# A Review of Factors Affecting Microbial Survival in Ground Water

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## Introduction

Ground water resources are heavily used for domestic drinking water supplies in the United States and most of the world. Nationally, 40% of the U.S. domestic water supply originates from ground water. Furthermore, over 40 million people use ground water to supply their drinking water via domestic wells (Alley *et al.*, 1999). Aquifers have until the last several decades been generally considered protected from potential sources of microbial or chemical contamination typically found in surface waters. Due to increasing population densities, development and industrialization, and increased withdrawals from aquifers, however, the quality of ground water is increasingly a concern. Along with a heightened state of awareness about potential ground water contamination has come interest within the regulatory, public health, and research communities to gain more information about the sources, transport, and fate of waterborne microorganisms in relation to aquifers and ground water. In particular, a large body of research has examined the transport of viruses through the vadose zone and within aquifers. However, also of concern is the fate or survival of all groups of microorganisms once in aquifers. Numerous studies in the published literature have examined this as well.

Waterborne microorganisms of public-health concern can enter aquifers via several sources and mechanisms, including percolation via surface water, sinkholes, septic systems, leaky sewer lines or direct injection of wastewater effluent or surface water. One technology of rising importance that involves injection of surface water to aquifers for storage and later recovery for use is termed aquifer storage recovery (ASR). At least 53 ASR systems were operational in the United States in January, 2002, with about 100 more in development or planning stages (Pyne, 2002). This technology is considered a proven, viable and cost effective means of storing water for later withdrawal and use. As such, ASR systems can be a valuable tool for water supply management officials to effectively manage supplies for drinking water, irrigation, or ecosystem preservation and restoration. Currently, the U.S. EPA and other local agencies require that water injected via ASR wells meets all primary and secondary drinking water standards (Drew, 2001). However, considerable interest among proponents of ASR technology exists in determining the feasibility of relaxing pre-treatment requirements for stored water, assuming that natural attenuation of potentially harmful microorganisms that may be introduced occurs due to biological, physical, and geochemical factors present in the subsurface environment. Since recovered water is again treated before use as drinking water, the major concern among opponents of these proposals to relax pre-treatment requirements lies in the possibility of transport of introduced pathogenic microbes to domestic wells serving small scale users who do not treat water prior to use. Thus, in order to enable informed decisions on the level of treatment required for ASR water, as well as to better evaluate risks associated with contamination of aquifers by microbes, comprehensive information on the survival and attenuation of potentially harmful viruses, bacteria, and protozoa is highly desired.

This review seeks to summarize the current state of knowledge on inactivation of many organisms of concern in ground water from a quantitative perspective. Since no standard exists for reporting results of studies on microbial inactivation, data have been reported by various authors in many different ways. The purpose of this review was to facilitate some level of comparison amongst numerous disparate studies in order to evaluate any consistencies and trends within the body of published research on the topic.

A standard variable was chosen to enable quantitative comparisons, the rate of inactivation. Since many authors have not presented data in their publications as inactivation rates, rates for this review were in many cases approximated based on the data that were presented. The information presented within summarizes methods and findings of many studies on microbial survival in ground water and in some cases surface water, and collates these findings into expressions of inactivation rates in terms of $\log_{10}$ decline in the viable or culturable organisms per day. Rates are thus summarized here as the -log of the ratio of viable or culturable organisms at a given time in the experiment over the initial number, over time in days. For example, an inactivation rate of 0.1 log/day would indicate a decline of 1 log or 90% of said organisms in 10 days. In many cases, authors have reported inactivation data in these terms, in other cases the data as presented were converted to log/day declines based on times to achieve a given level of reduction or approximated from graphical data. Observations on kinetics of inactivation from various studies have been noted when possible, and in general, rates converted from graphical data express an average rate resulting from the total decline in viable counts observed. Finally, summary tables are given which combine data for the organisms studied in the reports reviewed here to express ranges and other summary statistics.

**Studies on Viruses**

Seven published studies that examined the survival of only viruses were reviewed, spanning the years 1983 – 2000. In addition to a variety of virus types, which included coliphage, poliovirus, hepatitis A and echovirus, physical and chemical factors were evaluated for their impact on viral inactivation. These included pressure, temperature, total dissolved solids, hardness, and in one case soil type.

The effect of hydrostatic pressure on poliovirus-1 survival was evaluated by Bitton *et al.* (1983) using ground water and seawater. Ground water survival experiments were stored for 24 hours at 24° C (75° F) at initial pressures of atmospheric pressure (control), 500, 1000, 2000, 3000, and 4000 psi (range of 34-272 atm). Conditions of the ground water reported were pH = 7.9 and conductivity of 475 μmhos/cm, relating to an approximate TDS of 235 mg/l.

The effect of pressure was determined as the ratio of recovered virus concentration in the pressurized condition experiment over the control. For example, at 500 psi, after 24 hours the surviving poliovirus concentration was 90.7% of that in the un-pressurized control. Little effect was observed in the ground water samples as a result of pressure. Survival of poliovirus-1 ranged from 82.5% of the control at 3000 psi to 100% of the control at 4000 psi. A significant effect was observed in seawater samples stored at 2° C at 1000 psi for up to 24 hours. In that instance, only 15.6% of the control virus concentration was surviving at 24 hours.

Studies published by Yates and others in 1985 and 1990 have reported the effect of numerous parameters on virus survival in ground water. An analysis of the effect of chemical and physical factors, namely TDS, hardness, turbidity, pH, and nitrate concentrations, on virus survival in ground water was reported by Yates, *et al.* (1985). Several ground water samples were obtained from across the United States and MS-2, poliovirus 1, and echovirus 1 survival were analyzed in each of the water samples. Incubation temperatures generally matched that of the native aquifer from which the sample was obtained. In several cases, MS-2 survival was determined at additional temperatures. All ground water samples were analyzed in their natural state without treatment. The duration of all experiments extended to 30 days. Resulting inactivation rates as reported in the study are shown here in Tables 1a – 1c. Multiple regression analysis of the data by the authors revealed that incubation temperature was the only factor significantly correlated to inactivation rate (P=0.05) of all viruses; increased temperatures also increased viral inactivation rates. In addition, calcium hardness was also correlated to the decay rate of MS-2, with increasing calcium concentrations correlating to increased MS-2 inactivation. TDS, which ranged from 37 to 1,110 mg/l, was not found to significantly affect inactivation rates.

**Table 1. Inactivation rates for Poliovirus 1 (a), Echovirus 1 (b) and MS-2 (c) in a variety of ground waters as reported in Yates *et al.* (1985).**

**a.**

| Organism | Groundwater | T ( o C) | TDS* | Ca hard. (mg/l) | Mg hard. (mg/l) | pH | Rate (log10/d) |
|---|---|---|---|---|---|---|---|
| Polio 1 | New York 1 | 12 | 37 | 44 | 44 | 6.0 | 0.035 |
| | N. Carolina 2 | 12 | 95 | 100 | 4 | 8.3 | 0.114 |
| | New York 2 | 12 | 145 | 138 | 56 | 7.3 | 0.051 |
| | Wisconsin | 12 | 260 | 208 | 216 | 8.0 | 0.06 |
| | N. Carolina 1 | 12 | 430 | 138 | 24 | 7.9 | 0.138 |
| | Texas 1 | 13 | 850 | 224 | 224 | 8.0 | 0.036 |
| | Texas 2 | 13 | 950 | 354 | 572 | 7.7 | 0.137 |
| | California 2 | 17 | 200 | 216 | 56 | 8.1 | 0.081 |
| | California 1 | 18 | 235 | 216 | 70 | 8.0 | 0.185 |
| | Arizona 2 | 23 | 190 | 92 | 10 | 8.2 | 0.676 |
| | Arizona 1 | 23 | 1100 | 600 | 140 | 8.1 | 0.357 |

**b.**

| Organism | Groundwater | T ( o C) | TDS ( mg/l) | Ca hard. (mg/l) | Mg hard. (mg/l) | pH | Rate (log10/d) |
|---|---|---|---|---|---|---|---|
| Echo 1 | New York 1 | 12 | 37 | 44 | 44 | 6.0 | 0.054 |
| | N. Carolina 2 | 12 | 95 | 100 | 4 | 8.3 | 0.174 |
| | New York 2 | 12 | 145 | 138 | 56 | 7.3 | 0.051 |
| | Wisconsin | 12 | 260 | 208 | 216 | 8.0 | 0.066 |
| | N. Carolina 1 | 12 | 430 | 138 | 24 | 7.9 | 0.186 |
| | Texas 1 | 13 | 850 | 224 | 224 | 8.0 | 0.138 |
| | Texas 2 | 13 | 950 | 354 | 572 | 7.7 | 0.079 |
| | California 2 | 17 | 200 | 216 | 56 | 8.1 | 0.091 |
| | California 1 | 18 | 235 | 216 | 70 | 8.0 | 0.151 |
| | Arizona 2 | 23 | 190 | 92 | 10 | 8.2 | 0.628 |
| | Arizona 1 | 23 | 1100 | 600 | 140 | 8.1 | 0.188 |

