLE 1. (CONTINUED)

TEST #3 CHLORINE (MG/L): 1 KCL (MG/L): 526 CONTROL (PFU/ML): 250000  
INITIAL CHLORINE (MG/L): 1.22

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 6400                 | 9200                 | 7800                | 3.12            | 96.88               |
| 1              | 5200                 | 4400                 | 4800                | 1.9199          | 98.0801             |
| 1.5            | 1400                 | 1520                 | 1460                | .584            | 99.416              |
| 2              | 1000                 | 640                  | 820                 | .3279           | 99.6721             |
| 2.5            | 480                  | 400                  | 440                 | .1759           | 99.8241             |
| 3              | 216                  | 176                  | 196                 | .0783           | 99.9217             |
| 3.25           | 132                  | 128                  | 130                 | .0519           | 99.9481             |
| 3.5            | 88                   | 68                   | 78                  | .0311           | 99.9689             |
| 3.75           | 32                   | 32                   | 32                  | .0128           | 99.9872             |
| 4              | 8                    | 8                    | 8                   | 3.2E-03         | 99.9968             |

TEST #4 CHLORINE (MG/L): .97 KCL (MG/L): 1,262 CONTROL (PFU/ML): 250000  
INITIAL CHLORINE (MG/L): 1.22

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 6400                 | 2800                 | 4600                | 1.8399          | 98.1601             |
| 1              | 3600                 | 3600                 | 3600                | 1.4399          | 98.5601             |
| 1.5            | 280                  | 280                  | 280                 | .1119           | 99.8881             |
| 2              | 216                  | 172                  | 194                 | .0775           | 99.9225             |
| 2.25           | 172                  | 172                  | 172                 | .0688           | 99.9312             |
| 2.5            | 80                   | 88                   | 84                  | .0335           | 99.9665             |
| 2.75           | 56                   | 40                   | 48                  | .0192           | 99.9808             |
| 3              | 32                   | 16                   | 24                  | 9.6E-03         | 99.9904             |
| 3.25           | 8                    | 12                   | 10                  | 4E-03           | 99.996              |
| 3.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 2. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN PHTHALATE-BUFFERED  
PURIFIED WATER AT PH 4.5 BY APPROXIMATELY 5 MG/L  
OF FREE CHLORINE IN THE PRESENCE & ABSENCE OF KCL

TEST #1 CHLORINE (MG/L): 5.27 KCL (MG/L): 0 CONTROL (PFU/ML): 128000  
INITIAL CHLORINE (MG/L): 5.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .25            | 1640                 | 1320                 | 1480                | 1.1562          | 98.8438             |
| .5             | 1080                 | 440                  | 760                 | .5937           | 99.4063             |
| .75            | 88                   | 64                   | 76                  | .0593           | 99.9407             |
| 1              | 4                    | 8                    | 6                   | 4.6E-03         | 99.9954             |
| 1.25           | 0                    | 4                    | 2                   | 1.5E-03         | 99.9985             |
| 1.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 1.75           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2              | 4                    | 4                    | 4                   | 3.1E-03         | 99.9969             |
| 2.25           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST #2 CHLORINE (MG/L): 5.25 KCL (MG/L): 21 CONTROL (PFU/ML): 128000  
INITIAL CHLORINE (MG/L): 5.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .25            | 1400                 | 1160                 | 1280                | 1               | 99                  |
| .5             | 440                  | 480                  | 460                 | .3393           | 99.6407             |
| .75            | 120                  | 108                  | 114                 | .089            | 99.911              |
| 1              | 36                   | 8                    | 22                  | .0171           | 99.9829             |
| 1.25           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 1.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 1.75           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.25           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 2. (CONTINUED)

TEST #3 CHLORINE (MG/L): 5.2 KCL (MG/L): 526 CONTROL (PFU/ML): 128000  
INITIAL CHLORINE (MG/L): 5.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .25            | 1360                 | 1800                 | 1580                | 1.2343          | 98.7657             |
| .5             | 196                  | 224                  | 210                 | .164            | 99.836              |
| .75            | 40                   | 20                   | 30                  | .0234           | 99.9766             |
| 1              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 1.25           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 1.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 1.75           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.25           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST #4 CHLORINE (MG/L): 5.15 KCL (MG/L): 1,262 CONTROL (PFU/ML): 128000  
INITIAL CHLORINE (MG/L): 5.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .25            | 160                  | 176                  | 168                 | .1312           | 99.8688             |
| .5             | 16                   | 16                   | 16                  | .0125           | 99.9875             |
| .75            | 4                    | 4                    | 4                   | 3.1E-03         | 99.9969             |
| 1              | 0                    | 4                    | 2                   | 1.5E-03         | 99.9985             |
| 1.25           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 1.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 1.75           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.25           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 3. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED  
PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 1 MG/L  
OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST #1 CHLORINE (MG/L): 1.22 KCL (MG/L): 0 CONTROL (PFU/ML): 167500  
INITIAL CHLORINE (MG/L): 1.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 5              | 40000                | 56000                | 48000               | 28.6567         | 71.3433             |
| 10             | 2400                 | 5200                 | 3800                | 2.2686          | 97.7314             |
| 15             | 2400                 | 2000                 | 2200                | 1.3134          | 98.6866             |
| 20             | 24                   | 44                   | 34                  | .0202           | 99.9798             |
| 25             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 30             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 35             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 40             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 45             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 50             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST #2 CHLORINE (MG/L): 1.16 KCL (MG/L): 21 CONTROL (PFU/ML): 167500  
INITIAL CHLORINE (MG/L): 1.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 4              | 64000                | 68000                | 66000               | 39.4029         | 60.5971             |
| 8              | 24000                | 28000                | 26000               | 15.5223         | 84.4777             |
| 12             | 6800                 | 6400                 | 5600                | 3.3432          | 96.6568             |
| 16             | 920                  | 1040                 | 980                 | .585            | 99.415              |
| 20             | 0                    | 8                    | 4                   | 2.3E-03         | 99.9977             |
| 24             | 12                   | 4                    | 8                   | 4.7E-03         | 99.9953             |
| 28             | 4                    | 0                    | 2                   | 1.1E-03         | 99.9989             |
| 32             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 36             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 40             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 3. (CONTINUED)

TEST #3 CHLORINE (MG/L): 1.14 KCL (MG/L): 526 CONTROL (PFU/ML): 167500  
INITIAL CHLORINE (MG/L): 1.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 4              | 68000                | 64000                | 66000               | 39.4029         | 60.5971             |
| 8              | 2000                 | 2480                 | 2240                | 1.3373          | 98.6627             |
| 12             | 60                   | 80                   | 70                  | .0417           | 99.9583             |
| 16             | 28                   | 20                   | 24                  | .0143           | 99.9857             |
| 20             | 16                   | 16                   | 16                  | 9.5E-03         | 99.9905             |
| 24             | 4                    | 0                    | 2                   | 1.1E-03         | 99.9989             |
| 28             | 0                    | 4                    | 2                   | 1.1E-03         | 99.9989             |
| 32             | 4                    | 12                   | 8                   | 4.7E-03         | 99.9953             |
| 36             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 40             | 12                   | 12                   | 12                  | 7.1E-03         | 99.9929             |

TEST #4 CHLORINE (MG/L): 1.2 KCL (MG/L): 1,262 CONTROL (PFU/ML): 167500  
INITIAL CHLORINE (MG/L): 1.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 4              | 640                  | 720                  | 680                 | .4059           | 99.5941             |
| 8              | 80                   | 40                   | 60                  | .0358           | 99.9642             |
| 12             | 32                   | 20                   | 26                  | .0133           | 99.9845             |
| 16             | 0                    | 16                   | 8                   | 4.7E-03         | 99.9953             |
| 20             | 0                    | 8                    | 4                   | 2.3E-03         | 99.9977             |
| 24             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 28             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 32             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 36             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 40             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 4. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED  
PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 1 MG/L  
OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL.

TEST #1 CHLORINE (MG/L): 1.08 KCL (MG/L): 0 CONTROL (PFU/ML): 117000  
INITIAL CHLORINE (MG/L): 1.12

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 2              | 52000                | 52000                | 52000               | 44.4444         | 55.5556             |
| 4              | 44000                | 104000               | 74000               | 63.2478         | 36.7522             |
| 6              | 21200                | 21200                | 21200               | 18.1194         | 81.8804             |
| 8              | 19200                | 20000                | 19600               | 16.7521         | 83.2479             |
| 10             | 9600                 | 6400                 | 8000                | 6.8376          | 93.1624             |
| 12             | 3200                 | 7200                 | 5200                | 4.4444          | 95.5556             |
| 14             | 1200                 | 1440                 | 1320                | 1.1282          | 98.8718             |
| 16             | 228                  | 116                  | 172                 | .147            | 99.853              |
| 18             | 108                  | 76                   | 92                  | .0786           | 99.9214             |
| 20             | 80                   | 68                   | 74                  | .0632           | 99.9368             |

TEST #2 CHLORINE (MG/L): 1.05 KCL (MG/L): 21 CONTROL (PFU/ML): 117000  
INITIAL CHLORINE (MG/L): 1.12

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 2              | 92000                | 100000               | 96000               | 82.0512001      | 17.9488             |
| 4              | 52000                | 48000                | 50000               | 42.733          | 57.265              |
| 6              | 16800                | 16800                | 16800               | 14.3589         | 85.6411             |
| 8              | 14400                | 16000                | 15200               | 12.9914         | 87.0086             |
| 10             | 11600                | 11600                | 11600               | 9.9145          | 90.0855             |
| 12             | 8000                 | 6000                 | 7000                | 5.9829          | 94.0171             |
| 14             | 2400                 | 2080                 | 2240                | 1.9145          | 98.0855             |
| 16             | 1000                 | 720                  | 860                 | .733            | 99.265              |
| 18             | 108                  | 144                  | 126                 | .1076           | 99.8924             |
| 20             | 12                   | 12                   | 12                  | .0102           | 99.9898             |

TABLE 4. (CONTINUED)

TEST #3 CHLORINE (MG/L): 1.04 KCl (MG/L): 526 CONTROL (PFU/ML): 117000  
INITIAL CHLORINE (MG/L): 1.12

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 2              | 116000               | 120000               | 118000              | 100.8347        | -.834699999         |
| 4              | 140000               | 108000               | 124000              | 105.9829        | -5.9829             |
| 6              | 72000                | 72000                | 72000               | 61.5384         | 38.4616             |
| 8              | 4800                 | 4400                 | 4600                | 3.9216          | 96.0684             |
| 10             | 9600                 | 11200                | 10400               | 8.8888          | 91.1112             |
| 12             | 2400                 | 2000                 | 2200                | 1.8803          | 98.1197             |
| 14             | 1360                 | 1040                 | 1200                | 1.0256          | 98.9744             |
| 16             | 156                  | 124                  | 140                 | .1196           | 99.8804             |
| 18             | 20                   | 20                   | 20                  | .017            | 99.983              |
| 20             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST #4 CHLORINE (MG/L): 1.01 KCl (MG/L): 1,262 CONTROL (PFU/ML): 117000  
INITIAL CHLORINE (MG/L): 1.12

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1.5            | 60000                | 96000                | 78000               | 66.6666         | 33.3334             |
| 3              | 92000                | 84000                | 88000               | 75.2136001      | 24.7864             |
| 4.5            | 8000                 | 20000                | 14000               | 11.9658         | 88.0342             |
| 6              | 15200                | 11600                | 13400               | 11.4529         | 88.5471             |
| 7.5            | 6400                 | 1600                 | 4000                | 3.4188          | 96.5812             |
| 9              | 600                  | 920                  | 760                 | .6495           | 99.3505             |
| 10.5           | 132                  | 88                   | 110                 | .094            | 99.906              |
| 12             | 8                    | 16                   | 12                  | .0102           | 99.9898             |
| 13.5           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 15             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 5. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED  
PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L  
OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCl

TEST #1 CHLORINE (MG/L): 5.05 KCl (MG/L): 0 CONTROL (PFU/ML): 111000  
INITIAL CHLORINE (MG/L): 5.25

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 80000                | 56000                | 68000               | 61.2612         | 38.7388             |
| 1              | 20800                | 24000                | 22400               | 20.1801         | 79.8199             |
| 1.5            | 18400                | 14000                | 16200               | 14.5945         | 85.4055             |
| 2              | 14800                | 12400                | 13600               | 12.2522         | 87.7478             |
| 2.5            | 8400                 | 10400                | 9400                | 8.4684          | 91.5316             |
| 3              | 8400                 | 6400                 | 7400                | 6.6666          | 93.3334             |
| 3.5            | 7600                 | 6000                 | 6800                | 6.1261          | 93.8739             |
| 4              | 1440                 | 1160                 | 1300                | 1.1711          | 98.8289             |
| 4.5            | 1040                 | 960                  | 1000                | .9009           | 99.0991             |
| 5              | 1400                 | 1160                 | 1280                | 1.1531          | 98.8469             |

TEST #2 CHLORINE (MG/L): 5 KCl (MG/L): 21 CONTROL (PFU/ML): 111000  
INITIAL CHLORINE (MG/L): 5.25

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 1              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 1.5            | 48000                | 48000                | 48000               | 43.2432         | 56.7568             |
| 2              | 17200                | 18800                | 18000               | 16.2162         | 83.7838             |
| 2.5            | 15200                | 12800                | 14000               | 12.6126         | 87.3874             |
| 3              | 7200                 | 9200                 | 8200                | 7.3873          | 92.6127             |
| 3.5            | 7200                 | 7200                 | 7200                | 6.4864          | 93.5136             |
| 4              | 4400                 | 5600                 | 5000                | 4.5045          | 95.4955             |
| 4.5            | 1560                 | 1560                 | 1560                | 1.4054          | 98.5946             |
| 5              | 880                  | 840                  | 860                 | .7747           | 99.2253             |

TABLE 5. (CONTINUED)

TEST #3 CHLORINE (MG/L): 5.05 KCl (MG/L): 526 CONTROL (PFU/ML): 111000  
INITIAL CHLORINE (MG/L): 5.25

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 16800                | 17600                | 17200               | 15.4954         | 84.5046             |
| 1              | 13600                | 12000                | 12800               | 11.5313         | 88.4687             |
| 1.5            | 1960                 | 2400                 | 2180                | 1.9639          | 98.0361             |
| 2              | 680                  | 440                  | 560                 | .5045           | 99.4955             |
| 2.5            | 80                   | 64                   | 72                  | .0648           | 99.9352             |
| 3              | 36                   | 16                   | 26                  | .0234           | 99.9766             |
| 3.5            | 4                    | 4                    | 4                   | 3.6E-03         | 99.9964             |
| 4              | 8                    | 24                   | 16                  | .0144           | 99.9856             |
| 4.5            | 4                    | 4                    | 4                   | 3.6E-03         | 99.9964             |
| 5              | 12                   | 12                   | 12                  | .0108           | 99.9892             |

TEST #4 CHLORINE (MG/L): 5 KCl (MG/L): 1,262 CONTROL (PFU/ML): 111000  
INITIAL CHLORINE (MG/L): 5.25

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 19200                | 24400                | 21800               | 19.6396         | 80.3604             |
| 1              | 1520                 | 1360                 | 1440                | 1.2972          | 98.7028             |
| 1.25           | 800                  | 520                  | 660                 | .5945           | 99.4055             |
| 1.5            | 116                  | 88                   | 102                 | .0918           | 99.9082             |
| 1.75           | 36                   | 8                    | 22                  | .0198           | 99.9802             |
| 2              | 32                   | 36                   | 34                  | .0306           | 99.9694             |
| 2.25           | 4                    | 16                   | 10                  | 9E-03           | 99.991              |
| 2.5            | 8                    | 12                   | 10                  | 9E-03           | 99.991              |
| 2.75           | 8                    | 4                    | 6                   | 5.4E-03         | 99.9946             |
| 3              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 6. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED  
TAP WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L OF FREE CHLORINE  
IN THE PRESENCE AND ABSENCE OF KCl

TEST #1 CHLORINE (MG/L): 5.2 KCl (MG/L): 0 CONTROL (PFU/ML): 60000  
INITIAL CHLORINE (MG/L): 5.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | 4400                 | 3200                 | 3800                | 6.3333          | 93.6667             |
| 2              | 40                   | 200                  | 120                 | .2              | 99.8                |
| 3              | 16                   | 24                   | 20                  | .0333           | 99.9667             |
| 4              | 4                    | 0                    | 2                   | 3.3E-03         | 99.9967             |
| 5              | 0                    | 4                    | 2                   | 3.3E-03         | 99.9967             |
| 6              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 7              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 8              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 9              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 10             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST #2 CHLORINE (MG/L): 5.17 KCl (MG/L): 21 CONTROL (PFU/ML): 60000  
INITIAL CHLORINE (MG/L): 5.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | 4400                 | 6800                 | 5600                | 9.3333          | 90.6667             |
| 2              | 144                  | 184                  | 164                 | .2733           | 99.7267             |
| 3              | 32                   | 20                   | 26                  | .0433           | 99.9567             |
| 4              | 0                    | 4                    | 2                   | 3.3E-03         | 99.9967             |
| 5              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 6              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 7              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 8              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 9              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 10             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 6. (CONTINUED)

TEST #3 CHLORINE (MG/L): 5.1 KCl (MG/L): 526 CONTROL (PFU/ML): 60000  
INITIAL CHLORINE (MG/L): 5.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 3440                 | 3200                 | 3320                | 5.5333          | 94.4667             |
| 1              | 160                  | 160                  | 160                 | .2666           | 99.7334             |
| 1.5            | 64                   | 64                   | 64                  | .1066           | 99.8934             |
| 2              | 20                   | 32                   | 26                  | .0433           | 99.9567             |
| 2.5            | 16                   | 8                    | 12                  | .0199           | 99.9801             |
| 3              | 12                   | 12                   | 12                  | .0199           | 99.9801             |
| 3.5            | 8                    | 12                   | 10                  | .0166           | 99.9834             |
| 4              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 4.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 5              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST #4 CHLORINE (MG/L): 5 KCl (MG/L): 1,262 CONTROL (PFU/ML): 60000  
INITIAL CHLORINE (MG/L): 5.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 680                  | 680                  | 680                 | 1.1333          | 98.8667             |
| 1              | 72                   | 88                   | 80                  | .1333           | 99.8667             |
| 1.25           | 48                   | 52                   | 50                  | .0833           | 99.9167             |
| 1.5            | 56                   | 52                   | 54                  | .0899           | 99.9101             |
| 1.75           | 32                   | 16                   | 24                  | .0399           | 99.9601             |
| 2              | 32                   | 20                   | 26                  | .0433           | 99.9567             |
| 2.25           | 12                   | 16                   | 14                  | .0233           | 99.9767             |
| 2.5            | 0                    | 8                    | 4                   | 6.6E-03         | 99.9934             |
| 2.75           | 12                   | 8                    | 10                  | .0166           | 99.9834             |
| 3              | 4                    | 12                   | 8                   | .0133           | 99.9867             |

TABLE 7. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED  
PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 1 MG/L OF  
FREE CHLORINE IN THE PRESENCE & ABSENCE OF KCl.

TEST #1 CHLORINE (MG/L): .95 KCl (MG/L): 0 CONTROL (PFU/ML): 145000  
INITIAL CHLORINE (MG/L): 1.03

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 4              | 88000                | 144000               | 116000              | 80              | 20                  |
| 6.5            | 88000                | 88000                | 88000               | 60.6896         | 39.3104             |
| 12             | 68000                | 72000                | 70000               | 48.2758         | 51.7242             |
| 16             | 64000                | 28000                | 46000               | 31.7241         | 68.2759             |
| 20             | 15600                | 17200                | 16400               | 11.3103         | 88.6897             |
| 24             | 15600                | 18400                | 17000               | 11.7241         | 88.2759             |
| 28             | 16800                | 16400                | 16600               | 11.4482         | 88.5518             |
| 32             | 8800                 | 9200                 | 9000                | 6.2068          | 93.7932             |
| 36             | 4400                 | 4000                 | 4200                | 2.8965          | 97.1035             |
| 40             | 4400                 | 3600                 | 4000                | 2.7586          | 97.2414             |
| 44             | 4800                 | 6000                 | 5400                | 3.7241          | 96.2759             |
| 48             | 2000                 | 1840                 | 1920                | 1.3241          | 98.6759             |

TEST #2 CHLORINE (MG/L): .93 KCl (MG/L): 21 CONTROL (PFU/ML): 145000  
INITIAL CHLORINE (MG/L): 1.03

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 4              | 120000               | 124000               | 122000              | 84.1379         | 15.8621             |
| 8              | 40000                | 40000                | 40000               | 27.5862         | 72.4138             |
| 12             | 44000                | 44000                | 44000               | 30.3448         | 69.6552             |
| 16             | 15200                | 14400                | 14800               | 10.2068         | 89.7932             |
| 20             | 14400                | 16400                | 15400               | 10.6206         | 89.3794             |
| 24             | 7600                 | 10800                | 9200                | 6.3448          | 93.6552             |
| 28             | 4000                 | 4000                 | 4000                | 2.7586          | 97.2414             |
| 32             | 1600                 | 1880                 | 1740                | 1.2             | 98.8                |
| 36             | 1080                 | 800                  | 940                 | .6482           | 99.3518             |
| 40             | 760                  | 480                  | 620                 | .4275           | 99.5725             |
| 44             | 360                  | 360                  | 360                 | .2482           | 99.7518             |
| 48             | 68                   | 60                   | 64                  | .0441           | 99.9559             |

TABLE 7. (CONTINUED)

TEST #3 CHLORINE (MG/L): .9 KCl (MG/L): 526 CONTROL (PFU/ML): 145000  
INITIAL CHLORINE (MG/L): 1.03

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 2              | 108000               | 132000               | 120000              | 82.7586         | 17.2414             |
| 4              | 48000                | 52000                | 50000               | 34.4827         | 65.5173             |
| 6              | 68000                | 28000                | 48000               | 33.1034         | 66.8966             |
| 8              | 12800                | 10800                | 11800               | 8.1379          | 91.8621             |
| 10             | 3200                 | 3600                 | 3400                | 2.3448          | 97.6552             |
| 12             | 720                  | 520                  | 620                 | .4275           | 99.5725             |
| 14             | 96                   | 84                   | 90                  | .062            | 99.938              |
| 16             | 12                   | 16                   | 14                  | 9.6E-03         | 99.9904             |
| 18             | 8                    | 0                    | 4                   | 2.7E-03         | 99.9973             |
| 20             | 4                    | 4                    | 4                   | 2.7E-03         | 99.9973             |
| 22             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 24             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |

TEST #4 CHLORINE (MG/L): .85 KCl (MG/L): 1,262 CONTROL (PFU/ML): 145000  
INITIAL CHLORINE (MG/L): 1.03

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | 76000                | 40000                | 58000               | 40              | 60                  |
| 2              | 1840                 | 2000                 | 1920                | 1.3241          | 98.6759             |
| 3              | 120                  | 112                  | 116                 | .0799           | 99.9201             |
| 4              | 28                   | 36                   | 32                  | .022            | 99.978              |
| 5              | 16                   | 4                    | 10                  | 6.8E-03         | 99.9932             |
| 6              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 7              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 8              | 4                    | 8                    | 6                   | 4.1E-03         | 99.9959             |
| 9              | 8                    | 0                    | 4                   | 2.7E-03         | 99.9973             |
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 11             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 12             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 8. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED  
PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L OF  
FREE CHLORINE IN THE PRESENCE & ABSENCE OF KCl.