**c.**

| Organism | Groundwater | T ( o C) | TDS | Ca hard. (mg/l) | Mg hard. (mg/l) | pH | Rate (log10/d) |
|---|---|---|---|---|---|---|---|
| MS-2 | N. Carolina 2 | 4 | 95 | 100 | 4 | 8.3 | 0.012 |
| | Arizona 2 | 4 | 190 | 92 | 10 | 8.2 | 0.025 |
| | Wisconsin | 4 | 260 | 208 | 216 | 8.0 | 0.02 |
| | N. Carolina 1 | 4 | 430 | 138 | 24 | 7.9 | 0.014 |
| | Arizona 1 | 4 | 1100 | 600 | 140 | 8.1 | 0.064 |
| | New York 1 | 12 | 37 | 44 | 44 | 6.0 | 0.034 |
| | N. Carolina 2 | 12 | 95 | 100 | 4 | 8.3 | 0.095 |
| | New York 2 | 12 | 145 | 138 | 56 | 7.3 | 0.037 |
| | Arizona 2 | 12 | 190 | 92 | 10 | 8.2 | 0.04 |
| | Wisconsin | 12 | 260 | 208 | 216 | 8.0 | 0.093 |
| | N. Carolina 1 | 12 | 430 | 138 | 24 | 7.9 | 0.03 |
| | Arizona 1 | 12 | 1100 | 600 | 140 | 8.1 | 0.093 |
| | Texas 1 | 13 | 850 | 224 | 224 | 8.0 | 0.077 |
| | Texas 2 | 13 | 950 | 354 | 572 | 7.7 | 0.144 |
| | California 2 | 17 | 200 | 216 | 56 | 8.1 | 0.075 |
| | California 1 | 18 | 235 | 216 | 70 | 8.0 | 0.082 |
| | N. Carolina 2 | 23 | 95 | 100 | 4 | 8.3 | 0.262 |
| | Arizona 2 | 23 | 190 | 92 | 10 | 8.2 | 0.325 |
| | Wisconsin | 23 | 260 | 208 | 216 | 8.0 | 0.244 |
| | N. Carolina 1 | 23 | 430 | 138 | 24 | 7.9 | 0.187 |
| | Arizona 1 | 23 | 1100 | 600 | 140 | 8.1 | 0.244 |

A related paper by Yates and Gerba (1985) incorporated a comparison of the impact of indigenous ground water bacteria on MS-2 survival in ground water. Ground water samples as reported in Yates *et al.*, 1985 were used for survival microcosms, but for five of the samples, ground water in its raw state was compared to a subsample filtered through 0.22 µm pore filter to remove indigenous bacteria. Physio-chemical parameters of the water were also evaluated for their impact on MS-2 survival as components of multiple regression analysis. The addition of filter sterilized vs. raw ground water as a parameter did not change the analysis of significant factors for MS-2 survival from that described in the previous paper. Still, temperature and calcium hardness were the only factors significantly correlated

to die-off rates for MS-2. Table 2 contains inactivation rates from the filtered vs. non-filtered water. Since the original paper expresses only $\log_{10} N/N_0$ ratios after 30 days, rate values in Table 2 were approximated from the ratio of these two values.

**Table 2. Approximate MS-2 inactivation rates in filtered (0.22 μm) vs. non-filtered ground water at 12º C (Yates and Gerba, 1985).**

| | | | Rate ($\log_{10}$/d) | |
|---|---|---|---|---|
| Water | Treatment | TDS (mg/l) | filtered | non-filtered |
| New York 1 | filtered | 37 | 0.032 | 0.033 |
| N. Carolina 2 | filtered | 95 | 0.029 | 0.167 |
| Arizona 2 | filtered | 190 | 0.107 | 0.053 |
| New York 2 | filtered | 145 | 0.05 | 0.032 |
| N. Carolina 1 | filtered | 430 | 0.06 | 0.028 |

To comprehensively compare the effect of indigenous bacteria on survival of introduced viruses in ground water, and evaluate the effect of numerous physical, chemical and microbiological factors on viral persistence, a third study by Yates (Yates *et al.*, 1990) compared multiple samples with differing environmental variables in microcosm survival studies. Essentially, two lines of experimentation were reported. In one, survival in a total of nine samples from four separate states were analyzed and incubated at the natural water temperature of the aquifer, which ranged from 12° to 23° C (54° - 73° F), while comparing duplicates of each ground water sample after filtering through a 0.22 μm filter to remove bacteria. In addition, 19 samples of well water from the Tucson, AZ basin were compared in light of numerous parameters measured for each sample, including pH, turbidity, sulfate, nitrate, ammonia, magnesium hardness, iron, calcium hardness, total hardness, TDS, and heterotrophic bacteria. These microcosm survival studies were also incubated at the natural water temperature, but given they are from the same general area, the temperatures are

assumed to be similar (actual temperatures for the 19 water samples were not reported in the study).

Results of survival studies using MS-2 bacteriophage and poliovirus-1 were reported as days to achieve a 1-$\log_{10}$ reduction in titer, however, these values were extrapolated from linear regression inactivation rates as described in the methods of the study. For this review, then, these rates were back-calculated to determine the inactivation rate in $\log_{10}$ per day by dividing 1 by the days for 1 log reduction. Table 3 contains the inactivation rates determined in this way for the nine samples from North Carolina, Arizona, New York, Texas, and California when comparing filtered vs. raw ground water. For the 19 Tucson-area ground water samples evaluated, the ranges of inactivation rates in $\log_{10}$ reduction per day are as follows: MS-2 in unfiltered water 0.208-1.11, MS-2 in filtered water 0.130-1.43, poliovirus-1 in unfiltered water 0.161-2.00, and poliovirus-1 in filtered water 0.179-0.667.

Statistical correlations were performed by the authors in order to establish if any factors could be significantly associated to trends in inactivation rates. Temperature was again the only factor to consistently correlate to inactivation, with faster inactivation at higher temperatures. Subsequent studies using the same Tucson-area ground water samples to evaluate inactivation along with changes in bacterial population densities over the experimental time frame did reveal that MS-2 reduction was significantly correlated with an increase in bacterial numbers. However, the presence or absence of bacteria (raw vs. filtered) was not found to significantly affect decay rates of either MS-2 or poliovirus. This generalization applied when considering all water samples examined. But large variations in decay rates did exist between samples incubated at the same temperatures; for some inactivation was more rapid in unfiltered water while for others it was more rapid in filtered

waters. In others still, no significant difference existed between filtered and unfiltered waters. The authors conclude that the lack of a consistent trend for the factors examined in all samples except for temperature may thus indicate interactions exist which could vary considerably between different water sources, and may make drawing generalizations for virus inactivation in ground water prohibitively difficult.

**Table 3. Inactivation rates of MS-2 bacteriophage and poliovirus-1 from ground water sources incubated at native temperatures, contrasting filtered vs. raw water (Yates *et al.*, 1990).**

| | | Inactivation Rate ($log_{10}$/d) | | | |
|---|---|---|---|---|---|
| | | MS-2 | | Poliovirus-1 | |
| Sample Origin | T ($^oC$) | Filtered | Raw | Filtered | Raw |
| North Carolina 1 | 12 | 0.4 | 0.03 | 0.185 | 0.137 |
| North Carolina 2 | 12 | 0.28 | 0.094 | 0.161 | 0.114 |
| New York 1 | 12 | 0.031 | 0.034 | 0.026 | 0.035 |
| New York 2 | 12 | 0.054 | 0.037 | 0.049 | 0.052 |
| Texas 1 | 13 | 0.083 | 0.077 | 0.072 | 0.036 |
| Texas 2 | 13 | 0.213 | 0.114 | 0.074 | 0.137 |
| California 2 | 17 | 0.204 | 0.075 | 0.094 | 0.081 |
| California 1 | 18 | 0.039 | 0.081 | 0.079 | 0.185 |
| Arizona | 23 | 0.385 | 0.323 | 0.625 | 0.667 |
| Average | | 0.188 | 0.096 | 0.152 | 0.160 |

Survival of hepatitis A virus (HAV), poliovirus, and echovirus in ground water was evaluated with respect to the effect of temperature, aquifer substrate, and presence of autochthonous microorganisms by Sobsey *et al.* (1986). Virus survival was evaluated in ground water alone or in ground water with one of several soil substrates suspended in it. Soils used were, in wt./vol. concentrations, bentonite clay (3%), kaolinite clay (3%), sandy clay (5%), organic muck (25%), loamy sand (25%), and sand (75%). All soil suspensions

and ground water were at pH 7.0, and experiments were performed using both sterilized and non-sterile preparations of each category. A sterile PBS control was also used, without addition of soils. Survival experiments using primary or secondary treated effluent were also conducted, but those results are not reviewed here. Each trial was done at 5° and 25° C (41° and 77° F). The ground water source was from a deep well in Chapel Hill, NC. Total dissolved solids were not reported, although turbidity was <1 NTU, and nutrient concentrations were <0.1 mg/L ammonia nitrogen and 0.17 mg/L $PO_4$. Survival results were reported by the authors as time in weeks to attain a 2 $\log_{10}$ (99%) inactivation of virus particles. In many cases, less than 2 $\log_{10}$ inactivation was observed, and no data for experiments at 5° C are reported as little inactivation was observed for the ground water or soils suspensions, regardless of the presence of microbes. Table 4 contains approximate inactivation rates calculated by determining the average rate which would result in the total degree of inactivation observed over the study period. For Table 4, weeks were converted to days to provide consistency with other results reviewed here.

**Table 4. Estimated inactivation rates for hepatitis A virus in ground water and with suspended soils, and poliovirus 1 and echovirus 1 in soil suspensions at 25º C (Sobsey *et al.*, 1986).**

| Organism | Suspension | Sterility | Rate (log10/d) | Duration (d) |
|---|---|---|---|---|
| Hepatitis A | g-w only | sterile | 0.036 | 56 |
| | g-w only | non-sterile | 0.14 | 14 |
| | kaolinite clay | sterile | <0.036 | 56 |
| | kaolinite clay | non-sterile | <0.036 | 56 |
| | clay loam | sterile | <0.024 | 84 |
| | clay loam | non-sterile | <0.024 | 84 |
| | organic muck | sterile | <0.024 | 84 |
| | organic muck | non-sterile | 0.032 | 63 |
| | loamy sand | sterile | 0.036 | 56 |
| | loamy sand | non-sterile | 0.095 | 21 |
| | sand | sterile | <0.036 | 56 |
| | sand | non-sterile | 0.036 | 56 |
| | PBS only | sterile | <0.024 | 84 |
| Polio 1 | clay loam | sterile | 0.036 | 56 |
| | clay loam | non-sterile | 0.032 | 63 |
| | organic muck | sterile | 0.071 | 28 |
| | organic muck | non-sterile | 0.095 | 21 |
| | PBS only | sterile | 0.032 | 63 |
| Echo 1 | clay loam | sterile | 0.057 | 35 |
| | clay loam | non-sterile | 0.071 | 28 |
| | organic muck | sterile | 0.057 | 35 |
| | organic muck | non-sterile | 0.071 | 28 |
| | PBS only | sterile | 0.032 | 63 |

Inferences drawn from these data by the authors are that HAV appeared to survive longer in soil suspension than echovirus 1 and perhaps poliovirus 1 at 25° C, regardless of soil type, while all viruses survived well at 5° C. It is also worth noting that among the sterile/non-sterile pair comparisons, the non-sterile replicate resulted in more rapid inactivation for 4 out of 6 HAV experiments, 1 out of 2 poliovirus experiments, and 2 out of 2 echovirus experiments, or 7 out of 10 pairs total showed more rapid inactivation in the non-sterile water or soil-water suspension. The authors also concluded that HAV was affected to a lesser extent than the other viruses by temperature and the presence of native microbes in the ground water and/or ground water-soil suspensions, thus poliovirus 1 and echovirus 1 are not effective indicators for predicting the survival of hepatitis A virus.

A review by Hurst (1988) compiled data from other published reports on factors that influenced survival or inactivation rates of enteroviruses and rotaviruses in surface fresh waters. Quantitative data such as rates were not presented, although a figure of temperature effects was given. However, the temperature effect for data summarized by Hurst varies and no average is given. The author believes that for these studies there are likely other factors beyond those analyzed accounting for differences in temperature effects. Factors which were determined to have a statistically significant effect on waterborne virus survival include:

- chloride concentration over the range of < 0.5 to 16.3 mg/L
- pH over the range of 6.0 to 7.8
- total organic carbon from <1 to 17 mg/L
- hardness from 29 to 339 mg $CaCO_3$
- temperature from 4 - 37° C (39° - 98.6° F)
- turbidity from <2.5 to 36 NTU.

Sunlight also has a significant effect on survival but this is not a consideration in ground water survival, except to say there is a lack of it which would allow longer survival than viral particles in surface water.

A brief study by Yahya, *et al.* (1993) evaluated inactivation of the bacteriophages MS-2 and PRD-1 in four different ground water samples, incubated at the ambient temperature of the aquifer for each sample. Ground water samples came from Arizona (3) and Canada. No parameters of ground water samples were reported and no mention was made of any treatment to the ground water such as filtration, so the water samples were assumed to be in a raw state. Since inactivation results were reported graphically as the

decline of viable phage over time, rate values reported below are estimations from the total decline in infectious concentrations over the duration of the sampling period, thus expressing an average rate of decline. However, die-off curves did have an approximately linear rate on a semi-log scale. Inactivation rates estimated from this study are shown in Table 5.

**Table 5. Estimated bacteriophage inactivation rates in native ground water at ambient aquifer temperatures (Yahya *et al.*, 1993).**

| Organism | Water | T ( $^{o}$C) | Rate ($log_{10}$/d) | Duration (d) |
|---|---|---|---|---|
| MS-2 | Pinetop, AZ | 7 | 0.04 | 55 |
| MS-2 | Tricel, Canada | 7 | no decline | 80 |
| PRD-1 | Pinetop, AZ | 7 | 0.038 | 55 |
| PRD-1 | Tricel, Canada | 7 | no decline | 80 |
| MS-2 | Tucson, AZ (a) | 23 | 0.55 | 10 |
| MS-2 | Tucson, AZ (b) | 23 | 0.325 | 16 |
| PRD-1 | Tucson, AZ (a) | 23 | 0.052 | 75 |
| PRD-1 | Tucson, AZ (b) | 23 | 0.12 | 36 |

Conclusions derived from this study are that little difference in inactivation rates between MS-2 and PRD-1 was observed at lower temperatures, while elevated temperatures affected the survival of MS-2 much more than PRD-1. There was a more pronounced increase in the decay rate of MS-2 at higher temperatures than was observed for PRD-1, although it too showed faster rates of decline at 23º C than at 7º C.

Alvarez, *et al.* (2000) evaluated inactivation of MS-2 and poliovirus in ground water samples. MS-2 were seeded as both a crude lysate or purified by centrifuging with a CsCl density gradient and dialyzing against PBS to remove CsCl. Poliovirus were purified by concentrating via centrifuge and re-suspending in PBS, followed by centrifugation with glycerol gradients. The concentration of PBS was not reported. Ground water was either

filtered through a 0.22 μm pore filter or used raw. In addition, a deionized water control was employed. No TDS, TOC or other parameters of the ground water were reported. Microcosms were incubated at 27° C (80.6º F) with shaking at 100 rpm. Reactivation of viral particles was also examined by reducing the incubation temperature to 4° C (39.2º F) after no pfu were detected from a given sample. Data were given as figures only, so inactivation rates were estimated from the curves. In general, inactivation did not follow first order kinetics, so the rates shown here express average approximate rates of inactivation over the total duration of the experiment or until <1 pfu/ml was detected. Table 6 summarizes the rates estimated from this report. Almost 2 $\log_{10}$ reactivation occurred with the crude MS2 lysate by 3 days after switching to 4° C incubation in both filtered and unfiltered ground water. This reactivation disappeared within 1 day. No reactivation occurred with any of the other microcosms.

**Table 6. Approximate average inactivation rates from Alvarez, *et al.* (2000) for MS2 and poliovirus in ground water at 27º C.**

| Organism | Org. Tmt. | Water | Rate ($\log_{10}$/d) | Duration (d) |
|---|---|---|---|---|
| MS-2 | purified | Filtered g-w | 2.5 | 3 |
| MS2 | purified | Raw g-w | 2.13 | 3 |
| MS2 | purified | deionized | 0.33 | 6 |
| MS2 | lyseate | Filt. g-w | 0.78 | 9 |
| MS2 | lyseate | raw g-w | 0.78 | 9 |
| MS2 | lyseate | deionized | 0.0057 | 14 |
| poliovirus | purified | Filtered g-w | 1.67 | 3 |
| poliovirus | purified | Raw g-w | 1.36 | 3 |
| poliovirus | purified | deionized | 0.26 | 9 |

**Studies on Viruses and Bacteria**

Five reviewed studies evaluated inactivation of both viruses and bacteria, and spanned the years 1982 – 1999. Organisms evaluated by these studies included the virus groups covered by papers reviewed in the last section, such as bacteriophages and enteroviruses (poliovirus) plus other viral groups including coxsackievirus and rotaviruses. In addition, bacterial groups including indicator bacteria (coliforms, *E. coli*, and enterococcocci) and potential pathogens (*Salmonella*, *Shigella*, *Staphylococcus*, and *Vibrio cholera*) were studied.