TEST #1 CHLORINE (MG/L): 5 KCl (MG/L): 0 CONTROL (PFU/ML): 110000  
INITIAL CHLORINE (MG/L): 5.13

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 2              | 64000                | 32000                | 48000               | 43.6363         | 56.3637             |
| 4              | 14800                | 8000                 | 11400               | 10.3636         | 89.6364             |
| 6              | 11200                | 10400                | 10800               | 9.8181          | 90.1819             |
| 8              | 6400                 | 4800                 | 5600                | 5.0909          | 94.9091             |
| 10             | 1960                 | 1600                 | 1780                | 1.6181          | 98.3819             |
| 12             | 1480                 | 1400                 | 1440                | 1.309           | 98.691              |
| 14             | 480                  | 640                  | 560                 | .509            | 99.491              |
| 16             | 156                  | 124                  | 140                 | .1272           | 99.8728             |
| 18             | 68                   | 88                   | 78                  | .0709           | 99.9291             |
| 20             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |

TEST #2 CHLORINE (MG/L): 5 KCl (MG/L): 21 CONTROL (PFU/ML): 110000  
INITIAL CHLORINE (MG/L): 5.13

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 2              | 52000                | 32000                | 42000               | 38.1818         | 61.8182             |
| 4              | 15600                | 15200                | 15400               | 14              | 86                  |
| 6              | 10800                | 12400                | 11600               | 10.5454         | 89.4546             |
| 8              | 6800                 | 5200                 | 6000                | 5.45450001      | 94.5455             |
| 10             | 1600                 | 1800                 | 1700                | 1.5454          | 98.4546             |
| 12             | 800                  | 1040                 | 920                 | .8363           | 99.1637             |
| 14             | 196                  | 208                  | 202                 | .1836           | 99.8164             |
| 16             | 84                   | 84                   | 84                  | .0763           | 99.9237             |
| 18             | 8                    | 40                   | 24                  | .0218           | 99.9782             |
| 20             | 12                   | 16                   | 14                  | .6127           | 99.9873             |

TABLE 8. (CONTINUED)

TEST #3 CHLORINE (MG/L): 4.88 KCl (MG/L): 526 CONTROL (PFU/ML): 110000  
INITIAL CHLORINE (MG/L): 5.13

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 40000                | 44000                | 42000               | 38.1818         | 61.8182             |
| .75            | 12400                | 10400                | 11400               | 10.3636         | 89.6364             |
| 1              | 10800                | 9600                 | 10200               | 9.2727          | 90.7273             |
| 1.25           | 7600                 | 10400                | 9000                | 8.1818          | 91.8182             |
| 1.5            | 4400                 | 5600                 | 5000                | 4.5454          | 95.4546             |
| 1.75           | 1720                 | 1600                 | 1660                | 1.509           | 98.491              |
| 2              | 1560                 | 1400                 | 1480                | 1.3454          | 98.6546             |
| 2.25           | 680                  | 1040                 | 860                 | .7818           | 99.2182             |
| 2.5            | 440                  | 440                  | 440                 | .4              | 99.6                |
| 3              | 520                  | 440                  | 480                 | .4363           | 99.5637             |

TEST #4 CHLORINE (MG/L): 4.83 KCl (MG/L): 1,262 CONTROL (PFU/ML): 110000  
INITIAL CHLORINE (MG/L): 5.13

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .25            | 19600                | 20000                | 19800               | 18              | 82                  |
| .5             | 12000                | 8800                 | 10400               | 9.4545          | 90.5455             |
| .75            | 8000                 | 4000                 | 6000                | 5.45450001      | 94.5455             |
| 1              | 1840                 | 1720                 | 1780                | 1.6181          | 98.3819             |
| 1.25           | 840                  | 680                  | 760                 | .6909           | 99.3091             |
| 1.5            | 440                  | 600                  | 520                 | .4727           | 99.5273             |
| 1.75           | 252                  | 240                  | 246                 | .2236           | 99.7764             |
| 2              | 96                   | 84                   | 90                  | .0818           | 99.9182             |
| 2.25           | 40                   | 44                   | 42                  | .0381           | 99.9619             |
| 2.5            | 24                   | 36                   | 30                  | .0272           | 99.9728             |

TABLE 9. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED  
PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L  
FREE CHLORINE IN THE PRESENCE & ABSENCE OF KCl.

TEST #1 CHLORINE (MG/L): 5 KCl (MG/L): 0 CONTROL (PFU/ML): 110000  
INITIAL CHLORINE (MG/L): 5.05

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | 24000                | 48000                | 36000               | 32.7272         | 67.2728             |
| 2              | 18400                | 16800                | 17600               | 16              | 84                  |
| 3              | 16000                | 32000                | 24000               | 21.8181         | 78.1819             |
| 4              | 8800                 | 12000                | 10400               | 9.4545          | 90.5455             |
| 5              | 11600                | 9200                 | 10400               | 9.4545          | 90.5455             |
| 6              | 5600                 | 7600                 | 6600                | 6               | 94                  |
| 7              | 6000                 | 7200                 | 6600                | 6               | 94                  |
| 8              | 4000                 | 4800                 | 4400                | 4               | 96                  |
| 9              | 2800                 | 2560                 | 2680                | 2.4363          | 97.5637             |
| 10             | 6800                 | 6800                 | 6800                | 6.1818          | 93.8182             |
| 12             | 1640                 | 1640                 | 1640                | 1.4909          | 98.5091             |
| 14             | 1400                 | 1320                 | 1460                | 1.3272          | 98.6728             |

TEST #2 CHLORINE (MG/L): 5.05 KCl (MG/L): 21 CONTROL (PFU/ML): 110000  
INITIAL CHLORINE (MG/L): 5.05

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | 40000                | 80000                | 60000               | 54.5454         | 45.4546             |
| 2              | 44000                | 36000                | 40000               | 36.3636         | 63.6364             |
| 3              | 15200                | 16400                | 15800               | 14.3636         | 85.6364             |
| 4              | 10800                | 11600                | 11200               | 10.1818         | 89.8182             |
| 5              | 9600                 | 9600                 | 9600                | 8.72720001      | 91.2728             |
| 6              | 7600                 | 8000                 | 7800                | 7.0909          | 92.9091             |
| 7              | 12000                | 11600                | 11800               | 10.7272         | 89.2728             |
| 8              | 8400                 | 9200                 | 8800                | 8               | 92                  |
| 9              | 6000                 | 4400                 | 5200                | 4.7272          | 95.2728             |
| 10             | 2560                 | 2440                 | 2500                | 2.2727          | 97.7273             |
| 12             | 1320                 | 320                  | 820                 | .7454           | 99.2546             |
| 14             | 268                  | 232                  | 250                 | .2272           | 99.7728             |

TABLE 9. (CONTINUED)

TEST #3 CHLORINE (MG/L): 4.95 KCl (MG/L): 526 CONTROL (PFU/ML): 110000  
INITIAL CHLORINE (MG/L): 5.05

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 17200                | 7600                 | 12400               | 11.2727         | 88.7273             |
| 1              | 5300                 | 3600                 | 5200                | 4.7272          | 95.2728             |
| 1.5            | 1200                 | 1200                 | 1200                | 1.0909          | 98.9091             |
| 2              | 196                  | 208                  | 202                 | .1836           | 99.8164             |
| 2.5            | 24                   | 36                   | 30                  | .0272           | 99.9728             |
| 3              | 24                   | 16                   | 20                  | .0181           | 99.9819             |
| 3.5            | 12                   | 12                   | 12                  | .0109           | 99.9891             |
| 4              | 28                   | 28                   | 28                  | .0254           | 99.9746             |
| 4.5            | 8                    | 8                    | 8                   | 7.2E-03         | 99.9928             |
| 5              | 12                   | 8                    | 10                  | 9E-03           | 99.991              |
| 5.5            | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 6              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |

TEST #4 CHLORINE (MG/L): 5 KCl (MG/L): 1,262 CONTROL (PFU/ML): 110000  
INITIAL CHLORINE (MG/L): 5.05

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| .75            | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 1              | 600                  | 560                  | 580                 | .5272           | 99.4728             |
| 1.25           | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 1.5            | 72                   | 32                   | 52                  | .0472           | 99.9528             |
| 1.75           | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 2              | 16                   | 20                   | 18                  | .0163           | 99.9837             |
| 2.25           | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 2.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.75           | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 3              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 3.25           | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |

TABLE 10. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED TAP  
WATER AT PH 9.0 BY APPROXIMATELY 1 MG/L OF FREE CHLORINE IN  
THE PRESENCE AND ABSENCE OF KCl.

TEST #1 CHLORINE (MG/L): .95 KCl (MG/L): 0 CONTROL (PFU/ML): 132000  
INITIAL CHLORINE (MG/L): 1.2

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 6              | 1640                 | 1840                 | 1740                | 1.3181          | 98.6819             |
| 12             | 8                    | 8                    | 8                   | 6E-03           | 99.994              |
| 18             | 4                    | 8                    | 6                   | 4.5E-03         | 99.9955             |
| 24             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 30             | 4                    | 0                    | 2                   | 1.5E-03         | 99.9985             |
| 36             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 42             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 48             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 54             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 60             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST #2 CHLORINE (MG/L): 1.02 KCl (MG/L): 21 CONTROL (PFU/ML): 132000  
INITIAL CHLORINE (MG/L): 1.2

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 6              | 6400                 | 5600                 | 6000                | 4.5454          | 95.4546             |
| 12             | 68                   | 88                   | 78                  | .059            | 99.941              |
| 18             | 32                   | 20                   | 26                  | .0196           | 99.9804             |
| 24             | 4                    | 16                   | 10                  | 7.5E-03         | 99.9925             |
| 30             | 4                    | 0                    | 2                   | 1.5E-03         | 99.9985             |
| 36             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 42             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 48             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 54             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 60             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 10. (CONTINUED)

TEST #3 CHLORINE (MG/L): 1 KCl (MG/L): 526 CONTROL (PFU/ML): 132000  
INITIAL CHLORINE (MG/L): 1.2

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 2              | 1720                 | 1400                 | 1560                | 1.1818          | 98.8182             |
| 4              | 1760                 | 1520                 | 1640                | 1.2424          | 98.7576             |
| 6              | 196                  | 164                  | 180                 | .1363           | 99.8637             |
| 8              | 84                   | 68                   | 76                  | .0573           | 99.9425             |
| 10             | 16                   | 12                   | 14                  | .0106           | 99.9894             |
| 12             | 80                   | 64                   | 72                  | .0543           | 99.9455             |
| 14             | 36                   | 12                   | 24                  | .0181           | 99.9819             |
| 16             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 18             | 8                    | 24                   | 16                  | .0121           | 99.9879             |
| 20             | 0                    | 4                    | 2                   | 1.5E-03         | 99.9983             |

TEST #4 CHLORINE (MG/L): 1.02 KCl (MG/L): 1,262 CONTROL (PFU/ML): 132000  
INITIAL CHLORINE (MG/L): 1.2

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 12400                | 6400                 | 9400                | 7.1212          | 92.8788             |
| 1              | 2600                 | 2800                 | 2700                | 2.0454          | 97.9546             |
| 1.5            | 4000                 | 4000                 | 4000                | 3.0303          | 96.9697             |
| 2              | 680                  | 640                  | 660                 | .5              | 99.5                |
| 2.5            | 192                  | 164                  | 178                 | .1343           | 99.8652             |
| 3              | 152                  | 164                  | 158                 | .1196           | 99.8804             |
| 3.5            | 760                  | 640                  | 700                 | .5303           | 99.4697             |
| 4              | 92                   | 108                  | 100                 | .0757           | 99.9243             |
| 4.5            | 36                   | 40                   | 38                  | .0287           | 99.9713             |
| 5              | 104                  | 96                   | 100                 | .0757           | 99.9243             |

TABLE 11. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED TAP WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCl.

TEST #1 CHLORINE (MG/L): 4.98 KCl (MG/L): 0 CONTROL (PFU/ML): 175000  
INITIAL CHLORINE (MG/L): 5.05

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 2              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 4              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 6              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 8              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 12             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 14             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 16             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 18             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 20             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |

TEST #2 CHLORINE (MG/L): 5 KCl (MG/L): 0 CONTROL (PFU/ML): 175000  
INITIAL CHLORINE (MG/L): 5.05

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1.5            | 1200                 | 1040                 | 1120                | .6399           | 99.3601             |
| 3              | 10                   | 8                    | 12                  | 6.8E-03         | 99.9932             |
| 4.5            | 4                    | 4                    | 4                   | 2.2E-03         | 99.9978             |
| 6              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 7.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 9              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 11             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 13             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 15             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 17             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TABLE 11. (CONTINUED)

TEST #3 CHLORINE (MG/L): 4.85 KCl (MG/L): 526 CONTROL (PFU/ML): 175000  
INITIAL CHLORINE (MG/L): 5.05

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 18800                | 22800                | 20800               | 11.8857         | 88.1143             |
| 1              | 2240                 | 1800                 | 2020                | 1.1542          | 98.8458             |
| 1.5            | 220                  | 236                  | 228                 | .1302           | 99.8698             |
| 2              | 20                   | 16                   | 18                  | .0102           | 99.9898             |
| 2.5            | 8                    | 20                   | 14                  | 8E-03           | 99.992              |
| 3              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 3.5            | 4                    | 0                    | 2                   | 1.1E-03         | 99.9989             |
| 4              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 4.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 5              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST #4 CHLORINE (MG/L): 4.8 KCl (MG/L): 1,262 CONTROL (PFU/ML): 175000  
INITIAL CHLORINE (MG/L): 5.05

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .25            | 84000                | 92000                | 88000               | 50.2857         | 49.7143             |
| .5             | 15200                | 19200                | 17200               | 9.8285          | 90.1715             |
| .75            | 6000                 | 8800                 | 7400                | 4.2285          | 95.7715             |
| 1              | 2120                 | 1960                 | 2040                | 1.1657          | 98.8343             |
| 1.25           | 208                  | 248                  | 228                 | .1302           | 99.8698             |
| 1.5            | 40                   | 40                   | 40                  | .0228           | 99.9772             |
| 1.75           | 4                    | 4                    | 4                   | 2.2E-03         | 99.9978             |
| 2              | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 2.25           | 4                    | 4                    | 4                   | 2.2E-03         | 99.9978             |
| 2.5            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |



**APPENDIX B**

**(LIST OF FIGURES [pH 4.5 and 9.0])**

FIGURE 1. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN PHTHALATE-BUFFERED  
PURIFIED WATER AT PH 4.5 BY APPROXIMATELY 1 MG/L  
OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

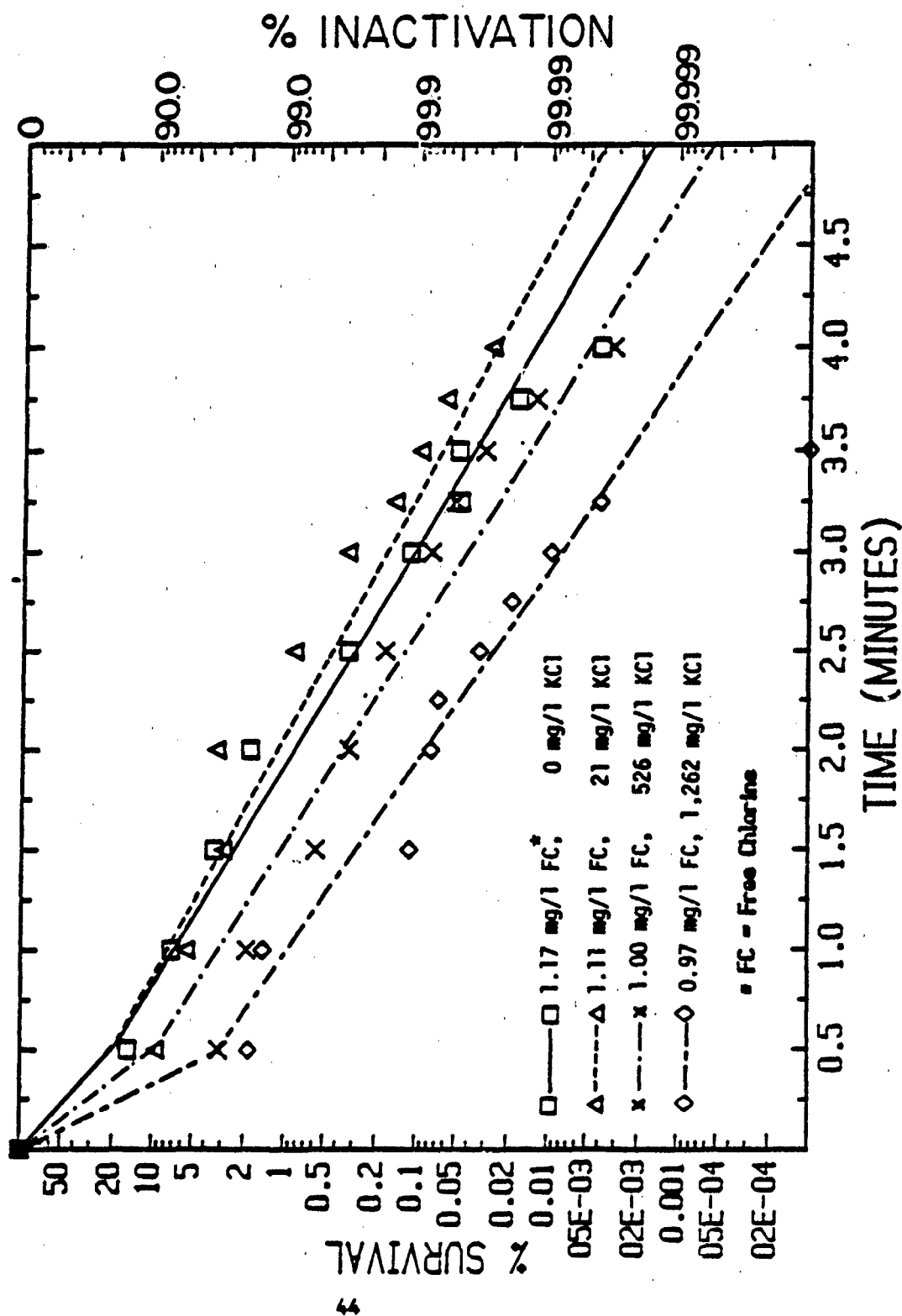


FIGURE 2. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN PHTHALATE-BUFFERED PURIFIED WATER AT PH 4.5 BY APPROXIMATELY 5 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