Keswick, *et al.* (1982) evaluated survival of several indicator organisms and animal viruses *in-situ* in well water using membrane survival chambers. Polycarbonate membranes in Plexiglas chambers allow exchange of water and dissolved compounds while retaining test organisms within the chamber and excluding autochthonous microbes. Organisms evaluated were coxsackievirus B3, poliovirus 1, echovirus 7, rotavirus SA-11, f2 bacteriophage, *E. coli*, *S. typhimurium*, and fecal streptococci (enterococci). *E. coli* was a laboratory strain, while the fecal streptococcus was a single isolate from domestic sewage. The source of *S. typhimurium* was not reported. Bacterial cultures were grown in nutrient broth and washed by centrifugation and re-suspension in PBS three times. Animal virus strains were all obtained from laboratories and propagated on various respective cell lines. Seeded chambers were placed in a covered container receiving a continuous flow of ground water. The water temperature over the 24-day duration of the experiment varied from 3° to 15° C (37.4º - 59º F). No other information of the ground water itself was reported. Inactivation rates as reported in this study are shown in Table 7, with the organisms ordered from the slowest to

fastest rates of decline. The indicator organisms bacteriophage f2 and *E. coli* both declined at a faster rate than two of the animal viruses (coxsackievirus B3 and poliovirus 1), while the sewage-isolate fecal streptococci exhibited a similar inactivation rate to poliovirus 1 and coxsackievirus B3.

**Table 7. Reported inactivation rates of several organisms in a continuous-flow ground water experiment by Keswick *et al.* (1982).**

| Organism | Rate ($log_{10}$/d) |
|---|---|
| coxsackievirus B3 | 0.19 |
| poliovirus 1 | 0.21 |
| fecal streptococci | 0.23 |
| *E. coli* | 0.32 |
| rotavirus SA-11 | 0.36 |
| bacteriophage f2 | 0.39 |

Bitton *et al.* (1983) evaluated survival of a number of indicator organisms and pathogens in a single Florida ground water source. Ground water from a 475 ft well serving the Gainesville, Fl area was used in microcosms to evaluate the die-off of *E. coli*, *Streptococcus (Enterococcus) faecalis*, *Salmonella typhimurium*, f2 bacteriophage, and poliovirus type 1, all laboratory strains. Reported characteristics of ground water samples included hardness at 198 mg/l $CaCO_3$, turbidity of 0.35 NTU, and a conductivity measurement of 355 µMhos/cm, equating to an approximate TDS concentration of 178 ppm. Principle ion concentrations were, in mg/l, $Ca^{2+}$ 59.2, $Mg^{2+}$ 12.2, $Cl^-$ 9.5, $SO_4^{2-}$ 29.8, $NO_3^-$ 3.5, and $S_2^-$ 0.088. Bacterial cultures were grown in nutrient broth and washed by centrifugation and re-suspension in ground water three times. Virus and phage were suspended in PBS and diluted in groundwater prior to seeding survival experiments. Seeded ground water flasks were incubated at 22° C (72º F) for 15 days. Inactivation rates were

reported as the slope of linear regression curves for plots of cultivatable organisms against time. These rates, units converted to $d^{-1}$ from $h^{-1}$, are shown in Table 8. In addition to laboratory microcosm experiments, a field study was reported in which samples were taken from 6 shallow monitoring wells tapping ground water underlying a cypress stand that received primary septic tank effluent from the city of Waldo, Fl. Septic discharge was halted due to excessively dry conditions in the spring of 1981, and sampling of the shallow wells was conducted with the cessation of discharge to evaluate the survival of indigenous bacteria that had infiltrated through the cypress stand. No physical or chemical parameters of this ground water were reported. *In situ* die-off rates of these bacteria, as reported by the authors but units converted to $d^{-1}$, are also shown in Table 8.

**Table 8. Inactivation rates in a Florida ground water sample at 22° C as reported in Bitton, *et al.* (1983).**

| Organism | Environment | Rate (log10/d) |
|---|---|---|
| *St. faecalis* | laboratory | 0.0288 |
| Poliovirus 1 | laboratory | 0.0456 |
| *Sa. typhimurium* | laboratory | 0.1296 |
| *E. coli* | laboratory | 0.1584 |
| f2 | laboratory | 1.416 |
| fecal streptococci | field | 0.0204 |
| fecal coliforms | field | 0.03 |
| total coliforms | field | 0.0384 |

These results indicate that *E. coli* and *S. typhimurium* were much more rapidly inactivated in this ground water than was poliovirus 1, but inactivation of *S. faecalis* was approximately similar to poliovirus 1. Thus, it appears from these results that enterococci may be a better indictor of enterovirus survival than *E. coli.* However, inactivation of indigenous fecal and total coliforms in the natural aquifer closely paralleled that of poliovirus 1 in the laboratory, so native indicator strains may be adequate for indicating enterovirus

361 survival. Also, native populations of bacteria may have longer survival times than laboratory
362 strains.
363
364 The survival of numerous indicator and pathogenic bacteria and coliphage as
365 impacted by several factors in fresh and sea water was reported by Evison (1988). The
366 following organisms were used in microcosm batch studies: *E. coli*, fecal streptococci,
367 maroon fecal streptococci, *Salmonella typhimurium* strains 12, 12a and 110, *Sal. anatum*,
368 *Shigella sonnei*, *Sh. flexneri*, *Yersinia enterocolitica*, *Campylobacter fetus*, MS-2
369 bacteriophage, and f2 bacteriophage. *E. coli*, fecal streptococci, and maroon fecal
370 streptococci were seeded by adding raw sewage to microcosms. Pathogenic bacteria were
371 clinical isolates from stool samples and were propagated using sterilized sewage as a nutrient
372 source, except for *C. fetus* which was grown with brain-heart infusion. Coliphage were
373 propagated using *E. coli*. Freshwater and seawater were both filter sterilized using 0.22 μm
374 pore-size filters prior to seeding with organisms and sewage. No physical or chemical
375 parameters such as TDS, salinity, or pH of the native water samples, fresh or marine, were
376 reported. Conditions evaluated for impacts on inactivation were temperature, salinity,
377 nutrient amendment using sterilized sewage, and dark vs. light intensity. As this review
378 focuses on ground water inactivation, only those studies performed under dark conditions are
379 summarized here. Neither the type of salt used to adjust salinity for salinity-controlled
380 experiments nor the actual salinity/TDS of water at 0.0 ppt was given. Inactivation results
381 were reported as hours to attain 1 $\log_{10}$ inactivation of viable organisms. These data were
382 converted to average inactivation rates in $\log_{10}$ reduction in titer/day, assuming more-or-less
383 linear inactivation kinetics. However, caution should be used in extrapolating these k values

beyond 1 $log_{10}$ inactivation. Tables 9a – 9c summarize the rates calculated from reported data under the various conditions.

**Table 9a. Inactivation rates from a range of temperatures, converted from hours to attain 1 $log_{10}$ reduction in titer (Evison, 1988).**

| Organism | Water | T ($^{o}$ C) | 2 | 5 | 10 | 15 | 20 | 25 | mean |
|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | fresh | Rates | 0.033 | 0.047 | 0.081 | 0.1 | 0.22 | 0.35 | 0.14 |
| *E. coli* | sea | ($log_{10}$/d) | 0.04 | 0.052 | 0.06 | 0.25 | 0.25 | 0.3 | 0.16 |
| fecal strep | fresh | | 0.076 | 0.13 | 0.21 | 0.15 | 0.43 | 0.36 | 0.23 |
| fecal strep | sea | | 0.014 | 0.015 | 0.027 | 0.034 | 0.12 | 0.13 | 0.06 |
| maroon fecal strep | fresh | | 0.04 | 0.054 | 0.11 | 0.071 | 0.36 | 0.33 | 0.16 |
| maroon fecal strep | sea | | 0.026 | 0.029 | 0.03 | 0.034 | 0.046 | 0.047 | 0.04 |
| MS-2 | fresh | | | 0.029 | 0.02 | 0.037 | 0.088 | 0.048 | 0.04 |
| MS-2 | sea | | | 0.045 | 0.074 | 0.048 | 0.1 | 0.066 | 0.07 |
| f2 | fresh | | | 0.1 | 0.27 | 0.38 | 0.63 | 1.6 | 0.60 |
| f2 | sea | | | 0.18 | 0.22 | 0.36 | 1.04 | 3 | 0.96 |
| *S. typhimurium 12a* | fresh | | 0.026 | 0.036 | 0.033 | 0.1 | 0.14 | 0.13 | 0.08 |
| *S. typhimurium 12a* | sea | | 0.052 | 0.079 | 0.077 | 0.081 | 0.13 | 0.14 | 0.09 |
| *S. anatum* | fresh | | 0.212 | 0.031 | 0.025 | 0.088 | 0.15 | 0.16 | 0.11 |
| *S. anatum* | sea | | 0.07 | 0.099 | 0.068 | 0.1 | 0.17 | 0.13 | 0.11 |
| *Sh. sonnei* | fresh | | 0.42 | 0.24 | 0.17 | 0.081 | 0.14 | 0.25 | 0.22 |
| *Sh. sonnei* | sea | | 0.82 | 1.04 | 1.5 | 1.5 | 0.081 | 0.027 | 0.83 |
| *Y. enterocolitica* | fresh | | | 0.038 | 0.023 | -0.22 | -0.06 | -0.13 | -0.07 |
| *Y. enterocolitica* | sea | | | 0.69 | 0.37 | 0.6 | 0.75 | 0.45 | 0.57 |
| *C. fetus* | fresh | | | 0.16 | 0.2 | 0.29 | 0.4 | 0.89 | 0.39 |
| *C. fetus* | sea | | | 0.5 | 0.48 | 0.83 | 0.71 | 1.26 | 0.76 |