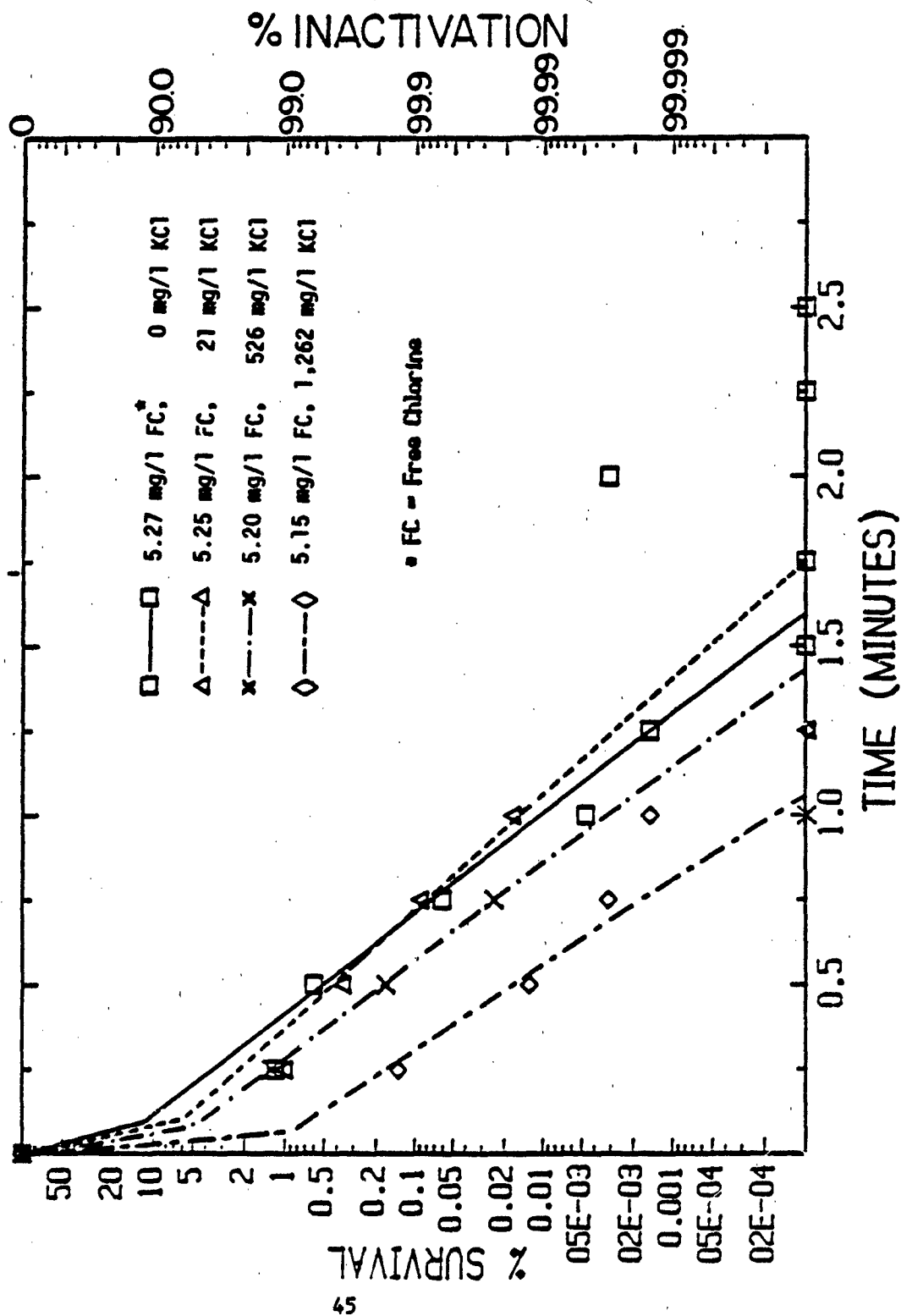


FIGURE 3. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED  
PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 1 MG/L OF  
FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

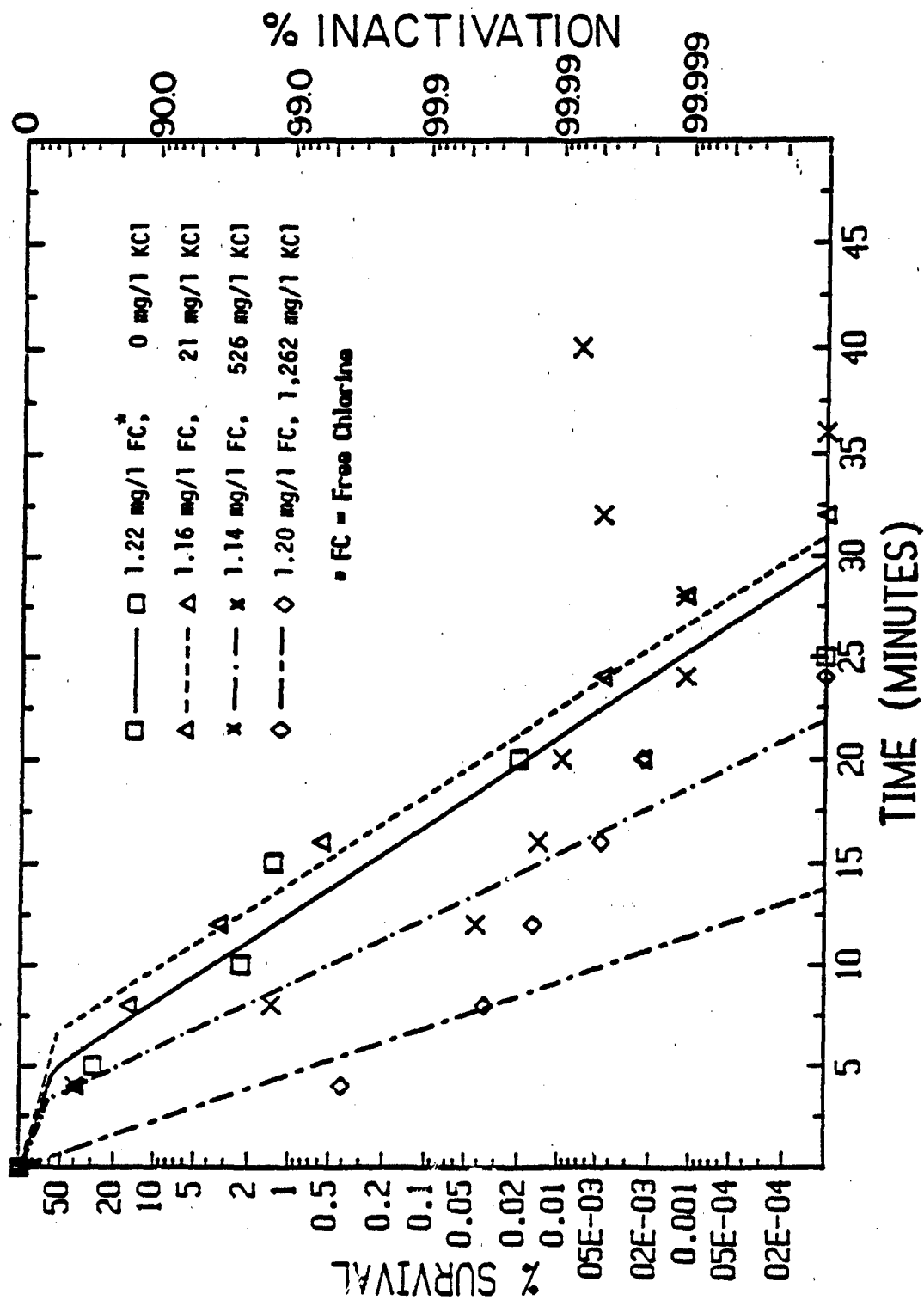


FIGURE 4. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 1 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

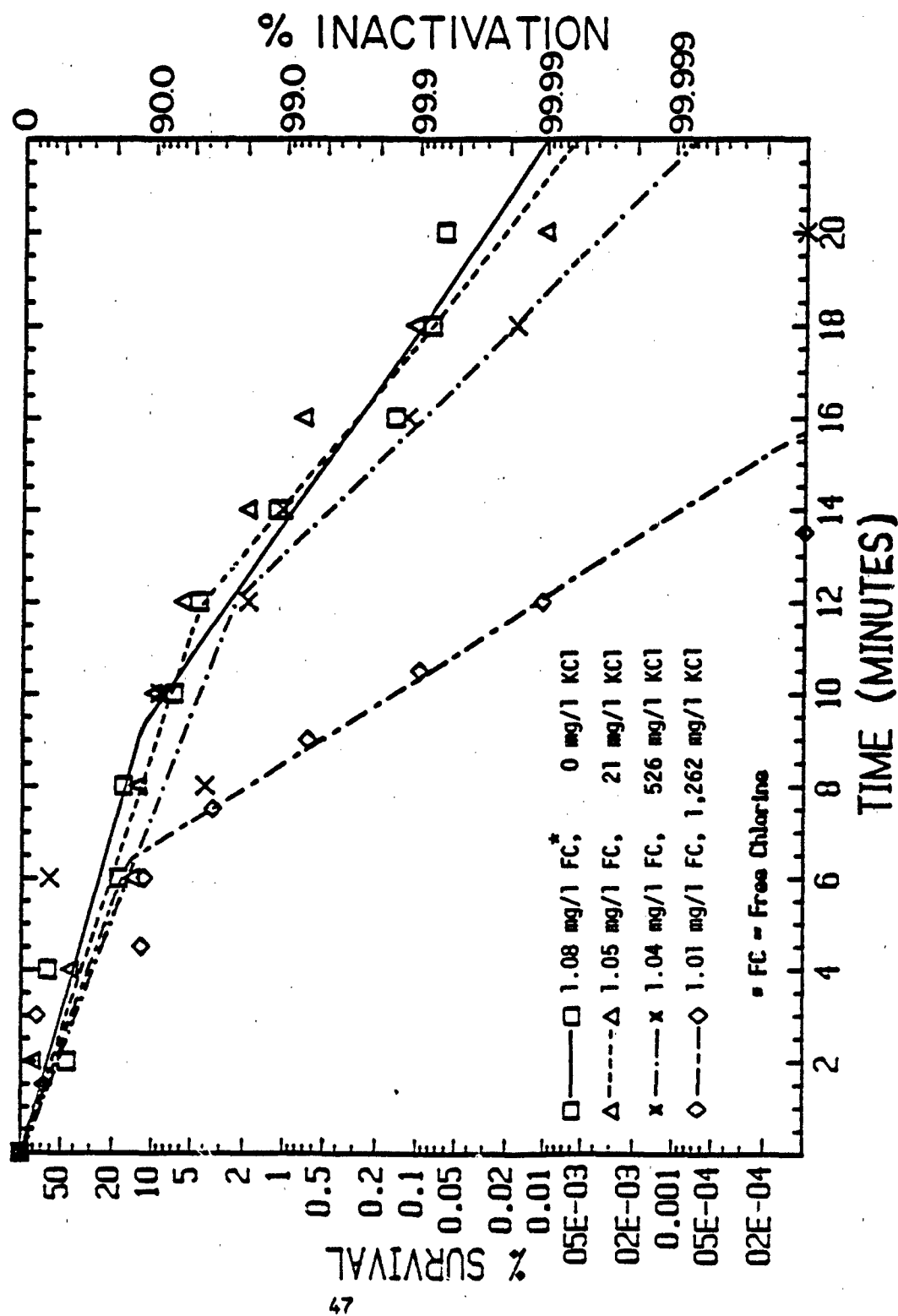


FIGURE 5. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

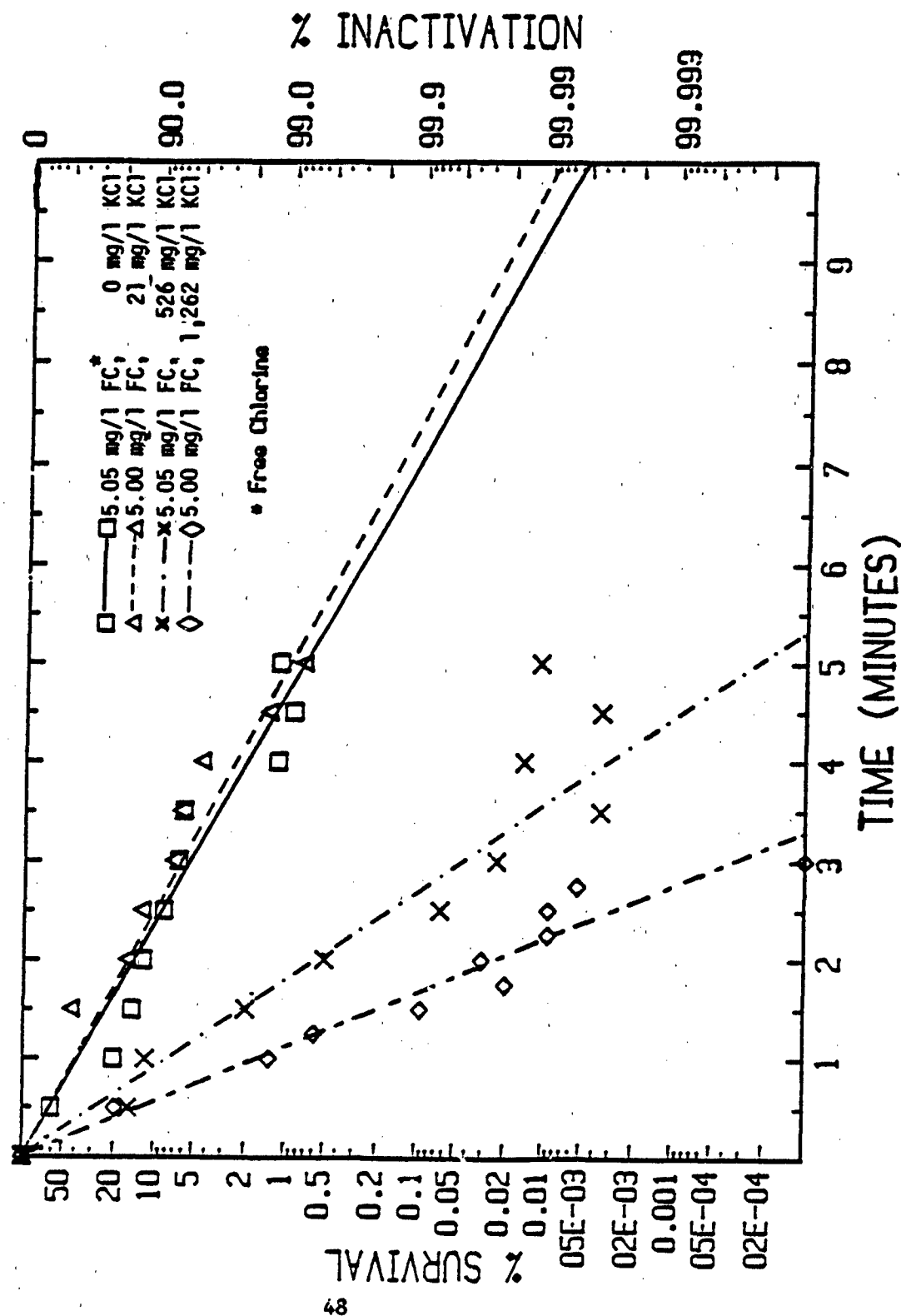


FIGURE 6. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED  
TAP WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L OF FREE CHLORINE  
IN THE PRESENCE AND ABSENCE OF KCL

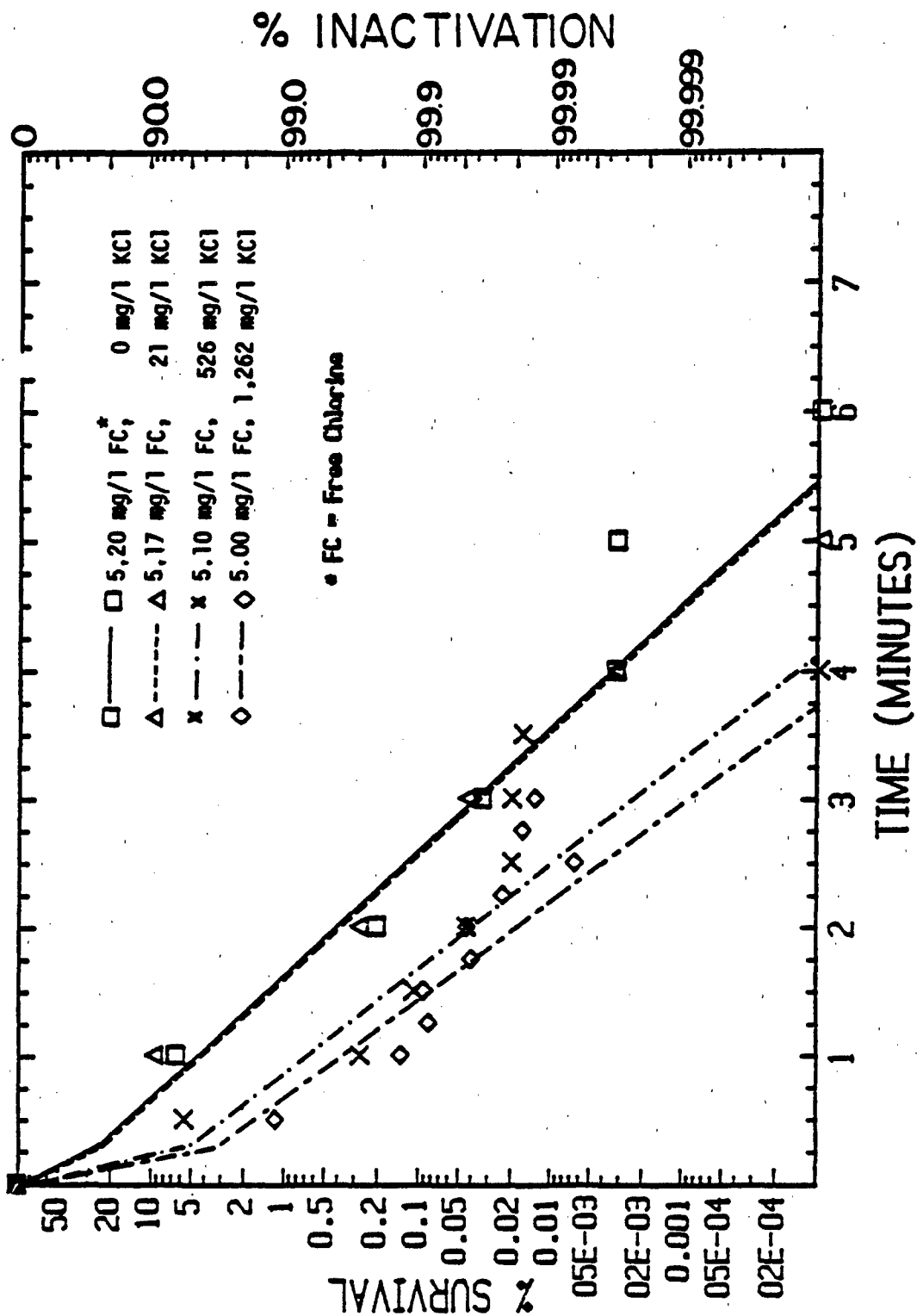


FIGURE 7. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 1 MG/L OF FREE CHLORINE IN THE PRESENCE & ABSENCE OF KCL

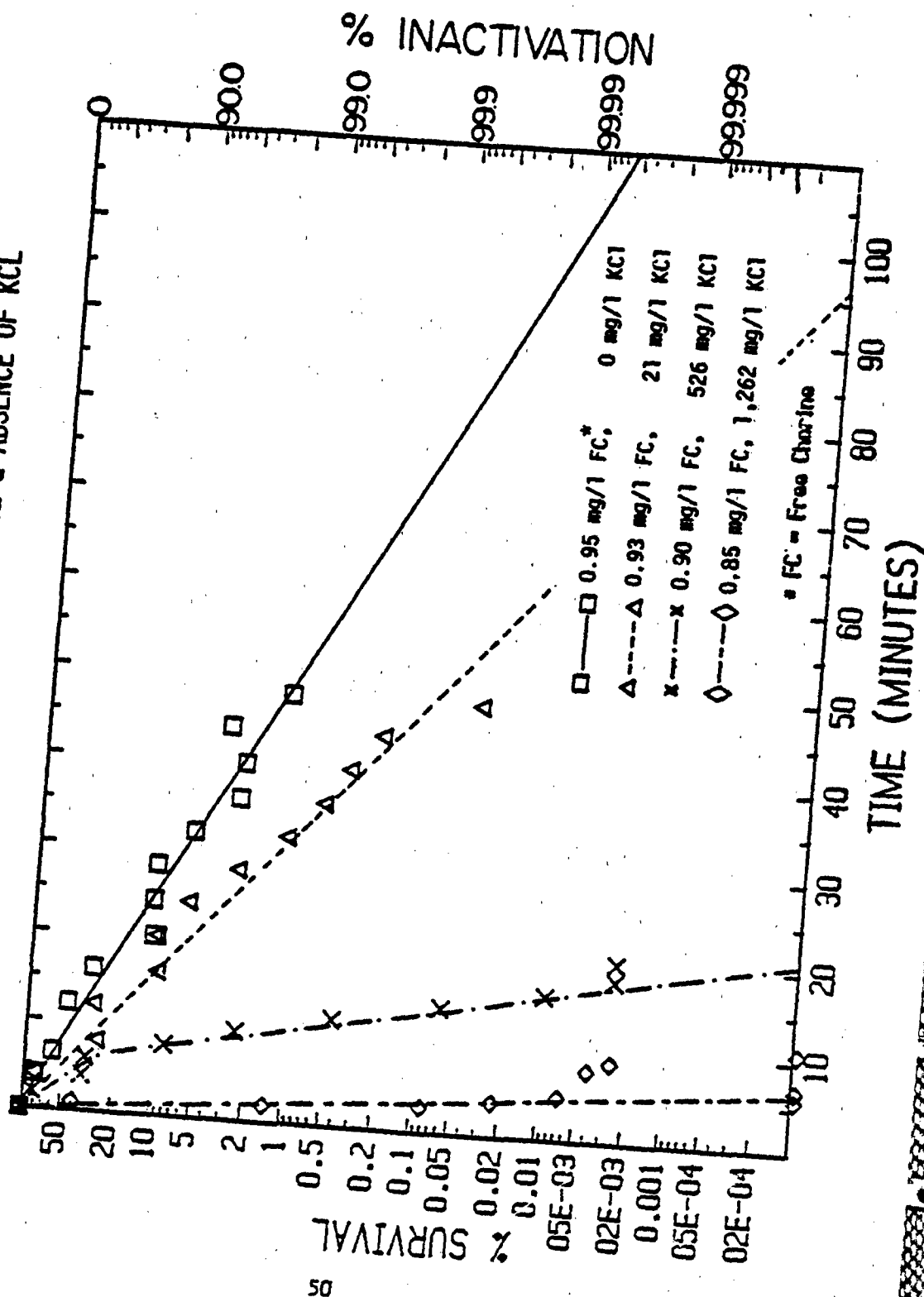


FIGURE 8. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L OF FREE CHLORINE IN THE PRESENCE & ABSENCE OF KCL

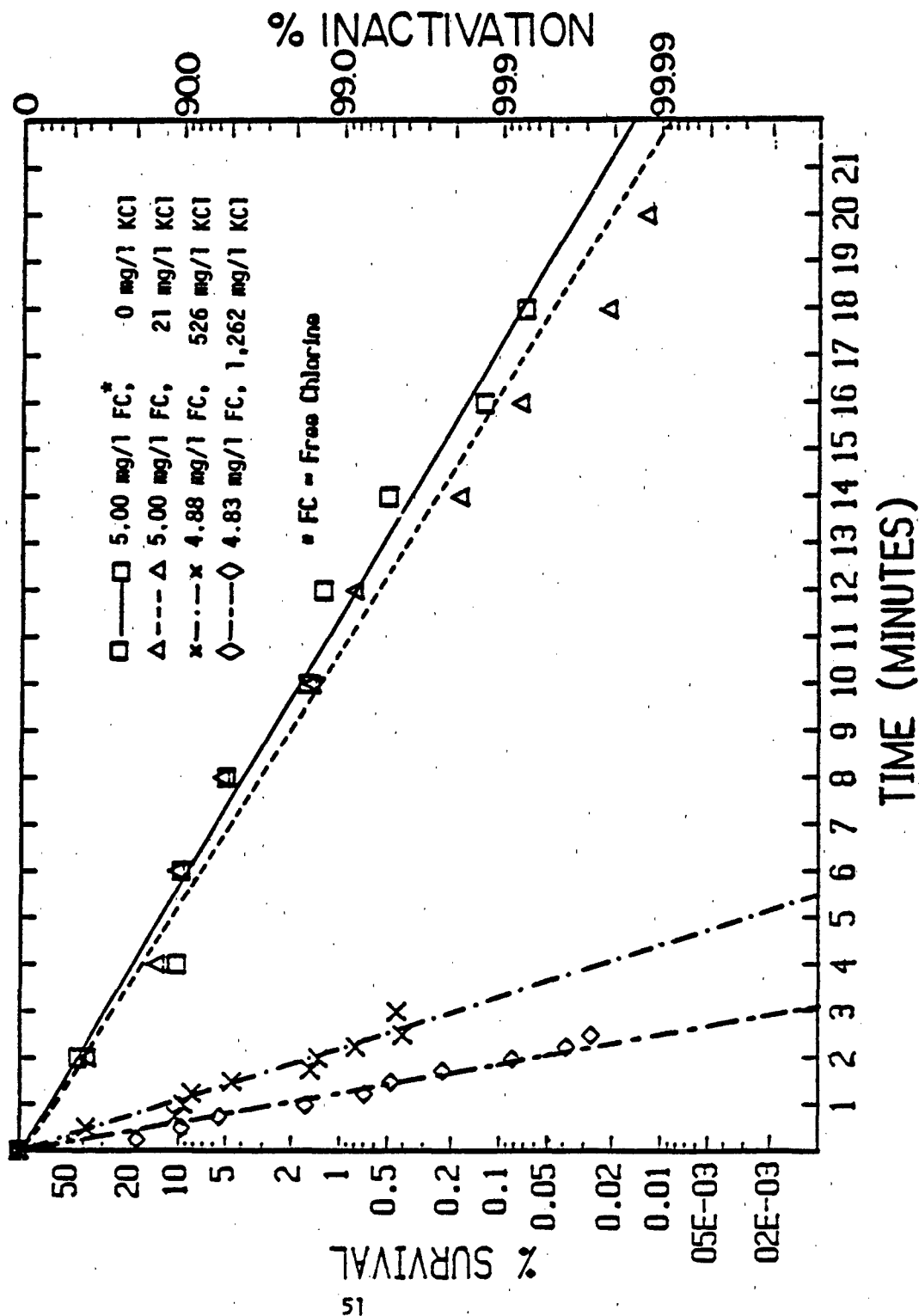


FIGURE 9. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED  
PURIFIED WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L  
OF FREE CHLORINE IN THE PRESENCE & ABSENCE OF KCL

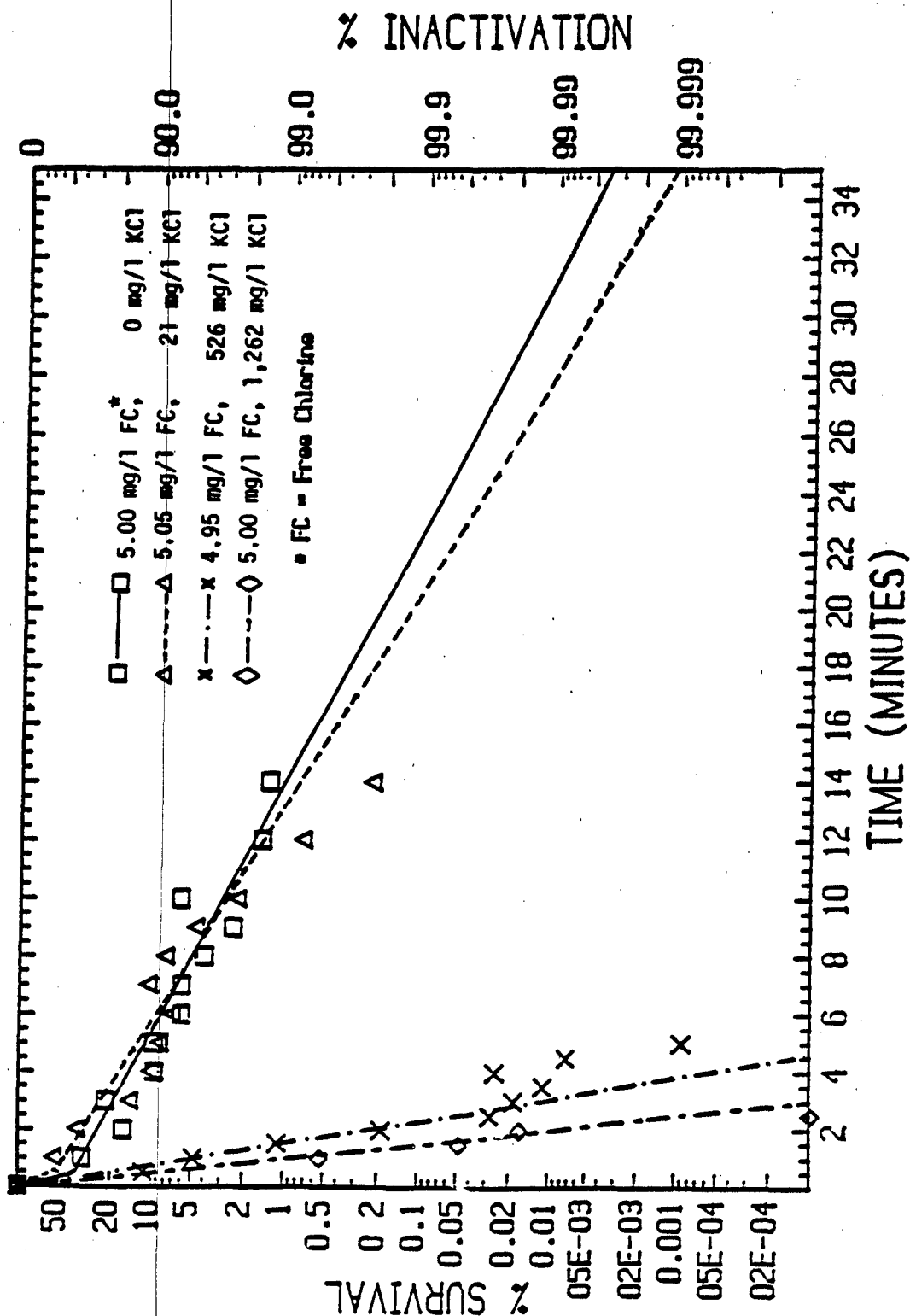


FIGURE 10. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED TAP WATER AT PH 9.0 BY APPROXIMATELY 1 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

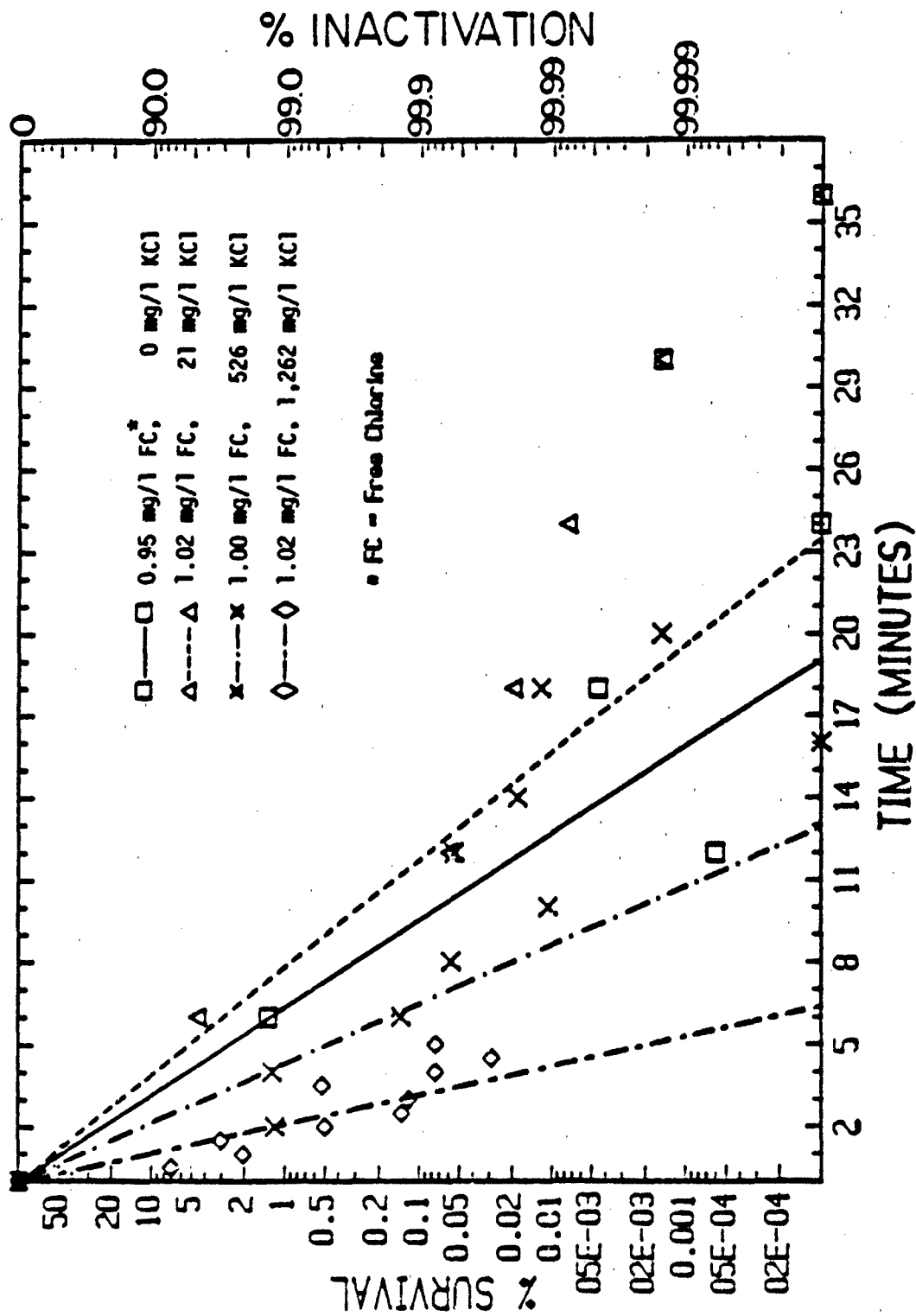


FIGURE 11. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN NONBUFFERED TAP WATER AT PH 9.0 BY APPROXIMATELY 5 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

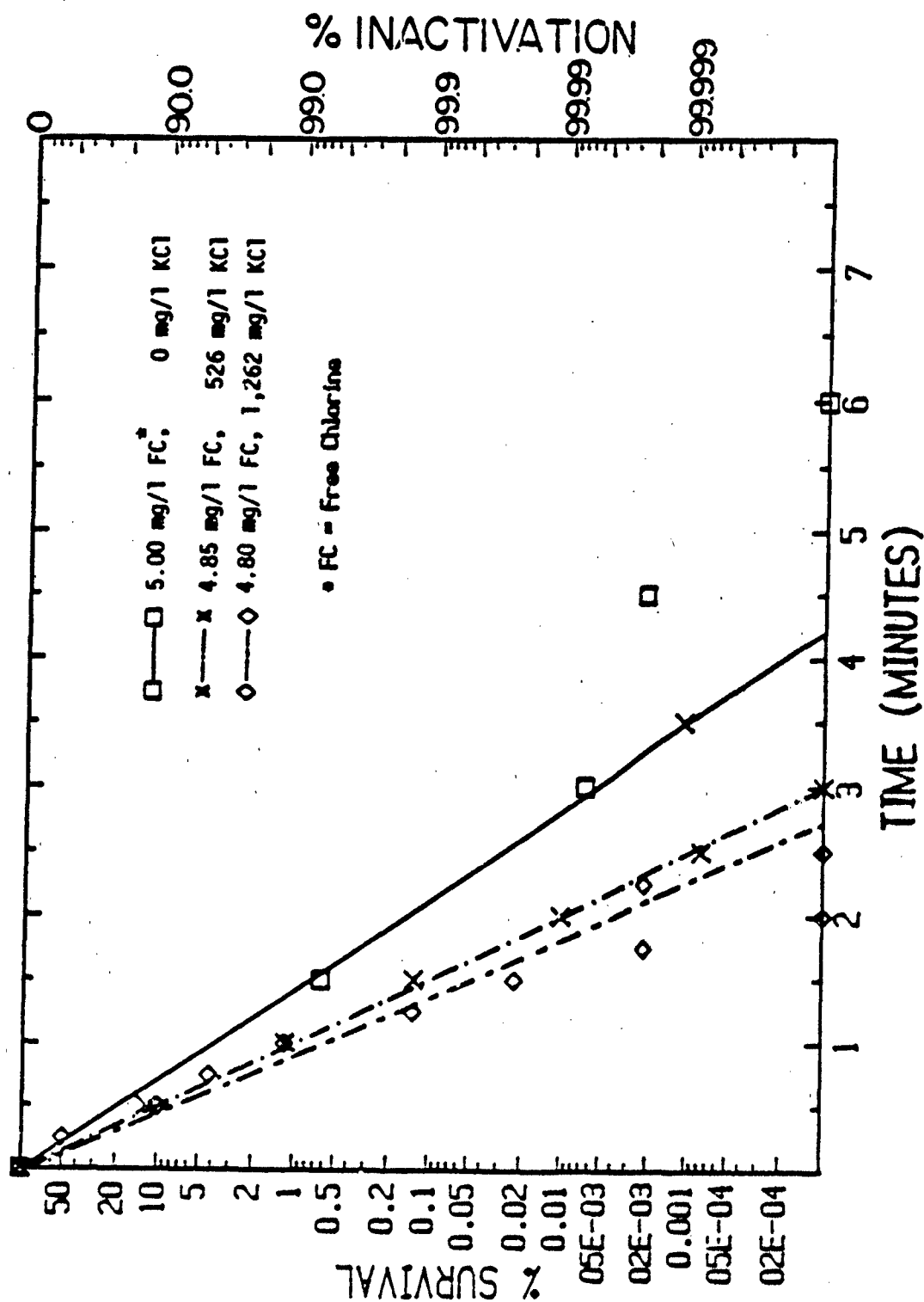


FIGURE 12. EFFECT OF  $\text{Cl}^-$  ON THE INACTIVATION OF POLIOVIRUS 1  
(99.99%) AT 5°C BY FREE CHLORINE AT PH 4.5 IN PHTHALATE-  
BUFFERED PURIFIED WATER

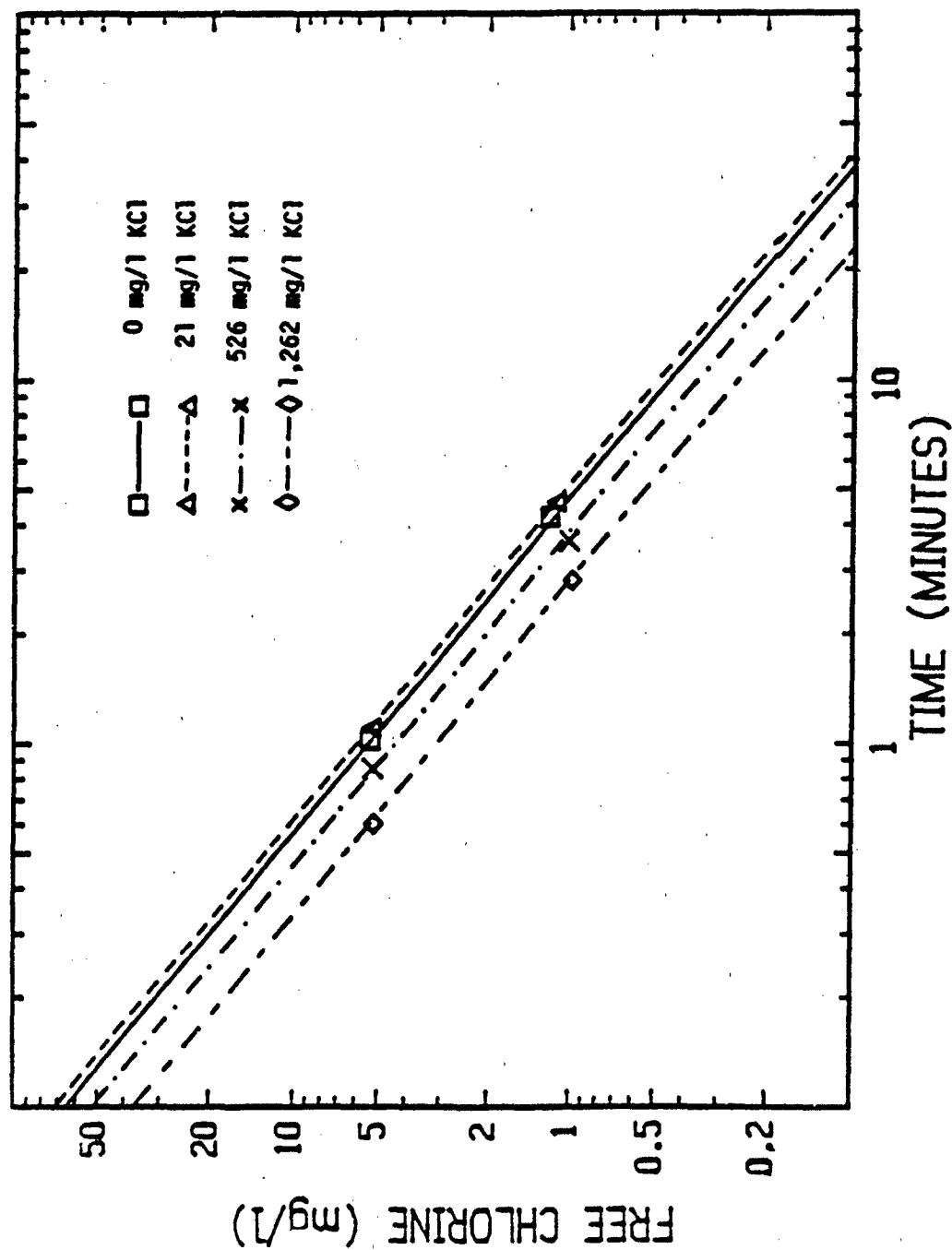


FIGURE 13, EFFECT OF KCL ON THE INACTIVATION OF POLIOVIRUS 1  
(99,99%) AT 5°C BY FREE CHLORINE AT PH 9.0 IN  
BORATE-BUFFERED PURIFIED WATER