**Table 9b. Inactivation rates across a range of salinities, converted from hours to attain 1 $log_{10}$ reduction in titer (Evison, 1988).**

| Organism | Inoculum source | Salinity (ppt) | 0.0 | 0.44 | 0.88 | 1.31 | 1.75 | 2.63 | 3.50 |
|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | sewage | Rates | 0.12 | 0.3 | 0.034 | 0.089 | 0.1 | 0.088 | 0.1 |
| fecal strep | sewage | ($log_{10}$/d) | 0.14 | 0.26 | 0.017 | 0.035 | 0.068 | 0.042 | 0.039 |
| maroon fecal strep | sewage | | 0.14 | 0.25 | 0.018 | 0.0094 | 0.04 | 0.027 | 0.0081 |
| *S. typhimurium 12* | clinical | | 0.077 | 0.2 | -0.0163 | 0.016 | 0.028 | 0.035 | 0.072 |
| *S. typhimurium 12a* | clinical | | 0.12 | 0.024 | 0.17 | 0.2 | 0.15 | 0.021 | 0.12 |
| *S. typhimurium 110* | clinical | | 0.14 | 0.011 | 0.27 | -0.055 | 0.027 | 0.029 | 0.092 |
| *Sh. flexneri* | clinical | | 0.015 | 0.59 | 0.59 | 0.92 | 0.86 | 0.65 | 0.169 |
| *Sh. sonnei* | clinical | | 0.059 | -0.075 | 0.067 | 0.86 | 1 | 0.57 | 0.043 |
| *Y. enterocolitica* | clinical | | -0.2 | -0.24 | 0.01 | 0.12 | 0.072 | 0.49 | 0.42 |
| mean for all organisms | | | 0.07 | 0.15 | 0.13 | 0.24 | 0.26 | 0.22 | 0.12 |

**Table 9c. Effect of nutrient amendment (as sterilized sewage) on inactivation rates estimated from hours to attain 1 $log_{10}$ inactivation (Evison, 1988).**

| Organism | Water | Amended sewage conc. (%) | 0.025 | 0.25 | 2.5 | 25 |
|---|---|---|---|---|---|---|
| *E. coli* | fresh | Rates ($log_{10}$/d) | 0.14 | 0.32 | 0.28 | 0.043 |
| *E. coli* | sea | | 0.15 | 0.12 | 0.13 | 0.023 |
| fecal strep | fresh | | 0.066 | 0.19 | 0.33 | 0.047 |
| fecal strep | sea | | 0.092 | 0.086 | 0.52 | 0.04 |
| maroon fecal strep | fresh | | 0.025 | 0.15 | 0.26 | 0.032 |
| maroon fecal strep | sea | | 0.018 | 0.0066 | 0.027 | -0.0058 |
| MS-2 | fresh | | 0.05 | 0.058 | 0.055 | 0.026 |
| MS-2 | sea | | 0.012 | 0.0061 | 0.0075 | 0.014 |
| *S. typhimurium 12a* | fresh | | 0.22 | 0.21 | 0.18 | -0.17 |
| *S. typhimurium 12a* | sea | | 0.0095 | 0.019 | 0.073 | -0.27 |
| *S. anatum* | fresh | | 0.3 | 0.28 | 0.21 | -0.5 |
| *S. anatum* | sea | | 0.0012 | 0.059 | 0.059 | -0.27 |
| *Sh. flexneri* | fresh | | 0.038 | 0.33 | 0.22 | 0.25 |
| *Sh. flexneri* | sea | | 0.63 | 0.15 | 0.08 | 0.23 |
| *Y. enterocolitica* | fresh | | 0.079 | 0.0053 | -0.28 | -1.26 |
| *Y. enterocolitica* | sea | | 0.56 | 0.26 | 0.076 | -0.0022 |
| *C. fetus* | fresh | | 0.42 | 0.36 | 0.38 | 0.6 |
| *C. fetus* | sea | | 0.23 | 0.16 | 0.15 | 0.8 |

While a statistical multi-way analysis of the factors affecting the rate of inactivation of theses organisms was not reported, comparisons of inactivation rates across ranges of conditions and of mean values allows some generalizations and trends to be noted. From

data shown in Table 9a, *E. coli* was inactivated slightly more rapidly in seawater than in freshwater on average, but fecal streptococci and maroon fecal streptococci inactivation was somewhat more rapid in fresh water than in sea water. While the response to salinity varied among the organisms, the author observes that the general trend from all organisms combined as evidenced by mean inactivation rates is for slowest inactivation at 0.88 ppt salinity. However, this observation was derived by omitting negative values from averages, and if all values are considered, including negative values reflecting growth, the slowest mean inactivation rate was in the 0.0 ppt salinity condition (Table 9b). Regarding the response to temperatures, the author states that for most organisms a linear relationship existed between temperature and inactivation, indicating more rapid inactivation at increasing temperatures. This relationship is visible by looking at the data in Table 9a.

Growth of some organisms was observed at some salinity values at 15º C (59º F). Growth was indicated by a negative number of hours to attain 1 $\log_{10}$ inactivation, although the author did not made clear if in these cases 1 $\log_{10}$ of growth was actually observed. Growth was frequently observed for *Y enterocolitica*, notably low salinity values at 15º C (59º F), in fresh water at 15º, 20º, and 25º C (59º, 68º and 77º F), and in nutrient amendment experiments with 2.5% and 25% amended sewage. Also, in the 25% added sewage conditions, *Salmonella* spp. in both sea water and fresh water, and maroon fecal streptococci in sea water demonstrated growth. These findings indicate that growth of some indicator and even pathogenic bacteria is possible given conditions such as high nutrient levels, as would be seen in heavily contaminated water. However, sterilized sewage was used as a nutrient source, so the impact of competing organisms that would be found in such a situation in the environment was negated. Overall, the author concluded that *E. coli* is an adequate indicator

for the presence of culturable pathogens in freshwater, but the fecal streptococci (enterococci) are a better indicator for seawater.

Nasser and Oman (1999) examined temperature effects on several organisms in ground water and in a PBS control. Organisms evaluated were hepatitis A virus (HAV), male specific bacteriophage (F+ phage), *E. coli*, and poliovirus 1. The bacteriophage were isolated from raw wastewater by PEG precipitation, the rest of the organisms were cultivated from laboratory organism stocks. Ground water samples were taken from a 53-m deep well in Israel. Parameters of the ground water reported were pH 7.4, conductivity 600 µMho/cm (~ 300 ppm TDS), and heterotrophic plate count bacteria <100 cfu /100 ml. Experimental reactors were incubated at temperatures of 4, 10, 20, 30, and 37 ° C (39º, 50º, 68º and 99º F). Samples were taken up to 90 days. Inactivation rates were expressed graphically as a function of temperature for each organism. Inactivation of organisms at 37° C was not shown. Approximate inactivation rates ($log_{10}$/d) were estimated for this review from figures and are given in Tables 10a -10d. It is interesting that inactivation of *E. coli* was most rapid at 4° C. Otherwise, survival was negatively impacted by temperature based on comparative observation of figures in this study.