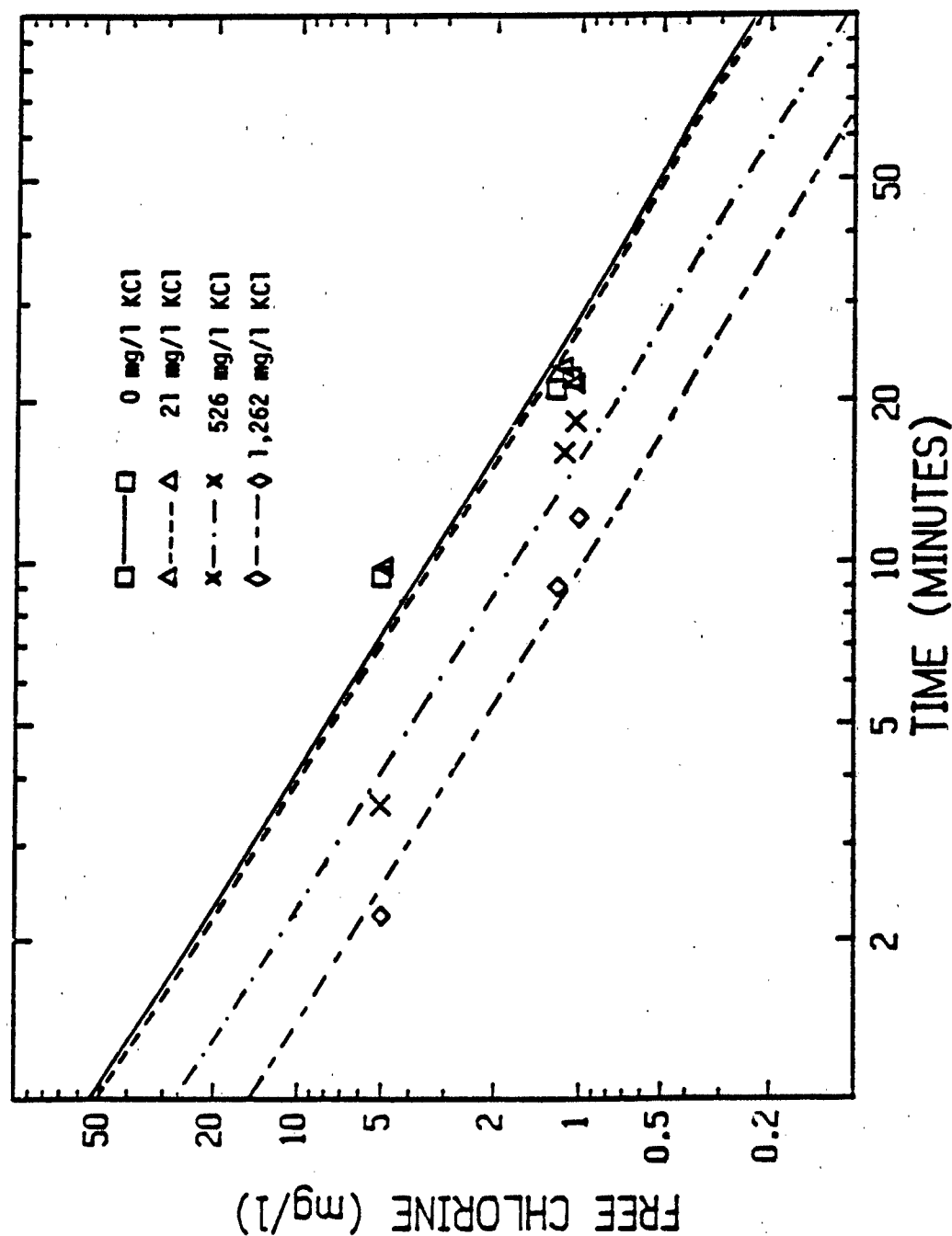


FIGURE 14. EFFECT OF KCL ON THE INACTIVATION OF POLIOVIRUS 1  
(99.99%) AT 5°C BY FREE CHLORINE AT PH 9.0 IN  
NONBUFFERED PURIFIED WATER

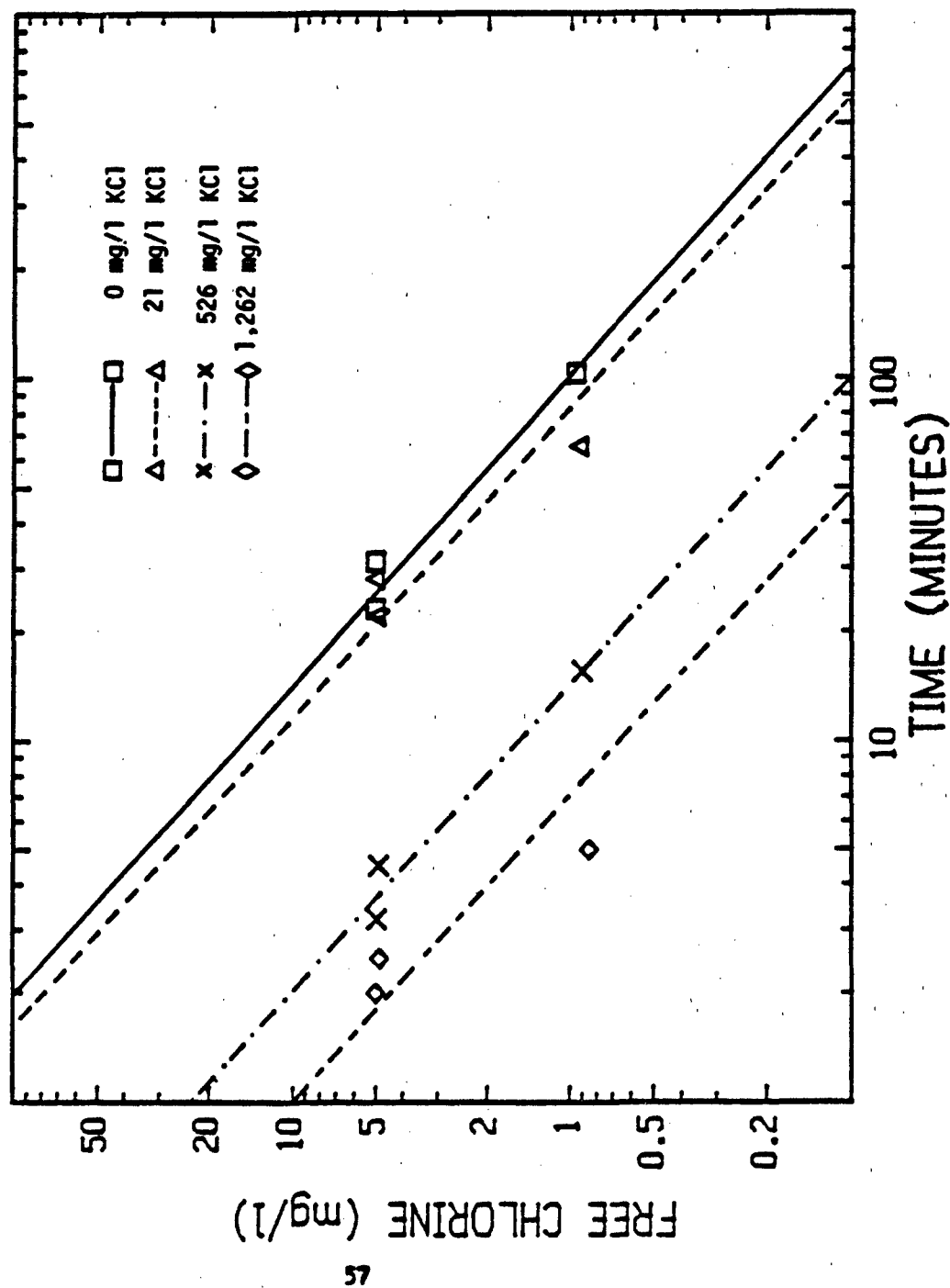


FIGURE 15. EFFECT OF 0.05 M BORATE BUFFER ON THE INACTIVATION OF POLIOVIRUS 1 (99.99%) AT 5°C BY FREE CHLORINE AT PH 9.0

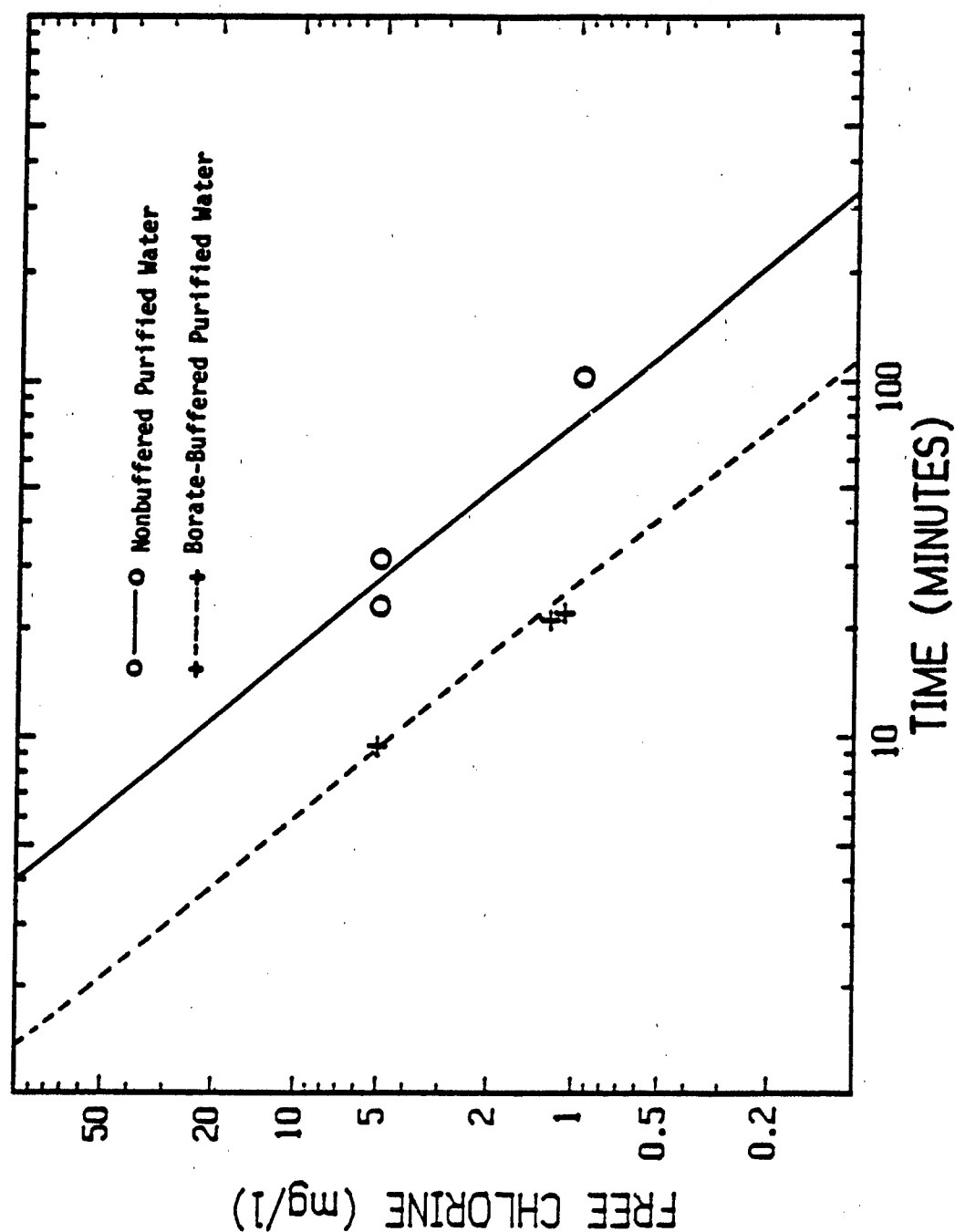


FIGURE 16. EFFECT OF KCL ON THE INACTIVATION OF POLIOVIRUS 1  
(99.99%) AT 5°C BY FREE CHLORINE AT PH 9.0 IN  
NONBUFFERED TAP WATER

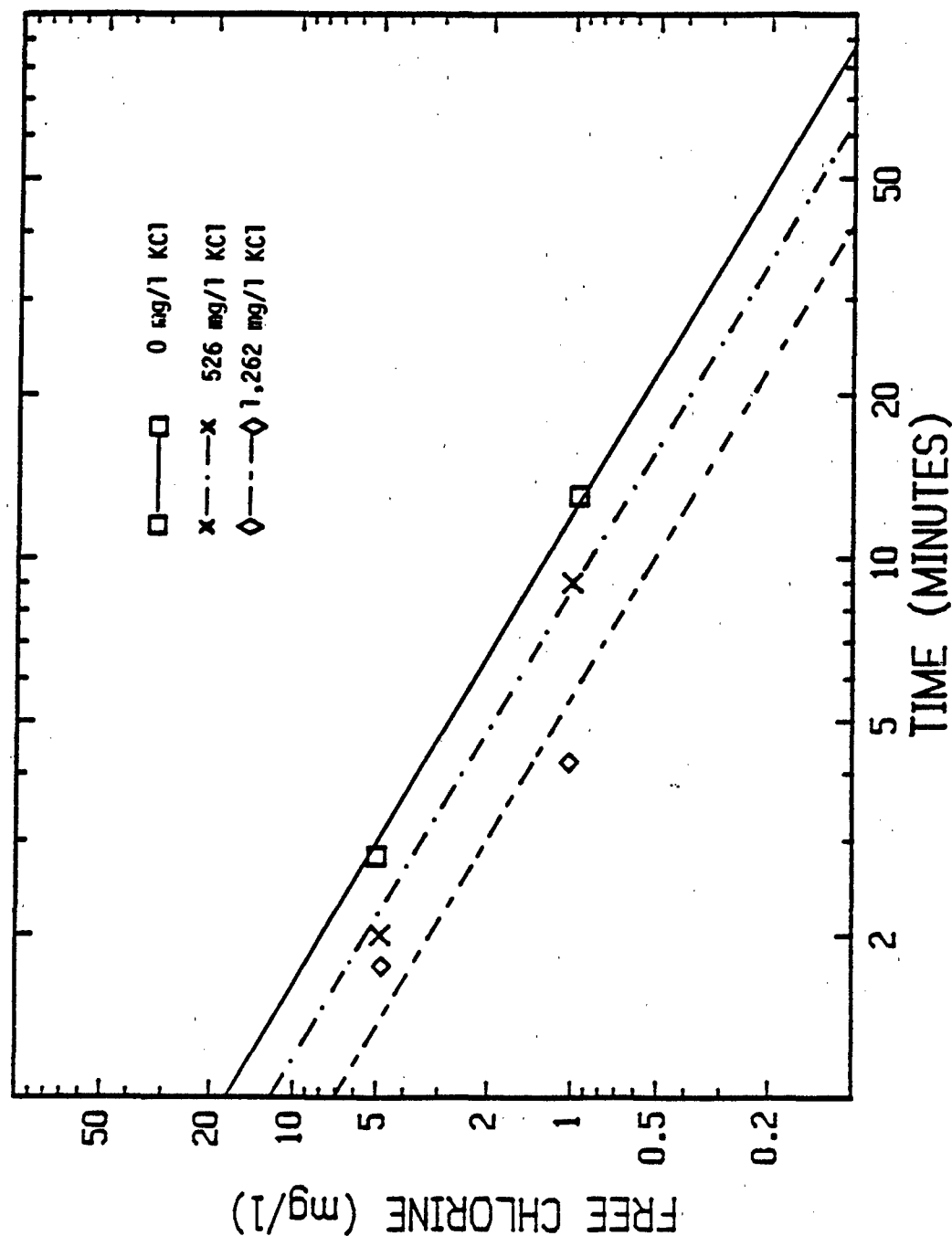
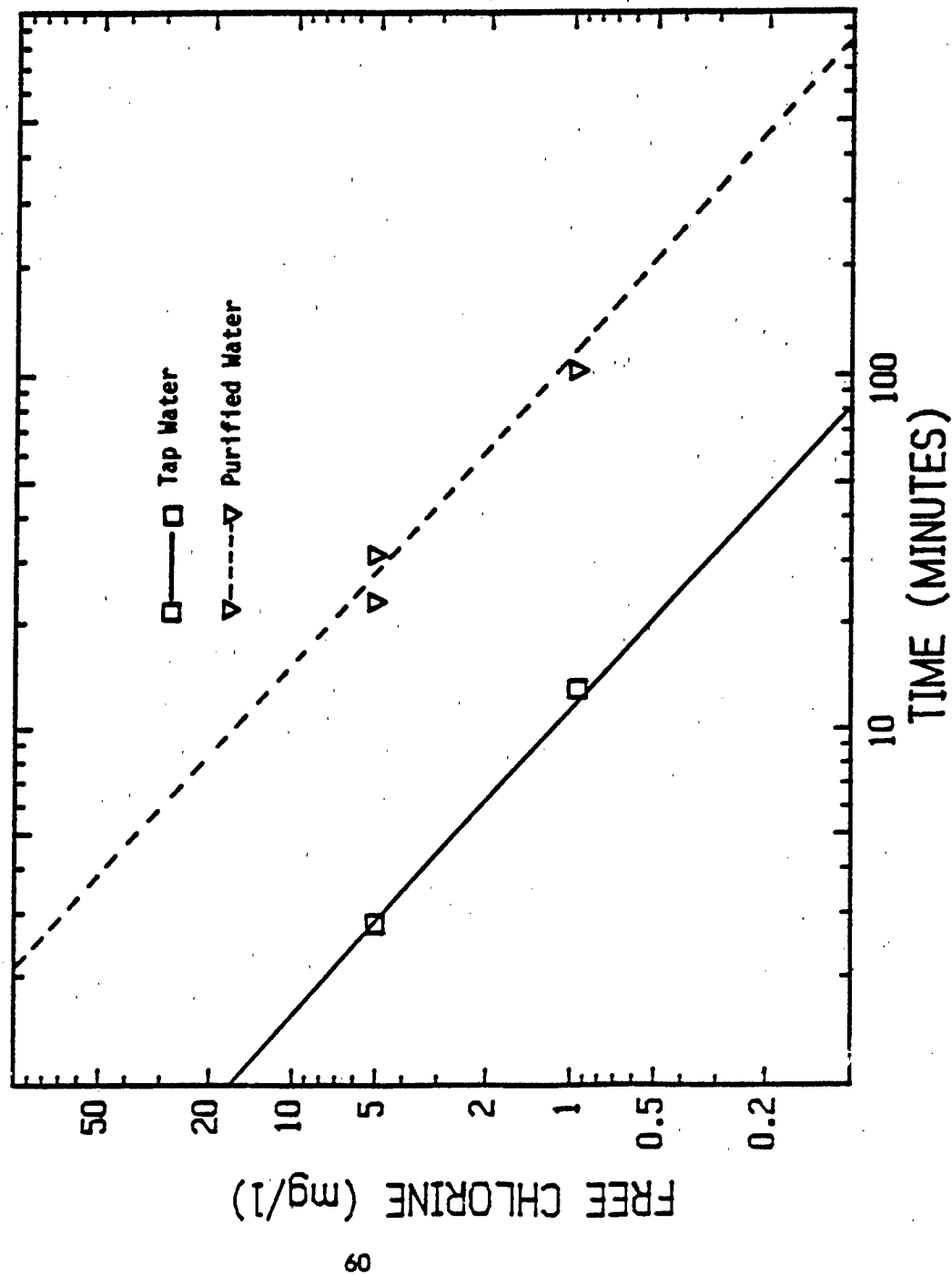


FIGURE 17. INACTIVATION OF POLIOVIRUS 1 (99,99%) AT 5°C BY  
FREE CHLORINE AT PH 5,0 IN NONBUFFERED PURIFIED  
WATER AND IN NONBUFFERED TAP WATER.



APPENDIX C  
(LIST OF TABLES, pH 10.0)]



TABLE A1. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.63-0.65 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL.

TEST # 1 CHLORINE (MG/L) : .65 KCL (MG/L) : 0 CONTROL (PFU/ML) : 152000  
INITIAL CHLORINE (MG/L) : .67

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | 184000               | 184000               | 184000              | 121.0526        | -21.0526            |
| 2              | 144000               | 104000               | 124000              | 81.5789         | 18.4211             |
| 3              | 144000               | 104000               | 124000              | 81.5789         | 18.4211             |
| 5              | 132000               | 116000               | 124000              | 81.5789         | 18.4211             |
| 8              | 116000               | 108000               | 112000              | 73.6842         | 26.3158             |
| 10             | 104000               | 108000               | 106000              | 69.7368         | 30.2632             |
| 15             | 104000               | 96000                | 100000              | 65.7894         | 34.2106             |
| 20             | 56000                | 36000                | 46000               | 30.2631         | 69.7369             |
| 25             | 37200                | 29200                | 33200               | 21.8421         | 78.1579             |
| 30             | 22800                | 25200                | 24000               | 15.7894         | 84.2106             |

TEST # 2 CHLORINE (MG/L) : .64 KCL (MG/L) : 21 CONTROL (PFU/ML) : 152000  
INITIAL CHLORINE (MG/L) : .67

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | 152000               | 200000               | 176000              | 115.7894        | -15.7894            |
| 2              | 274000               | 168000               | 186000              | 122.3684        | -22.3684            |
| 3              | 160000               | 112000               | 136000              | 89.4736         | 10.5264             |
| 5              | 132000               | 104000               | 118000              | 77.6315         | 22.3685             |
| 8              | 120000               | 136000               | 128000              | 84.2105         | 15.7895             |
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 15             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 20             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 25             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 30             | 17600                | 16000                | 16800               | 11.0526         | 88.9474             |

TABLE A2. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.63 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST # 1 CHLORINE (MG/L) : .63 KCL (MG/L) : 526 CONTROL (PFU/ML) : 149000  
INITIAL CHLORINE (MG/L) : .69

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 2              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 3              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 5              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 8              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 15             | 20000                | 44000                | 32000               | 21.4765         | 78.5235             |
| 20             | 12000                | 12000                | 12000               | 8.0536          | 91.9464             |
| 25             | 20000                | 20000                | 20000               | 13.4228         | 86.5772             |
| 30             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |

TEST # 2 CHLORINE (MG/L) : .62 KCL (MG/L) : 1,262 CONTROL (PFU/ML) : 149000  
INITIAL CHLORINE (MG/L) : .69

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 2              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 3              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 5              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 8              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 15             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 20             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 25             | 24000                | 36000                | 30000               | 20.1342         | 79.8658             |
| 30             | 4000                 | 8000                 | 6000                | 4.0268          | 95.9732             |

TABLE A3. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 1.3 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST # 1 CHLORINE (MG/L) : 1.27 KCL (MG/L) : 0 CONTROL (PFU/ML) : 178000  
INITIAL CHLORINE (MG/L) : 1.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 20             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 30             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 40             | 240                  | 360                  | 300                 | .1685           | 99.8315             |
| 50             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 60             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 70             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 80             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 90             | 56                   | 20                   | 38                  | .0213           | 99.9787             |
| 100            | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST # 2 CHLORINE (MG/L) : 1.22 KCL (MG/L) : 21 CONTROL (PFU/ML) : 178000  
INITIAL CHLORINE (MG/L) : 1.3

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 20             | 36000                | 52000                | 44000               | 24.7191         | 75.2809             |
| 30             | 2000                 | 0                    | 1000                | .5617           | 99.4383             |
| 40             | 800                  | 400                  | 600                 | .337            | 99.663              |
| 50             | 80                   | 120                  | 100                 | .0561           | 99.9439             |
| 60             | 80                   | 40                   | 60                  | .0337           | 99.9663             |
| 70             | 40                   | 40                   | 40                  | .0224           | 99.9776             |
| 80             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 90             | 160                  | 0                    | 80                  | .0449           | 99.9551             |
| 100            | 28                   | 0                    | 14                  | 7.8E-03         | 99.9922             |

TABLE A4. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 5.63-5.69 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

EST # 1 CHLORINE (MG/L) : 5.65 KCL (MG/L) : 0 CONTROL (PFU/ML) : 143000  
INITIAL CHLORINE (MG/L) : 5.55

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1.5            | 64000                | 56000                | 60000               | 41.958          | 58.042              |
| 3              | 28000                | 32000                | 30000               | 20.979          | 79.021              |
| 4.5            | 6400                 | 10400                | 8400                | 5.8741          | 94.1259             |
| 6              | 920                  | 960                  | 940                 | .6573           | 99.3427             |
| 7.5            | 880                  | 800                  | 840                 | .5874           | 99.4126             |
| 9              | 40                   | 72                   | 56                  | .0391           | 99.9609             |
| 10.5           | 28                   | 16                   | 22                  | .0153           | 99.9847             |
| 12             | 16                   | 4                    | 10                  | 6.9E-03         | 99.9931             |
| 13.5           | 8                    | 8                    | 8                   | 5.5E-03         | 99.9945             |
| 15             | 24                   | 24                   | 24                  | .0167           | 99.9833             |

EST # 2 CHLORINE (MG/L) : 5.64 KCL (MG/L) : 21 CONTROL (PFU/ML) : 143000  
INITIAL CHLORINE (MG/L) : 5.55

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1.5            | 52000                | 32000                | 42000               | 29.3706         | 70.6294             |
| 3              | 64000                | 48000                | 36000               | 39.1608         | 60.8392             |
| 4.5            | 8000                 | 8000                 | 8000                | 5.5944          | 94.4056             |
| 6              | 2800                 | 5200                 | 4000                | 2.7972          | 97.2028             |
| 7.5            | 600                  | 600                  | 600                 | .4195           | 99.5805             |
| 9              | 440                  | 400                  | 420                 | .2937           | 99.7063             |
| 10.5           | 112                  | 100                  | 106                 | .0741           | 99.9259             |
| 12             | 20                   | 32                   | 26                  | .0181           | 99.9819             |
| 13.5           | 32                   | 12                   | 22                  | .0153           | 99.9847             |
| 15             | 32                   | 16                   | 24                  | .0167           | 99.9833             |

TABLE A4. (Continued)

TEST # 3 CHLORINE (MG/L) : 5.69 KCL (MG/L) : 526 CONTROL (PFU/ML) : 143000  
INITIAL CHLORINE (MG/L) : 5.55

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1.5            | 84000                | 44000                | 64000               | 44.7552         | 55.2448             |
| 3              | 72000                | 40000                | 56000               | 39.1608         | 60.8392             |
| 4.5            | 11600                | 6800                 | 9200                | 6.4335          | 93.5665             |
| 6              | 1800                 | 1600                 | 1700                | 1.1888          | 98.8112             |
| 7.5            | 144                  | 32                   | 88                  | .0615           | 99.9385             |
| 9              | 40                   | 28                   | 34                  | .0237           | 99.9763             |
| 10.5           | 20                   | 36                   | 28                  | .0195           | 99.9805             |
| 12             | 20                   | 12                   | 16                  | .0111           | 99.9889             |
| 13.5           | 16                   | 12                   | 14                  | 9.7E-03         | 99.9903             |
| 15             | 12                   | 16                   | 14                  | 9.7E-03         | 99.9903             |

TEST # 4 CHLORINE (MG/L) : 5.64 KCL (MG/L) : 1,262 CONTROL (PFU/ML) : 143000  
INITIAL CHLORINE (MG/L) : 5.55

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1.5            | 60000                | 48000                | 54000               | 37.7622         | 62.2378             |
| 3              | 520                  | 560                  | 540                 | .3776           | 99.6224             |
| 4.5            | 100                  | 132                  | 116                 | .0811           | 99.9189             |
| 6              | 44                   | 48                   | 46                  | .0321           | 99.9679             |
| 7.5            | 32                   | 36                   | 34                  | .0237           | 99.9763             |
| 9              | 36                   | 36                   | 36                  | .0251           | 99.9749             |
| 10.5           | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 12             | 0                    | 12                   | 6                   | 4.1E-03         | 99.9959             |
| 13.5           | 12                   | 12                   | 12                  | 8.3E-03         | 99.9917             |
| 15             | 12                   | 8                    | 10                  | 6.9E-03         | 99.9931             |

TABLE A5A. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.81-0.83 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST # 1 CHLORINE (MG/L) : .81 KCL (MG/L) : 1,262 CONTROL (PFU/ML) : 214000  
INITIAL CHLORINE (MG/L) : .9

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 10             | 128000               | 104000               | 116000              | 54.2056         | 45.7944             |
| 20             | 120000               | 84000                | 102000              | 47.6635         | 52.3365             |
| 30             | 68000                | 44000                | 56000               | 26.1682         | 73.8318             |
| 40             | 24000                | 48000                | 36000               | 16.8224         | 83.1776001          |
| 50             | 8400                 | 6000                 | 7200                | 3.3644          | 96.6356             |
| 60             | 160                  | 800                  | 480                 | .2242           | 99.7758             |
| 70             | 68                   | 68                   | 68                  | .0317           | 99.9683             |
| 80             | 8                    | 4                    | 6                   | 2.8E-03         | 99.9972             |
| 90             | 4                    | 8                    | 6                   | 2.8E-03         | 99.9972             |
| 100            | 16                   | 16                   | 16                  | 7.4E-03         | 99.9926             |

TEST # 4 CHLORINE (MG/L) : .83 KCL (MG/L) : 0 CONTROL (PFU/ML) : 161000  
INITIAL CHLORINE (MG/L) : .9

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 10             | 68000                | 44000                | 56000               | 34.7826         | 65.2174001          |
| 20             | 44000                | 60000                | 52000               | 32.2981         | 67.7019             |
| 30             | 15600                | 13600                | 14600               | 9.0683          | 90.9317             |
| 40             | 4000                 | 5200                 | 4500                | 2.8571          | 97.1429             |
| 50             | 1160                 | 1680                 | 1420                | .8819           | 99.1181             |
| 60             | 280                  | 240                  | 260                 | .1614           | 99.8386             |
| 70             | 96                   | 76                   | 86                  | .0534           | 99.9466             |
| 80             | 80                   | 44                   | 62                  | .0385           | 99.9615             |
| 90             | 72                   | 40                   | 56                  | .0347           | 99.9653             |
| 100            | 40                   | 24                   | 32                  | .0198           | 99.9802             |

TABLE A5B. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.61-0.77 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST # 2 CHLORINE (MG/L) : .61 KCL (MG/L) : 3,728 CONTROL (PFU/ML) : 214000  
INITIAL CHLORINE (MG/L) : .9

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 108000               | 136000               | 122000              | 57.0093         | 42.9907             |
| 1              | 96000                | 164000               | 130000              | 60.7476         | 39.2524             |
| 1.5            | 116000               | 104000               | 110000              | 51.4018         | 48.5982             |
| 2              | 160000               | 148000               | 154000              | 71.9626         | 28.0374             |
| 3              | 140000               | 124000               | 132000              | 61.6822         | 38.3178             |
| 4              | 76000                | 108000               | 92000               | 42.9906         | 57.0094             |
| 5              | 96000                | 96000                | 96000               | 44.5598         | 55.1402             |
| 6              | 120000               | 128000               | 124000              | 57.9439         | 42.0561             |
| 7              | 116000               | 104000               | 110000              | 51.4018         | 48.5982             |
| 8              | 48000                | 80000                | 64000               | 29.9065         | 70.0935             |

TEST # 3 CHLORINE (MG/L) : .77 KCL (MG/L) : 3,728 CONTROL (PFU/ML) : 161000  
INITIAL CHLORINE (MG/L) : .9

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 120000               | 72000                | 96000               | 59.6273         | 40.3727             |
| 1              | 112000               | 124000               | 118000              | 73.2919         | 26.7081             |
| 1.5            | 60000                | 88000                | 74000               | 45.9627         | 54.0373             |
| 2              | 84000                | 84000                | 84000               | 52.1739         | 47.8261             |
| 3              | 84000                | 64000                | 74000               | 45.9627         | 54.0373             |
| 4              | 64000                | 80000                | 72000               | 44.7204         | 55.2796             |
| 5              | 44000                | 80000                | 62000               | 38.5093         | 61.4907             |
| 6              | 60000                | 56000                | 58000               | 36.0248         | 63.9752             |
| 7              | 60000                | 60000                | 60000               | 37.267          | 62.733              |
| 8              | 80000                | 44000                | 62000               | 38.5093         | 61.4907             |

TABLE A6. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.60-1.30 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST # 1 CHLORINE (MG/L) : 1.19 KCL (MG/L) : 7,456 CONTROL (PFU/ML) : 181000  
INITIAL CHLORINE (MG/L) : 1.5

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 1              | 104000               | 104000               | 104000              | 57.4585         | 42.5415             |
| 1.5            | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 2              | 92000                | 92000                | 92000               | 50.8287         | 49.1713             |
| 5              | 100000               | 120000               | 110000              | 60.7734         | 39.2266             |
| 10             | 104000               | 104000               | 104000              | 57.4585         | 42.5415             |
| 20             | 5200                 | 8800                 | 7000                | 3.8674          | 96.1326             |
| 30             | 36                   | 52                   | 44                  | .0243           | 99.9757             |
| 40             | 52                   | 52                   | 52                  | .0287           | 99.9713             |
| 50             | 16                   | 24                   | 20                  | .011            | 99.989              |

TEST # 2 CHLORINE (MG/L) : .6 KCL (MG/L) : 7,456 CONTROL (PFU/ML) : 181000  
INITIAL CHLORINE (MG/L) : 1.5

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 1              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 1.5            | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 2              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 5              | 108000               | 108000               | 108000              | 59.6685         | 40.3315             |
| 10             | 108000               | 108000               | 108000              | 59.6685         | 40.3315             |
| 20             | 100000               | 100000               | 100000              | 55.2486         | 44.7514             |
| 30             | 60000                | 44000                | 52000               | 28.7292         | 71.2708             |
| 40             | 4400                 | 7200                 | 5800                | 3.2044          | 96.7956             |
| 50             | 440                  | 520                  | 480                 | .2651           | 99.7349             |

TABLE A6. (Continued)

TEST # 3 CHLORINE (MG/L) : 1.11 KCl (MG/L) : 7,456 CONTROL (PFU/ML) : 107000  
 INITIAL CHLORINE (MG/L) : 1.5

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 88000                | 88000                | 88000               | 82.2429         | 17.7571             |
| 1              | 124000               | 96000                | 110000              | 102.8037        | -2.8037             |
| 1.5            | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 2              | 60000                | 96000                | 78000               | 72.8971         | 27.1029             |
| 5              | 60000                | 48000                | 54000               | 50.4672         | 49.5328             |
| 10             | 60000                | 56000                | 58000               | 54.2056         | 45.7944             |
| 20             | 720                  | 600                  | 660                 | .6168           | 99.3832             |
| 30             | 120                  | 120                  | 120                 | .1121           | 99.8879             |
| 40             | 40                   | 72                   | 56                  | .0523           | 99.9477             |
| 50             | 28                   | 44                   | 36                  | .0336           | 99.9664             |

TEST # 4 CHLORINE (MG/L) : 1.3 KCl (MG/L) : 0 CONTROL (PFU/ML) : 107000  
 INITIAL CHLORINE (MG/L) : 1.5

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 10             | 40000                | 20000                | 30000               | 28.0373         | 71.9627             |
| 20             | 44000                | 44000                | 44000               | 41.1214         | 58.8786             |
| 30             | 12400                | 8400                 | 10400               | 9.7196          | 90.2804             |
| 40             | 960                  | 840                  | 900                 | .8411           | 99.1589             |
| 50             | 60                   | 40                   | 50                  | .0467           | 99.9533             |
| 60             | 32                   | 52                   | 42                  | .0392           | 99.9608             |
| 70             | 32                   | 44                   | 38                  | .0355           | 99.9645             |
| 80             | 28                   | 16                   | 22                  | .0205           | 99.9795             |
| 90             | 28                   | 28                   | 28                  | .0261           | 99.9739             |
| 100            | 8                    | 8                    | 8                   | 7.4E-03         | 99.9926             |

TABLE A7. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.65-4.21 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST # 1 CHLORINE (MG/L) : .65 KCL (MG/L) : 7.456 CONTROL (PFU/ML) : 8200  
INITIAL CHLORINE (MG/L) : .98

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 3600                 | 4000                 | 3800                | 46.3414         | 53.6586             |
| 1              | 3600                 | 3200                 | 3400                | 41.4634         | 58.5366             |
| 1.5            | 4800                 | 1600                 | 3200                | 39.0243         | 60.9757             |
| 2              | 2400                 | 3200                 | 2800                | 34.1463         | 65.8537001          |
| 5              | 680                  | 640                  | 660                 | 8.0487          | 91.9513             |
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 20             | 32                   | 32                   | 32                  | .3902           | 99.6098             |
| 30             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 40             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 50             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST # 2 CHLORINE (MG/L) : .73 KCL (MG/L) : 0 CONTROL (PFU/ML) : 8200  
INITIAL CHLORINE (MG/L) : .98

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 10000                | 8400                 | 9200                | 112.1951        | -12.1951            |
| 1              | 11200                | 8000                 | 9600                | 117.0731        | -17.0731            |
| 1.5            | 4800                 | 1600                 | 3200                | 39.0243         | 60.9757             |
| 2              | 6400                 | 3200                 | 4800                | 58.5365         | 41.4635             |
| 5              | 3200                 | 800                  | 2000                | 24.3902         | 75.6098             |
| 10             | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 20             | 2400                 | 2000                 | 2200                | 26.8292         | 73.1708             |
| 30             | 200                  | 120                  | 160                 | 1.9512          | 98.0488             |
| 40             | 36                   | 60                   | 48                  | .5853           | 99.4147             |
| 50             | 16                   | 8                    | 12                  | .1463           | 99.8537             |

TABLE A7. (Continued)

TEST # 3 CHLORINE (MG/L) : 3.9 KCL (MG/L) : 7,456 CONTROL (PFU/ML) : 10500  
 INITIAL CHLORINE (MG/L) : .98

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 3200                 | 4000                 | 3600                | 34.2857         | 65.7143             |
| 1              | 800                  | 2800                 | 1800                | 17.1428         | 82.8572             |
| 1.5            | 800                  | 1200                 | 1000                | 9.5238          | 90.4762             |
| 2              | 320                  | 360                  | 340                 | 3.238           | 96.762              |
| 4              | 40                   | 56                   | 48                  | .4571           | 99.5429             |
| 6              | 48                   | 32                   | 40                  | .3809           | 99.6191             |
| 8              | 4                    | 4                    | 4                   | .038            | 99.962              |
| 10             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 12             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |
| 14             | 0                    | 0                    | 0                   | < 0.0001        | > 99.9999           |

TEST # 4 CHLORINE (MG/L) : 4.21 KCL (MG/L) : 0 CONTROL (PFU/ML) : 10500  
 INITIAL CHLORINE (MG/L) : .98

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| .5             | 8800                 | 6400                 | 7600                | 72.3809         | 27.6191             |
| 1              | 4400                 | 4000                 | 4200                | 40              | 60                  |
| 1.5            | 2800                 | 3200                 | 3000                | 28.5714         | 71.4286001          |
| 2              | 3200                 | 2800                 | 3000                | 28.5714         | 71.4286001          |
| 4              | 2000                 | 2400                 | 2200                | 20.9523         | 79.0477             |
| 6              | 360                  | 600                  | 480                 | 4.5714          | 95.4286             |
| 8              | 280                  | 280                  | 280                 | 2.6666          | 97.3334             |
| 10             | 68                   | 84                   | 76                  | .7238           | 99.2762             |
| 12             | 24                   | 28                   | 26                  | .2476           | 99.7524             |
| 14             | 8                    | 4                    | 6                   | .0571           | 99.9429             |

TABLE A8. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.95-1.15 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST # 1 CHLORINE (MG/L) : 1.15 KCL (MG/L) : 0 CONTROL (PFU/ML) : 181000  
INITIAL CHLORINE (MG/L) : 1.21

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 5              | 168000               | 156000               | 162000              | 89.5027         | 10.4973             |
| 10             | 72000                | 64000                | 68000               | 37.569          | 62.431              |
| 15             | 68000                | 48000                | 58000               | 32.0441         | 67.9559             |
| 20             | 40000                | 72000                | 56000               | 30.9392         | 69.0608001          |
| 25             | 8400                 | 9600                 | 9000                | 4.9723          | 95.0277             |
| 30             | 4000                 | 5600                 | 4800                | 2.6519          | 97.3481             |
| 35             | 1360                 | 1360                 | 1360                | .7513           | 99.2487             |
| 40             | 560                  | 560                  | 560                 | .3093           | 99.6907             |
| 45             | 116                  | 116                  | 116                 | .064            | 99.936              |
| 50             | 72                   | 72                   | 72                  | .0397           | 99.9603             |

TEST # 2 CHLORINE (MG/L) : .95 KCL (MG/L) : 7.456 CONTROL (PFU/ML) : 181000  
INITIAL CHLORINE (MG/L) : 1.21

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 3              | 40000                | 32000                | 36000               | 19.8895         | 80.1105             |
| 6              | 920                  | 1160                 | 1040                | .5745           | 99.4255             |
| 9              | 360                  | 440                  | 400                 | .2209           | 99.7791             |
| 12             | 360                  | 360                  | 360                 | .1988           | 99.8012             |
| 15             | 280                  | 240                  | 260                 | .1436           | 99.8564             |
| 18             | 84                   | 104                  | 94                  | .0519           | 99.9481             |
| 21             | 8400                 | 8400                 | 8400                | 4.6408          | 95.3592             |
| 24             | 68                   | 72                   | 70                  | .0386           | 99.9614             |
| 27             | 68                   | 60                   | 64                  | .0353           | 99.9647             |
| 30             | 56                   | 36                   | 46                  | .0254           | 99.9746             |

TABLE 8. (Continued)

TEST # 3 CHLORINE (MG/L) : 1.13 KCl (MG/L) : 21 CONTROL (PFU/ML) : 181000  
INITIAL CHLORINE (MG/L) : 1.21

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 4              | 28000                | 44000                | 36000               | 19.8895         | 80.1105             |
| 8              | 12800                | 15600                | 14200               | 7.8453          | 92.1547             |
| 12             | 11600                | 14800                | 13200               | 7.2928          | 92.7072             |
| 16             | 12000                | 10800                | 11400               | 6.2983          | 93.7017             |
| 20             | 6800                 | 9600                 | 8200                | 4.5303          | 95.4697             |
| 24             | 1600                 | 2000                 | 1800                | .9944           | 99.0056             |
| 28             | 1520                 | 1120                 | 1320                | .7292           | 99.2708             |
| 32             | 560                  | 800                  | 680                 | .3756           | 99.6244             |
| 36             | 132                  | 56                   | 94                  | .0519           | 99.9481             |
| 40             | 108                  | 140                  | 124                 | .0685           | 99.9315             |

TEST # 4 CHLORINE (MG/L) : 1.05 KCl (MG/L) : 1,262 CONTROL (PFU/ML) : 181000  
INITIAL CHLORINE (MG/L) : 1.21

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 4              | 32000                | 28000                | 30000               | 16.5745         | 83.4255             |
| 8              | 1200                 | 3200                 | 2200                | 1.2154          | 98.7846             |
| 12             | 240                  | 280                  | 260                 | .1436           | 99.8564             |
| 16             | 200                  | 320                  | 260                 | .1436           | 99.8564             |
| 20             | 104                  | 72                   | 88                  | .0486           | 99.9514             |
| 24             | 64                   | 44                   | 54                  | .0298           | 99.9702             |
| 28             | 64                   | 68                   | 66                  | .0364           | 99.9636             |
| 32             | 44                   | 40                   | 42                  | .0232           | 99.9768             |
| 36             | 36                   | 44                   | 40                  | .022            | 99.978              |
| 40             | 36                   | 32                   | 34                  | .0187           | 99.9813             |