**Table 10. Inactivation rates of *E. coli* (a) , F+ bacteriophage (b), poliovirus 1 (c) and hepatitis A virus (d) at multiple temperatures reported by Nasser and Oman (1999).**

**a. *E. coli*** **b. F+ phage**

| Water | T ( °C) | Rate ($log_{10}$/d) |
|---|---|---|
| groundwater | 4 | > 0.08 |
| PBS | 4 | 0.04 |
| groundwater | 10 | 0.06 |
| PBS | 10 | 0.002 |
| groundwater | 20 | 0.015 |
| PBS | 20 | 0.003 |
| groundwater | 30 | 0.035 |
| PBS | 30 | 0.01 |

| Water | T ( °C) | Rate ($log_{10}$/d) |
|---|---|---|
| groundwater | 4 | 0.015 |
| PBS | 4 | 0.005 |
| groundwater | 10 | 0.012 |
| PBS | 10 | 0.001 |
| groundwater | 20 | 0.02 |
| PBS | 20 | 0.005 |
| groundwater | 30 | 0.022 |
| PBS | 30 | 0.01 |

**c. poliovirus 1**

| Water | T ( °C) | Rate ($log_{10}$/d) |
|---|---|---|
| groundwater | 4 | 0.005 |
| PBS | 4 | 0.005 |
| groundwater | 10 | 0.01 |
| PBS | 10 | 0.002 |
| groundwater | 20 | 0.05 |
| PBS | 20 | 0.02 |
| groundwater | 30 | 0.055 |
| PBS | 30 | 0.05 |

**d. hepatitis a virus**

| Water | T ( °C) | Rate ($log_{10}$/d) |
|---|---|---|
| groundwater | 4 | 0.01 |
| PBS | 4 | 0.005 |
| groundwater | 10 | 0.001 |
| PBS | 10 | 0.005 |
| groundwater | 20 | 0.015 |
| PBS | 20 | 0.015 |
| groundwater | 30 | 0.035 |
| PBS | 30 | 0.04 |

Dowd and Pillai (1997) evaluated survival of two bacteria and two bacteriophage in ground water microcosms. Experiments were run with *S. typhimurium* and a *Klebsiella* species, both clinical isolates, and MS-2 and PRD-1, both laboratory strains. Bacterial suspensions were washed thoroughly, although the number of wash cycles was not reported. Ground water samples were pH 7.3, 0.0138% total organic carbon (TOC), 440 mg/L Na, 389 mg/L Cl, and 751 mg/L $SO_4$. Survival microcosms were incubated at 21° C (70º F) with shaking for up to 32 days. Inactivation results were reported graphically only for bacterial organisms, while the bacteriophage inactivation was stated as approximately 0.8 $log_{10}$/d for both phage strains. Thus, for Table 11, bacterial inactivation values are estimated from figures in the original article.

**Table 11. Inactivation rates of bacteria and bacteriophages in ground water at 21° C (Dowd and Pillai, 1997).**

| Organism | Rate ($log_{10}$/d) | Duration (d) |
|---|---|---|
| *Klebsiella* spp. | 0.094 | 32 |
| *S. typhimurium* | 0.517 | 12 |
| MS-2 | 0.8 | 8 |
| PRD-1 | 0.8 | 8 |

**Studies on Bacteria and *Cryptosporidium***

Six studies in which survival of bacteria and / or *Cryptosporidium* oocysts were evaluated were reviewed, which spanned the years 1974 - 1999. One of the first published reports on in-situ survival of microorganisms in an aquifer was authored by McFeters, *et al.* (1974), in which investigators used membrane chambers suspended in an overflow well with a flowing supply of ground water. Well water had a measured conductivity of 420 µmhos/cm (approximate TDS of 210 mg/L), pH 7.48, calcium ion concentration of 3.09 meq/L, and temperature of 9.5 – 12.5 ° C (49º - 54º F). Other ionic chemical constituent concentrations were reported as well. Organisms were derived from several sources and included both natural populations and laboratory organisms, including mixed cultures and enteric pathogens. Indicator bacteria were isolated from streams or fecal samples of various animals. These isolates were used to make purified cultures and characterized. Coliform cultures were identified to be one of the following: *E. coli*, *E. coli* II, *Enterobacter aerogenes*, or *Escherichia freundii*. Fecal streptococci (enterococci) isolates were identified as one of *Streptococcus faecalis, S. faecium, S liquifaciens, S. zymogenes, S. durans*, or *S. bovis*. In addition, an isolate of *S. equines* was used as supplied by the U.S. EPA. An

*Aeromonas* species was isolated form a lake in Washington state and used. Enteric pathogens were obtained form laboratories, and are listed in Table 12 with their inactivation rates. Mixed suspensions of indicator bacteria were obtained by bovine and elk droppings and raw domestic sewage. Each suspension of organisms was used to fill a separate sterile membrane chamber. Membranes allowed the passage of water and dissolved species, but retained bacteria within, and prevented intrusion by other bacteria naturally in the well water.

Survival results were reported in half-times (time for half of the organisms to die), and were converted to $\log_{10}$ inactivation rates for comparison as presented here in Table 12. Extra caution should be noted when considering these values. By extrapolating times in hours for reduction of the concentration of these organisms to inactivation rates in log/day, the resulting total duration over which these rates are estimated is less than 1 day for most. In addition, the total degree of inactivation considered is only a ratio of 0.5 or .3 log reduction, whereas most of the other data reviewed here examines larger degrees of inactivation over longer time periods.

**Table 12. Approximate inactivation rates for indicator and pathogenic bacteria in membrane chambers suspended in flowing well water, converted from time in hours for half the initial number of organisms to die (McFeters *et al.*, 1974). Experimental temperatures ranged from 9.5º to 12.5º C (49.1º - 54.5º F).**

| Organism | Rate ($Log_{10}$/d) | Duration (d) |
|---|---|---|
| Coliform isolates (avg.) | 0.425 | 0.71 |
| Enterococci isolates (avg.) | 0.328 | 0.917 |
| Coliform (sewage) | 0.413 | 0.73 |
| Enterococci (sewage) | 0.37 | 0.81 |
| *Strep. equinus* | 0.72 | 0.42 |
| *Strep. bovis* | 1.68 | 0.18 |
| *Shigella dysenteriae* | 0.323 | 0.93 |
| *Sh. sonnei* | 0.295 | 1 |
| *Sh. flexneri* | 0.27 | 1.1 |
| *Sal.enteriditis* paratyphi A | 0.452 | 0.67 |
| *Sal.enteriditis* paratyphi D | 0.376 | 0.8 |
| *Sal. enteriditis* typhimurium | 0.452 | 0.67 |
| *Sal. typhi* | 1.2 | 0.25 |
| *Vibrio colerae* | 1 | 0.3 |
| *Sal. enteriditis* paratyphi B | 3.01 | 0.1 |

These data indicate that enterococci as a group have a slightly longer survival time than coliforms, as has been shown by other reports. The results of studies on the *Aeromonas* isolate revealed no measurable decline over three days. As with laboratory based studies, extrapolation of these inactivation rates to field situations should be done with caution since the membrane chambers did not allow interaction with competing or predatory organisms that may be naturally in aquifer water. Also, the organisms, except those derived from sewage, were processed somewhat in order to purify the cell suspension. The authors report they found washing organisms (all enterococci and pathogens except *Sh. sonnei*) by re-suspension in refrigerated phosphate buffered saline decreased their survival potential by as much as an order of magnitude. Organisms for this study were thus washed using sterilized well water. Although data were not shown, considerable variability in die-off rates among the coliform group was observed, without respect to fecal vs. non-fecal coliform grouping. Meanwhile, enterococci showed a higher degree of agreement.

Survival of several pathogenic and indicator/facultative pathogenic bacteria in a single ground water source from Germany was evaluated by Filip, *et al.* (1988). The ground water source was well-characterized in terms of ionic and other chemical constituents; notably, pH was 7.3 and TDS were 486 mg/L as determined by evaporation. Experiments were performed with microcosms in 1-l sterile flasks, using filter sterilized water samples held at 10° C (50º F), the natural temperature of the originating aquifer. Organisms analyzed for their survival in this water were as follows: *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Yersinia enterocolitica, Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Bacillus megaterium,* and *Clostridium perfringens*. All organisms were derived as pure cultures from laboratory stocks except for *Sal. typhimurium* which was a surface water isolate. Experimental durations ranged to 100 days.

Results were reported graphically in the original article as survival in $\log_{10}$ units per ml as a function of time (days). To derive inactivation rates for this review, total inactivation by day 100 or at total extinction was divided by the number of days, and these average rates are shown in Table 13. Initial concentrations of these bacteria as estimated from the original figure varied but was in the range of $8 \times 10^5$ to $8 \times 10^6$ except for *C. perfringens* which was approximately $6 \times 10^3$. For those organisms that a rate is listed in Table 13, inactivation was approximately first order by visual inspection, while for others the exception is noted.

**Table 13. Estimated average inactivation rates of several pathogenic and indicator bacteria in a single, sterile ground water source at 10° C (50º F) (Filip *et al.*, 1988).**

| Organism | Rate ($Log_{10}$/d) | Duration (d) |
|---|---|---|
| *Str. faecalis* | 0.012 | 100 |
| *Y. enterocolitica* | 0.008 | 100 |
| *E. coli* | 0.027 | 100 |
| *Sal. typhimurium* | 0.042 | 100 |
| *Staph. aureus* | 0.19 | 25 |
| *B. megaterium* | 0.55 | 10 |
| *B. cereus* | N/A* | 100 |
| *C. perfringens* | N/A** | 100 |
| *P. aeruginosa* | N/A*** | 100 |

* declined ~ 3.6 log in 10 d, no subsequent decline to 100 d
** little to no decline to 100 d
*** increase in concentration to ~ 11 d, thereafter ~ 1 log decline to 100 d

The impact of indigenous microbiota on the survival potential of *Aeromonas hydrophila* in freshwater microcosms was evaluated by Kersters, *et al.* (1996). An *A. hydrophila* strain was genetically-marked with antibiotic resistance genes to differentiate it from naturally occurring aeromonads and seeded into survival microcosms of both filtered and autoclaved and raw surface water or ground water samples. Water samples were taken from drinking water source waters. The authors also evaluated survival in a number of low-nutrient waters such as bottled drinking water, tap water, and deionized water. The survival of *Spirillum* strain NOX (a spiral heterotrophic bacterium) and *Pseudomonas fluorescens* were also evaluated in the low-nutrient waters. Only the inactivation rates from *A. hydrophila* survival in surface water and ground water are reviewed here, however, as this bacterium was the only one evaluated in natural waters.

Physio-chemical parameters of the water samples were not reported. The location of origin for water samples was drinking water plants from four locations in the Netherlands. Two surface water sources and two ground water sources were evaluated. Survival experiments were conducted at room temperature and experimental durations extended for up

to 15 days. The authors report inactivation rates in tabular format, but specify that these rates indicate the decline over the linear portion of the inactivation curves. Graphical figures of *A. hydrophila* concentrations observed indicate an initial increase in concentration in some of the samples, particularly in one of the ground water samples. After two days, *A. hydrophila* concentrations declined in all water samples. Table 14 contains the inactivation rates in $lod_{10}$ /d as reported by the authors in the original publication. Significant differences were observed between sterilized samples and raw samples, with inactivation proceeding more rapidly in the raw samples on average. In addition, the interaction of water source and sterility was significant, meaning that the relative impact of sterilizing the water sample on survival varied between water sources. There was a larger increase in inactivation rates between sterile and raw water for surface water than for ground water. However, part of the reason for this difference is that for the ground water 2 sample, inactivation was slower in the raw water compared to sterile water.

**Table 14. Inactivation rates of *A. hydrophila* in filtered/autoclaved vs. raw surface water and ground water at approximately 22º C (72º F) (Kersters *et al.*, 1996).**

| Sample Origin | Treatment | Rate (log10/d) |
|---|---|---|
| ground water 1 | sterile | 0.08 |
| ground water 1 | raw | 0.61 |
| ground water 2 | sterile | 0.5 |
| ground water 2 | raw | 0.37 |
| surface water 1 | sterile | 0.03 |
| surface water 1 | raw | 0.5 |
| surface water 2 | sterile | 0.09 |
| surface water 2 | raw | 0.97 |

585 Survival of *Cryptosporidium parvum* oocysts in river water and in response to
586 numerous environmental pressures including freezing, drying, and metal exposure was
587 assessed by Robertson, *et al.* (1992). Determining viability of *Cryptosporidium* oocysts is
588 more complicated than for culturable bacteria and many viruses, and in this case authors used
589 a dye inclusion/exclusion method whereby oocysts which exclude propidium iodide and
590 include 4', 6-diamidino-2-phenylindole (DAPI) are considered viable based on correlation
591 with excystation results. For determining inactivation in river water, a semipermeable
592 chamber was seeded with purified oocysts and then suspended in a flowing river. Neither
593 temperature nor any other physio-chemical parameters of the river water were recorded. To
594 determine oocyst survival, samples were taken on various days up to 176 d, and the
595 percentage of oocysts exhibiting PI exclusion and DAPI inclusion were determined. In order
596 to interpret these data for inclusion in this review, it was noted that the percentage of DAPI+
597 oocysts declined from 74.4% at day 0 to 10.0% at day 176. That equates to an $N/N_0$ ratio of
598 approximately 0.134, thus $\log_{10} N/N_0 = -0.87$. Thus for expressing this decline as a rate as
599 elsewhere in this review, the inactivation rate is approximately 0.005 $\log_{10}$/d.
600
601 Medema *et al.* (1997) determined inactivation kinetics of several indicator species
602 and *C. parvum* in river water that was either sterilized or raw at two temperatures.
603 Organisms evaluated in this study were *C. parvum*, *E. coli*, *Enterococcus faecium*, and
604 *Clostridium perfringens*. The source of indicator organisms varied between microcosms
605 using autoclaved river water (Meuse River, Netherlands) or raw water. Autoclaved water
606 organisms were isolated from water and maintained as pure cultures in the laboratory.
607 Inoculi for raw water microcosms was primary sewage effluent, purified by Percoll-sucrose

flotation. Oocysts used in both types of water were a deer strain cultivated in lambs. Both water types were incubated at both 5° and 15° C (41º and 59º F). *Cryptosporidium* viability was assayed by both excystation and dye exclusion methods. Results were reported by the authors as inactivation rate constants ($log_{10}$/d), and occasionally bi-phasic kinetics were observed, in which case two rate constants were reported. Inactivation rates are summarized in Table 15.

**Table 15. Inactivation rates of indicator bacteria and *Cryptosporidium* in autoclaved and non-sterile river water (Medema *et al.*, 1997).**

| Organism | Inoculum source | Water | T ( °C) | Rate ($log_{10}$/d) | Duration (d) |
|---|---|---|---|---|---|
| *C. parvum* excystation | lamb | autoclaved | 5 | 0.01 | 35 |
| *C. parvum* dye excl. | lamb | autoclaved | 5 | 0.01 | 35 |
| *C. parvum* excystation | lamb | autoclaved | 15 | 0.006 | 35 |
| *C. parvum* dye excl. | lamb | autoclaved | 15 | 0.011 | 35 |
| *C. parvum* excystation | lamb | raw | 5 | 0.01 | 35 |
| *C. parvum* dye excl. | lamb | raw | 5 | 0.01 | 35 |
| *C. parvum* excystation | lamb | raw | 15 | 0.024 | 35 |
| *C. parvum* dye excl. | lamb | raw | 15 | 0.018 | 35 |
| *E. coli* | water isolate | autoclaved | 5 | 0.01 | 77 |
| *E. coli* | water isolate | autoclaved | 15 | -0.008 | 77 |
| *E. coli* | sewage | raw | 5 | 0.102 | 42 |
| *E. coli* | sewage | raw | 15 | 0.202 | 0-14 |
| *E. coli* | sewage | raw | 15 | 0.049 | 14-42 |
| *Ent. faecium* | water isolate | autoclaved | 5 | 0.014 | 77 |
| *Ent. faecium* | water isolate | autoclaved | 15 | 0.005 | 77 |
| fecal enterococci | sewage | raw | 5 | 0.077 | 42 |
| fecal enterococci | sewage | raw | 15 | 0.233 | 0-14 |
| fecal enterococci | sewage | raw | 15 | 0.025 | 14-42 |
| *C. perfringens* | water isolate | autoclaved | 5 | 0.012 | 77 |
| *C. perfringens* | water isolate | autoclaved | 15 | 0.027 | 77 |
| *C. perfringens* | sewage | raw | 5 | 0.003 | 42 |
| *C. perfringens* | sewage | raw | 15 | 0.005 | 42 |

To summarize these data, *C. parvum* exhibited no difference in inactivation between sterilized and non-sterile water at 5° C, but declined more rapidly in non-sterile water at 15° C. For *E. coli* and *Ent. faecium*, inactivation was generally slower in autoclaved water than non-sterile water. *E. coli* increased in titer in autoclaved water at 15 ° C then remained constant for the duration of the experiment. Decay of *Ent. faecium* was slower at 15° C than in 5° C water. *C. perfringens* was 3-4 times more persistent in natural (non-sterile) water than *Cryptosporidium* oocysts, but this trend was reversed in autoclaved water.

The effect of temperature and salinity on *Cryptosporidium parvum* infectivity was evaluated by Freire-Santos, *et al.* (1999) using mouse infectivity analyses. Conditions evaluated in a factorial experiment were storage times of 2, 21, and 40 days, temperatures of 4°, 11°, and 18° C (39º , 52º, and 64º F), and salinity of 0, 17 and 35 ppt. *C. parvum* oocysts stored under the respective conditions were inoculated into neonate mice and later mouse intestines were macerated and examined for the number of oocysts present. Analysis of variance on the factors affecting intensity of infection revealed that time, salinity, and the interaction of time-salinity produced a significant effect on the infectivity of oocysts. The effect of these factors was modeled into an infection intensity function, which revealed maximum infection potential with oocysts stored in the lowest salinity and for the shortest time. Oocysts stored for the longest time and at the highest salinity produced the lowest infection density. These data are of limited use for the context of this review since they do not express a measure of inactivation, only of effects on infection intensity as determined by evaluation of *Cryptosporidium* abundance in mouse intestinal tissue after inoculation. Therefore, a quantitative estimate of inactivation rates or a similar statistic was not possible.