TABLE A9. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 4.63-4.76 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

TEST # 1 CHLORINE (MG/L) : 4.75 KCL (MG/L) : 0 CONTROL (PFU/ML) : 155500  
INITIAL CHLORINE (MG/L) : 4.88

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | N/A                  | N/A                  | N/A                 | N/A             | N/A                 |
| 2              | 92000                | 124000               | 108000              | 69.4533         | 30.5467             |
| 4              | 72000                | 80000                | 76000               | 48.8745         | 51.1255             |
| 6              | 40000                | 48000                | 44000               | 28.2958         | 71.7042             |
| 8              | 40000                | 20000                | 30000               | 19.2926         | 80.7074             |
| 10             | 8000                 | 28000                | 18000               | 11.5755         | 88.4245             |
| 12             | 5200                 | 5600                 | 5400                | 3.4726          | 96.5274             |
| 14             | 0                    | 400                  | 200                 | .1286           | 99.8714             |
| 16             | 400                  | 120                  | 260                 | .1672           | 99.8328             |
| 18             | 28                   | 16                   | 22                  | .0141           | 99.9859             |

TEST # 2 CHLORINE (MG/L) : 4.72 KCL (MG/L) : 21 CONTROL (PFU/ML) : 155500  
INITIAL CHLORINE (MG/L) : 4.88

| TIME<br>(MIN.) | SAMPLE 1<br>(PFU/ML) | SAMPLE 2<br>(PFU/ML) | AVERAGE<br>(PFU/ML) | SURVIVAL<br>(%) | INACTIVATION<br>(%) |
|----------------|----------------------|----------------------|---------------------|-----------------|---------------------|
| 1              | 112000               | 60000                | 86000               | 55.3054         | 44.6946             |
| 2              | 100000               | 88000                | 94000               | 60.4501         | 39.5499             |
| 4              | 52000                | 28000                | 40000               | 25.7234         | 74.2766             |
| 6              | 452                  | 44000                | 22226               | 14.2932         | 85.7068             |
| 8              | 32000                | 32000                | 32000               | 20.5787         | 79.4213             |
| 10             | 8000                 | 16000                | 12000               | 7.717           | 92.283              |
| 12             | 7200                 | 3200                 | 5200                | 3.344           | 96.656              |
| 14             | 640                  | 520                  | 580                 | .3729           | 99.6271             |
| 16             | 12                   | 44                   | 28                  | .018            | 99.982              |
| 18             | 4                    | 8                    | 6                   | 3.8E-03         | 99.9962             |

APPENDIX D  
(LIST OF FIGURES [pH 10.0])

FIGURE 41. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN 0.01 M BICARBONATE-BUFFERED  
PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.63-0.65 MG/L OF FREE  
CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

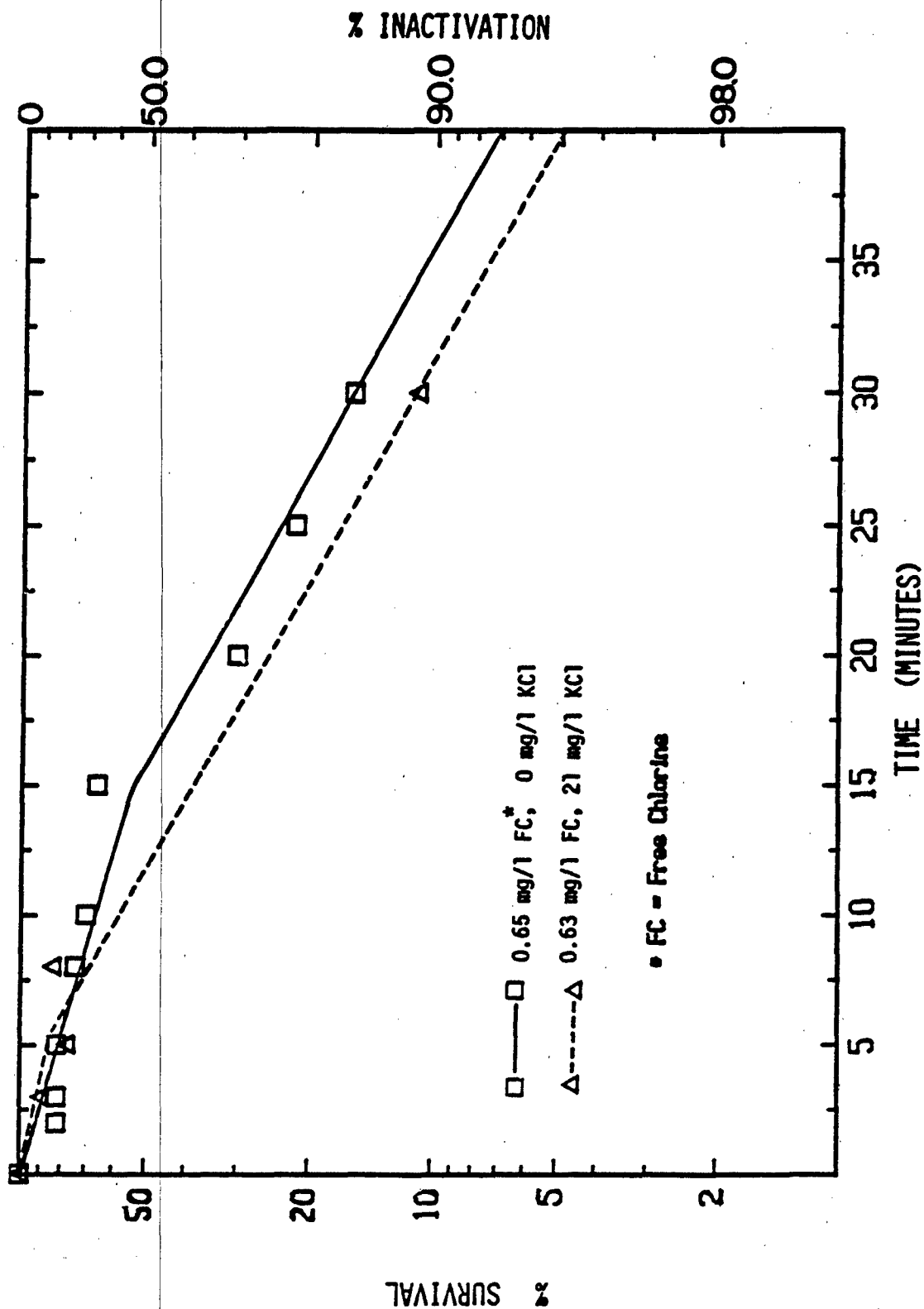
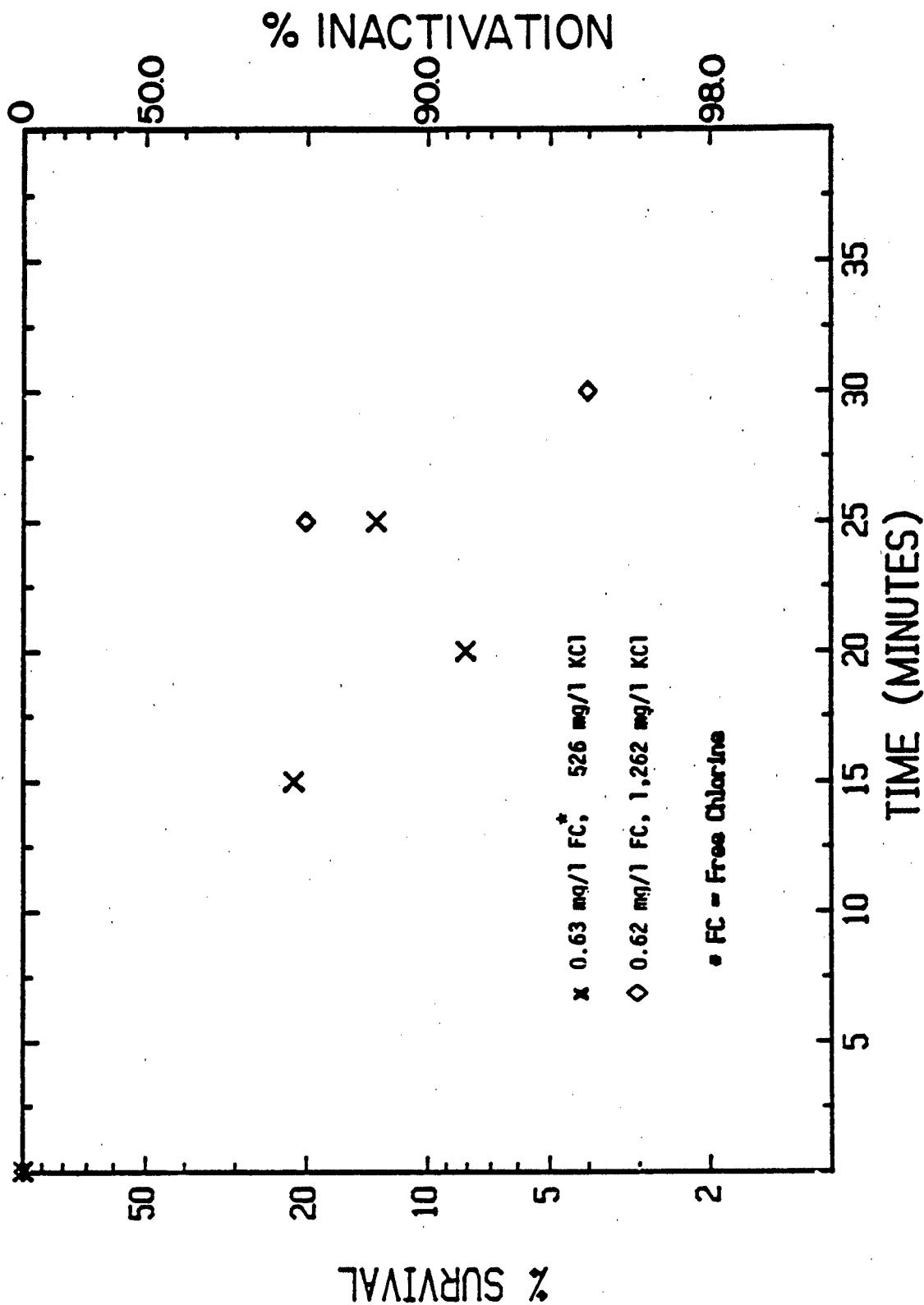


FIGURE A2. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED  
PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.63 MG/L OF FREE  
CHLORINE IN THE PRESENCE AND ABSENCE OF KCL



PURIFIED WATER AT PH 10.0 BY APPROXIMATELY 1.3 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

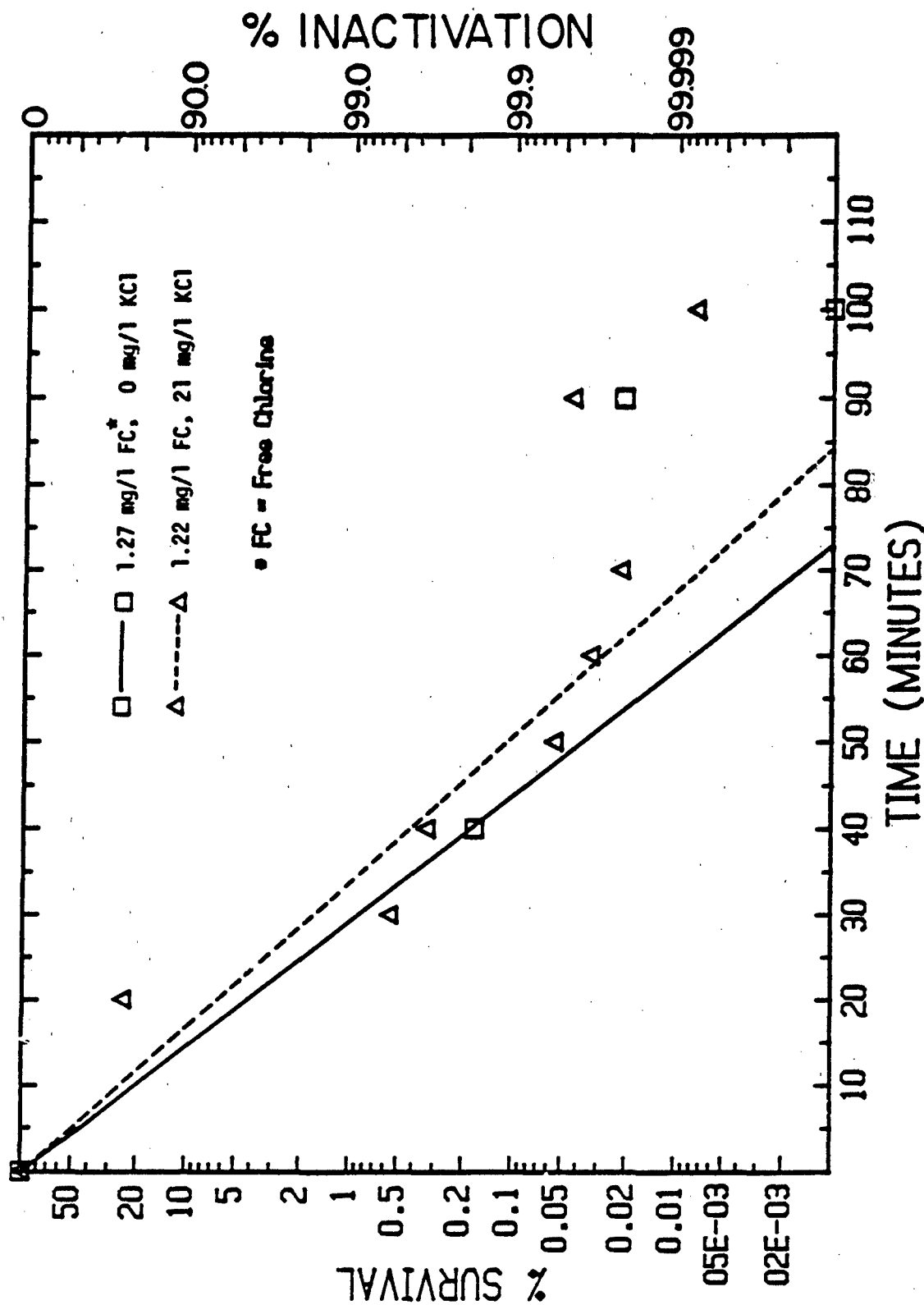


FIGURE A4. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT PH 10.0 BY APPROXIMATELY 5.65-5.69 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

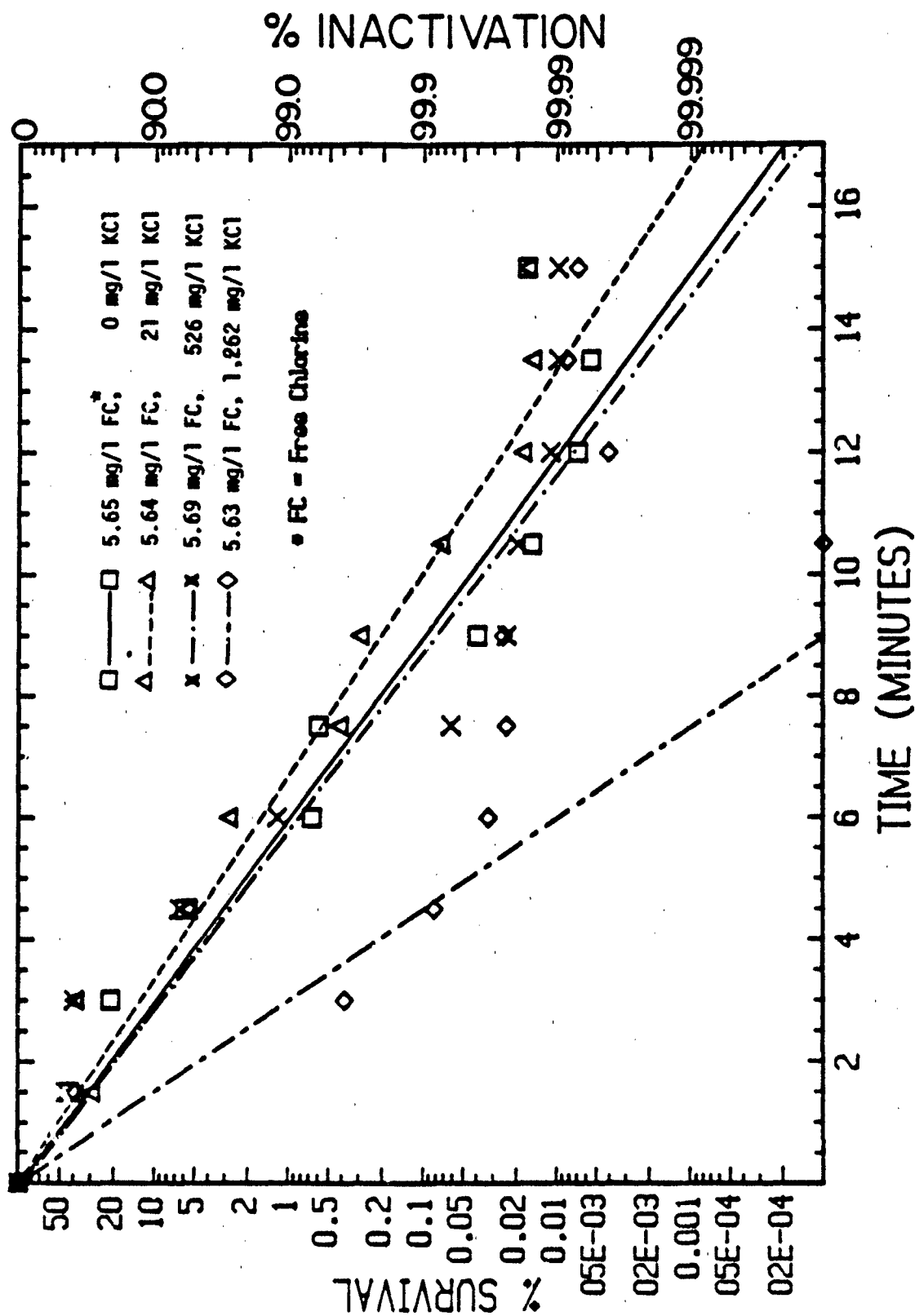


FIGURE A5A. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT PH 10.0 BY APPROXIMATELY 0.81-0.83 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCl

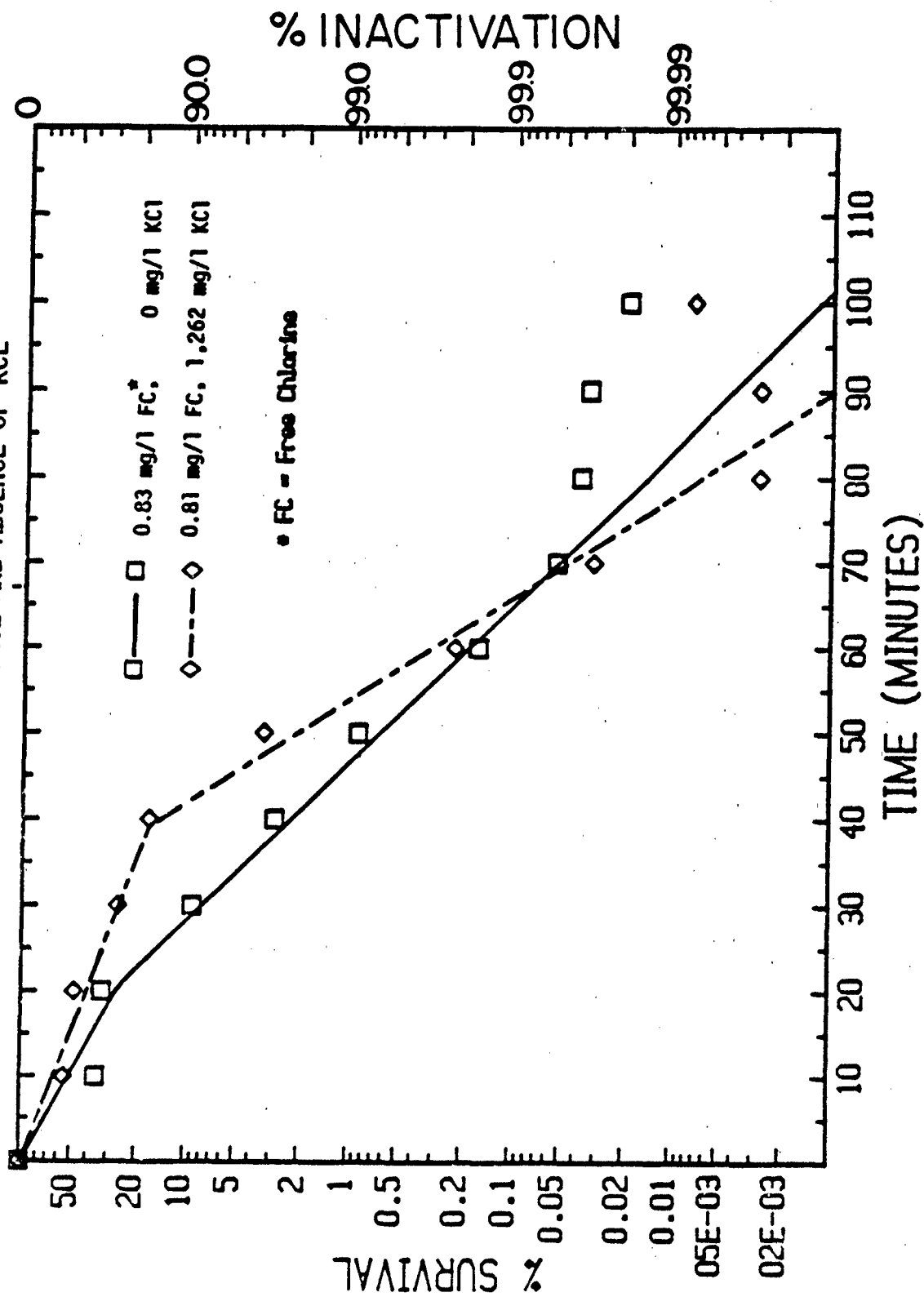


FIGURE A5B. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT PH 10.0 BY APPROXIMATELY 0.61-0.77 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

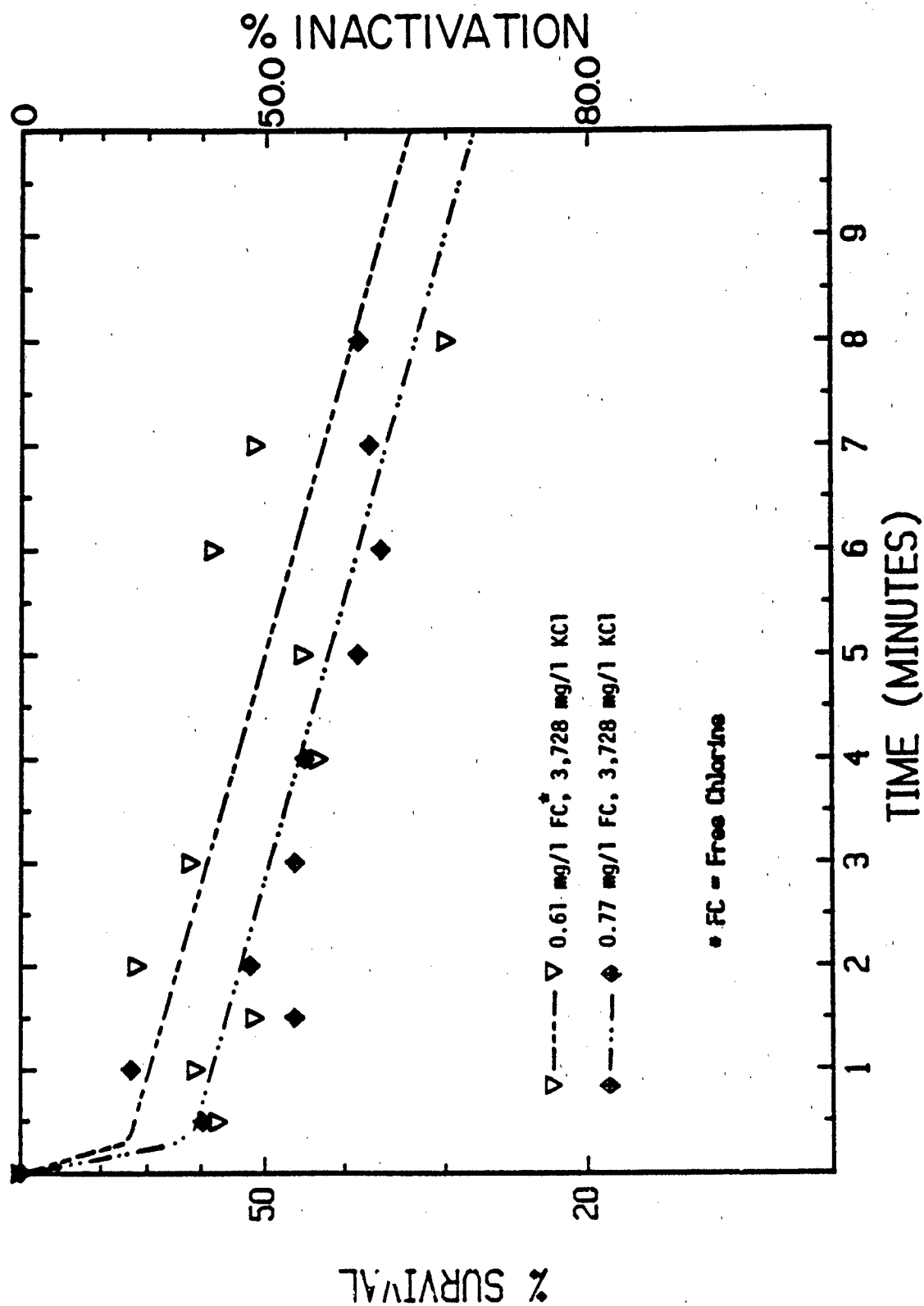


FIGURE A6. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT PH 10.0 BY APPROXIMATELY 0.60-1.30 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

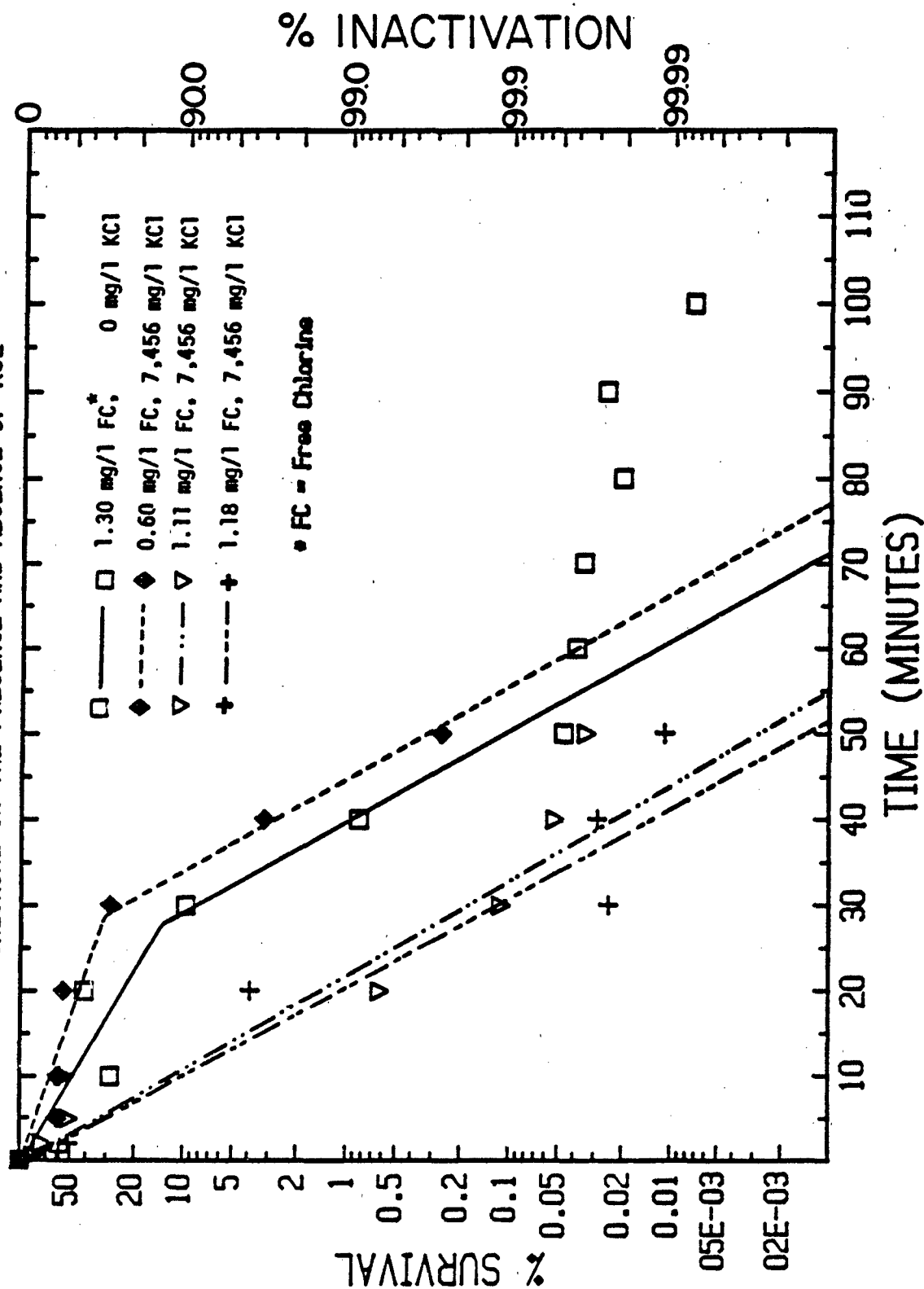


FIGURE A7. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.65-4.21 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

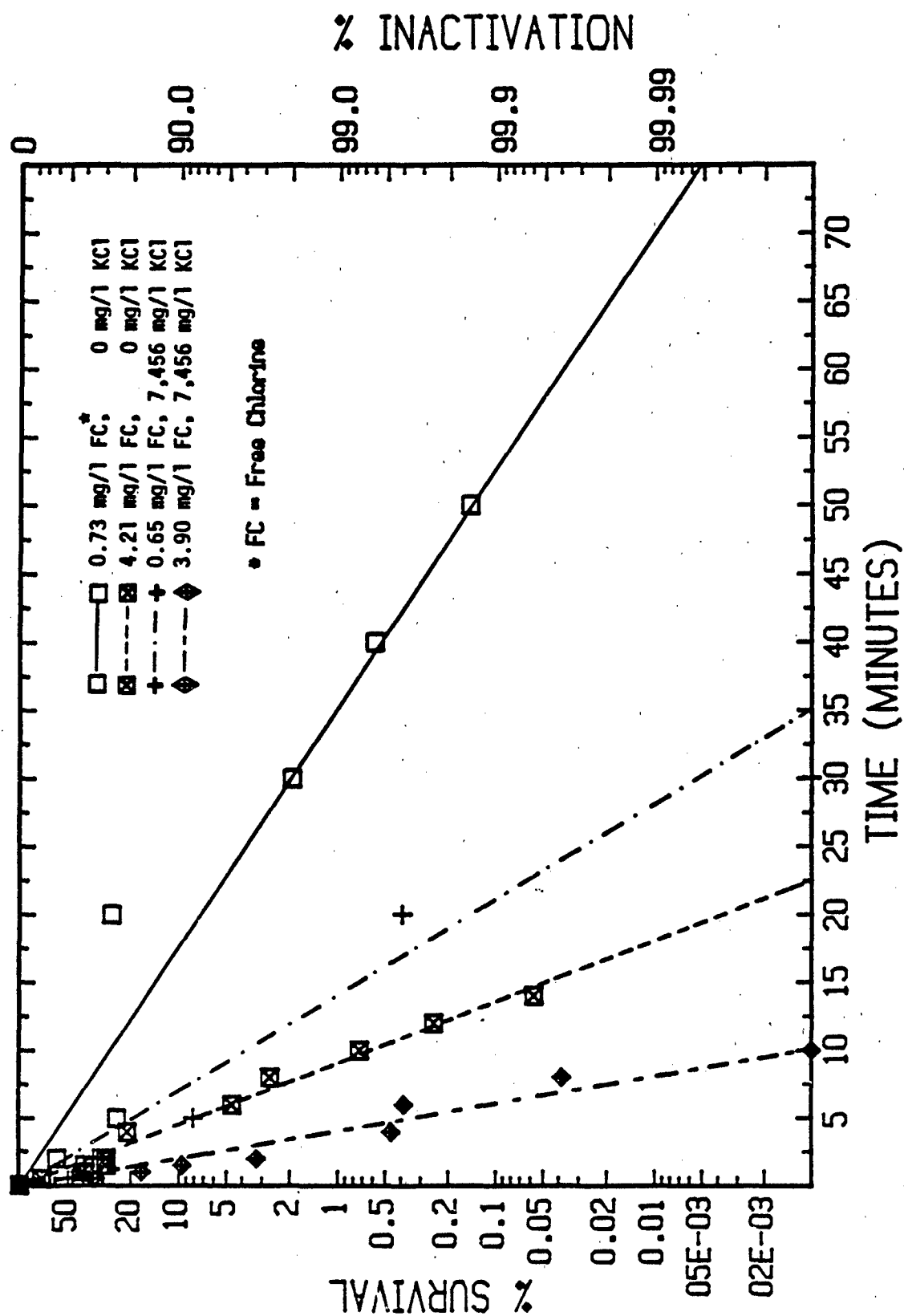


FIGURE A8. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT pH 10.0 BY APPROXIMATELY 0.95-1.15 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL

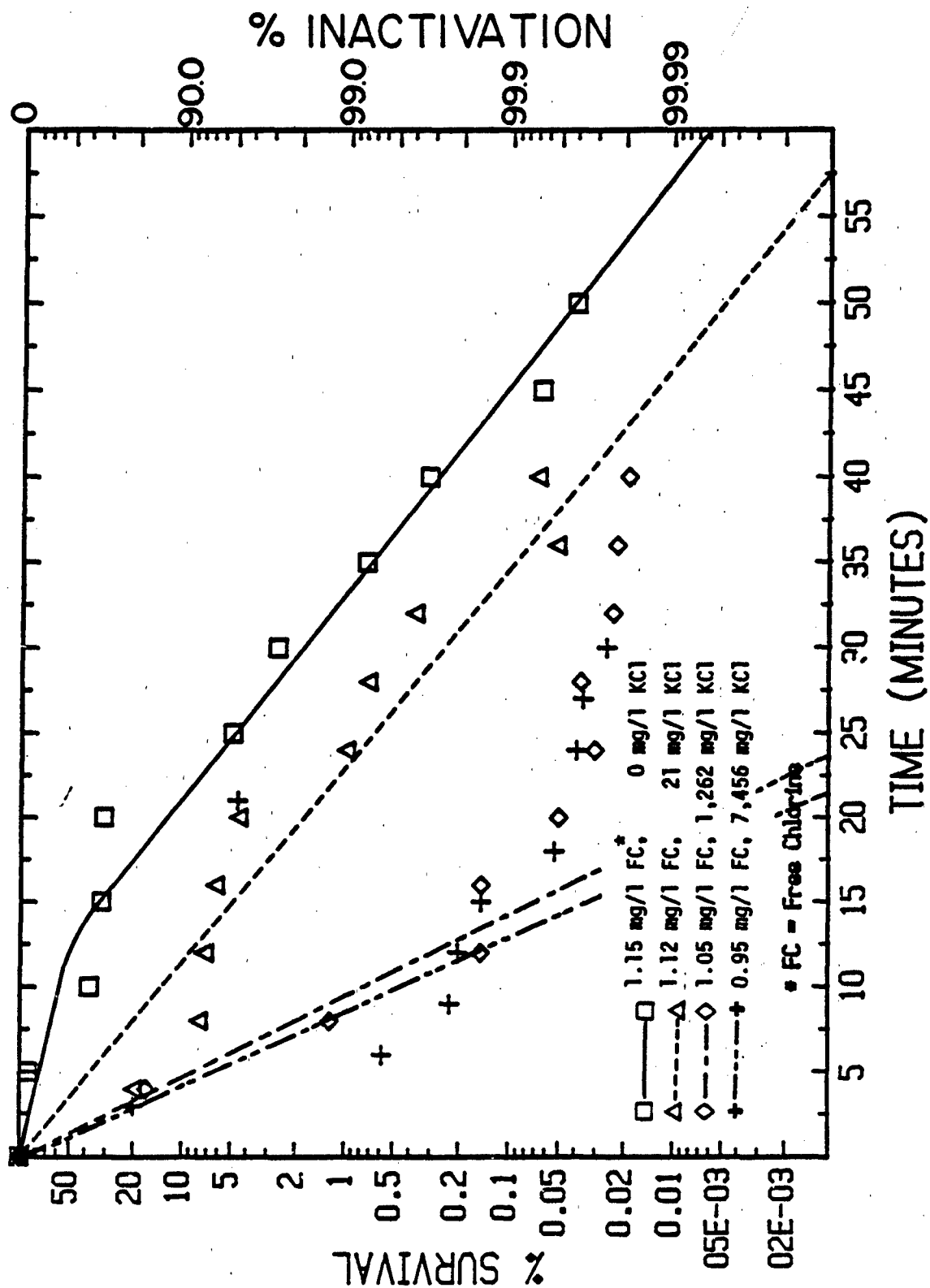
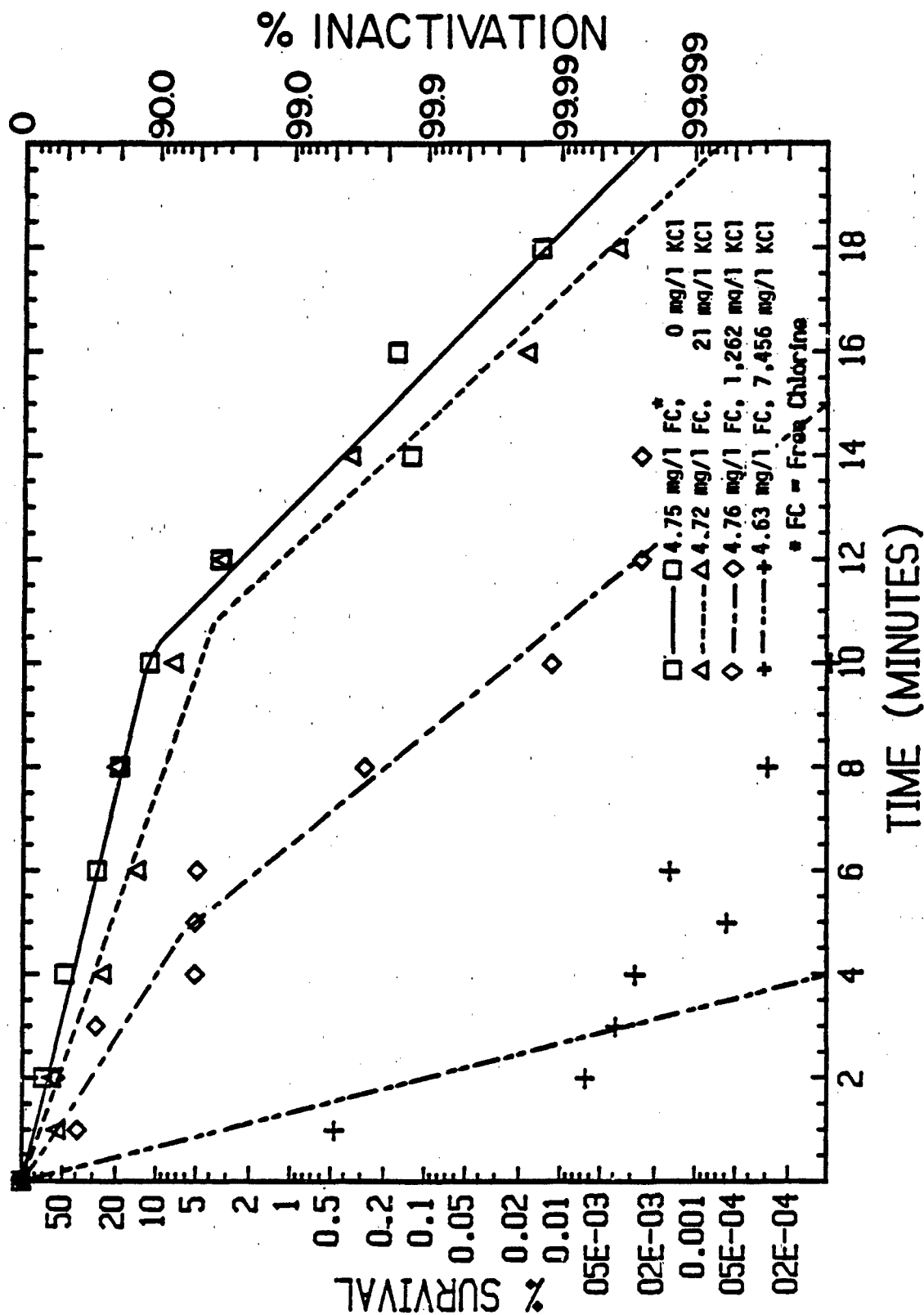


FIGURE A9. INACTIVATION OF POLIOVIRUS 1 AT 5°C IN BORATE-BUFFERED PURIFIED WATER AT PH 10.0 BY APPROXIMATELY 4.63-4.76 MG/L OF FREE CHLORINE IN THE PRESENCE AND ABSENCE OF KCL



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increased the polioviral effectiveness of FC by 1400%.

At 5°C, poliovirus 1 was inactivated by FC at pH 9.0 more than 9 times more rapidly in nonbuffered tap water than in non-buffered purified water suggesting that KCl with or without other ions in tap water potentiated the virucidal effectiveness of FC.

Potassium chloride did not potentiate the polioviral effectiveness of FC at pH 9.0 to nearly the degree in nonbuffered tap water that it did in non-buffered purified water suggesting that ions present in tap water potentiated the virucidal effectiveness of FC.

FC inactivated poliovirus 1 at pH 9.0 more than 3 times more rapidly in borate-buffered (0.05M) purified water than in nonbuffered purified water suggesting that the buffer ions potentiated the virucidal effectiveness of the FC. Clearly, disinfection studies should not be done in buffers without first determining the virucidal potentiating effect of the buffers.

Potassium chloride potentiated the virucidal effectiveness of FC in 0.05 M borate buffer to a lesser degree than in nonbuffered purified water further indicating that buffer ions potentiated the virucidal effectiveness of FC and that a maximum ion effect exists.

Potassium chloride, at a level 1, 262 mg/L, potentiated the virucidal effectiveness of FC at pH 4.5 in phthalate-buffered purified water, but to a lesser degree than it potentiated the virucidal effectiveness of FC at pH 9.0 in borate-buffered purified water.

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