642

643

644 **Summary and Conclusions**

645 To summarize inactivation rate data for these organisms, most rates as reported in or
646 approximated from reviewed studies and expressed here in Tables 1 – 15 were compiled for
647 each type of organism (Appendix 1). Some rates from study conditions were omitted if the
648 parameters were not deemed to be representative of conditions in aquifers, such as studies in
649 seawater or seawater-like salinity, those in sterile buffered saline, or those involving nutrient
650 amendment. From these compilations, some summary statistics were determined in order to
651 gauge the ranges and central tendencies of the data. Groupings of organisms for these
652 summary statistics were coliphage, poliovirus, echovirus, hepatitis A virus, PRD-1
653 bacteriophage, coliform bacteria, enterococci/streptococci, *Salmonella* spp., *Shigella* spp.,
654 *Clostridium perfringens*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, and
655 *Cryptosporidium parvum*. In addition, histograms of the frequency of rate values were
656 constructed in order to provide visual representation of the distribution of inactivation rates
657 (Appendix 2). Since several works that have been reviewed here report significant effects
658 due to temperature, rates were grouped for each organism into temperature ranges as well.

659 Summary statistics for each organism group are shown in Tables 16 and 17. Table 16
660 contains summaries of bacteria and *Cryptosporidium* inactivation data, while Table 17
661 contains information for the viruses and bacteriophage. In each table, statistics calculated
662 include the number of observations, means, medians, and standard deviations. Also, the
663 values corresponding to Q1 and Q3 (lower and upper bounds of the middle 50% of observed
664 values, respectively), interquartile ranges (iqr - range of the middle 50% of values), and

minimum and maximum inactivation rate values from the reviewed studies are listed. The location of the outer fences F1 and F3 were determined and given, which were based on the value of Q1 or Q3 -/+ (3 x iqr) respectively. The location of outer fences identifies any rate values that are strong outliers compared to the rest of the reviewed inactivation rates for that organism group, and the number of strong outliers is given for each group. Also, temperature ranges of the studies included and the number of studies evaluated for each organism group are shown. Statistics for the quartiles, fences, and outliers were only performed on organism groups for which there were at least 10 values.

**Table 16. Summary statistics for bacteria and *Cryptosporidium parvum* inactivation rates derived from reviewed studies. All rate statistics are in $log_{10}$ inactivation / d.**

| Organism | Coliform bacteria | Enterococci/ fecal streptococci | *Salmonella* spp. | *Shigella* spp. | *Clostridium perfringens* | *Yersinia enterocolitica* | *Aeromonas hydrophila* | *Cryptosporidium parvum* |
|---|---|---|---|---|---|---|---|---|
| n (values) | 22 | 25 | 20 | 9 | 5 | 6 | 9 | 9 |
| Temp range ($^{o}$ C) | 2 - 30 | 2 - 25 | 2 - 25 | 2 - 25 | 5 - 15 | 5 - 25 | 11 - 22 | 5 - 15 |
| Mean | 0.127 | 0.243 | 0.366 | 0.242 | 0.009 | -0.057 | 0.35 | 0.0116 |
| Median | 0.071 | 0.13 | 0.135 | 0.24 | 0.005 | -0.026 | 0.37 | 0.01 |
| Std Dev. | 0.132 | 0.34 | 0.681 | 0.096 | 0.011 | 0.093 | 0.31 | 0.0058 |
| Q1 | 0.033 | 0.035 | 0.039 | | | | | |
| Q3 | 0.203 | 0.345 | 0.414 | | | | | |
| iqr | 0.17 | 0.31 | 0.375 | | | | | |
| range | -0.008 - 0.425 | 0.005 - 1.68 | 0.025 - 3.01 | 0.081 - 0.42 | 0 - 0.027 | -0.22 - 0.038 | 0 - 0.970 | 0.005 - 0.024 |
| outer fence F1 | -0.477 | < 0 | < 0 | | | | | |
| outer fence F3 | 0.713 | 1.275 | 1.539 | | | | | |
| # strong outliers | 0 | 1 | 1 | | | | | |
| number studies | 8 | 6 | 5 | 2 | 2 | 2 | 2 | 2 |

**Table 17. Summary statistics for virus and coliphage inactivation rates derived from reviewed studies. All rate statistics are in $log_{10}$ inactivation / d.**

| Organism | Coliphage | Poliovirus | Echovirus | Hepatitis A virus[1] | PRD-1 |
|---|---|---|---|---|---|
| n (values) | 72 | 41 | 15 | 10 | 5 |
| Temp range ($^{o}$ C) | 4 - 30 | 4 - 30 | 12 - 25 | 4 - 30 | 7 - 23 |
| Mean | 0.251 | 0.203 | 0.138 | 0.044 | 0.202 |
| Median | 0.079 | 0.081 | 0.079 | 0.036 | 0.052 |
| Std Dev. | 0.457 | 0.34 | 0.14 | 0.04 | 0.337 |
| Q1 | 0.032 | 0.047 | 0.057 | 0.015 | |
| Q3 | 0.253 | 0.173 | 0.174 | 0.036 | |
| iqr | 0.221 | 0.126 | 0.117 | 0.021 | |
| range | 0 - 2.5 | 0.005 - 1.67 | 0.051 - 0.628 | 0.001 - 0.140 | 0 - 0.8 |
| outer fence F1 | < 0 | < 0 | < 0 | < 0 | |
| outer fence F3 | 0.916 | 0.551 | 0.525 | 0.099 | |
| # strong outliers | 4 | 5 | 1 | 1 | |
| number studies | 10 | 7 | 2 | 2 | 2 |

Notes:
1. six data points not included because inactivation was below minimum determinable

Table 18 contains information for all organism groups broken into temperature ranges. The temperature ranges were chosen to be 0 - 10, 11 - 15, 16 - 20, 21 - 25º, and 26 - 30° C, although for some these groupings were altered if data were sparse. Fahrenheit conversions of these groups are approximately 32 - 50°, 52 - 59°, 61 - 68°, 70 - 77°, and 79 - 86° F. Within each group, the mean inactivation rate and standard deviation were determined, and for brackets with 4 or more values, the median rate is given. In addition, minimum and maximum values are listed.

**Table 18. Inactivation rates from reviewed studies grouped into temperature ranges**

| Organism | Temperature Group ($^{o}$ C) | Mean rate log/d | Median rate log/d | Std. Dev. log/d | Range log/d | n |
|---|---|---|---|---|---|---|
| Poliovirus | 0 - 10 | 0.0075 | | 0.0035 | 0.005 - 0.01 | 2 |
| | 11 - 15 | 0.0868 | 0.072 | 0.0506 | 0.026 - 0.185 | 19 |
| | 16 - 20 | 0.108 | 0.081 | 0.054 | 0.05 - 0.185 | 7 |
| | 21 - 25 | 0.289 | 0.095 | 0.293 | 0.032 - 0.676 | 9 |
| | 26 - 30 | 1.03 | | 0.857 | 0.055 - 1.67 | 3 |
| hepatitis A | 0 - 10 | 0.0055 | 0.0055 | 0.006 | 0.001 - 0.01 | 2 |
| | 20 - 25 | 0.0557 | 0.036 | 0.0448 | 0.015 - 0.14 | 7 |
| | 26 - 30 | 0.0375 | | 0.00354 | 0.035 - 0.04 | 2 |
| echovirus | 11 - 15 | 0.107 | 0.079 | 0.0579 | 0.051 - 0.186 | 7 |
| | 16 - 20 | 0.121 | | 0.0424 | 0.091 - 0.151 | 2 |
| | 21 - 25 | 0.179 | 0.071 | 0.226 | 0.057 - 0.628 | 7 |
| coxsackievirus | 3 - 15 | 0.19 | | | | 1 |
| rotavirus | 3 - 15 | 0.36 | | | | 1 |
| coliphage | 0 - 10 | 0.029 | 0.02 | 0.0264 | 0 - 0.1 | 13 |
| | 11 - 15 | 0.097 | 0.06 | 0.0977 | 0.028 - 0.4 | 31 |
| | 16 - 20 | 0.143 | 0.081 | 0.189 | 0.02 - 0.63 | 9 |
| | 21 - 25 | 0.426 | 0.324 | 0.364 | 0.048 - 1.416 | 12 |
| | 26 - 30 | 1.242 | 0.78 | 1.035 | 0.022 - 2.5 | 5 |
| PRD-1 | 0 - 10 | 0.019 | | 0.0269 | 0 - 0.038 | 2 |
| | 21 - 25 | 0.324 | | 0.414 | 0.052 - 0.8 | 3 |
| Coliform bacteria | 0 - 10 | 0.0514 | 0.04 | 0.032 | 0.01 - 0.102 | 7 |
| | 11 - 15 | 0.197 | 0.152 | 0.186 | -0.008 - 0.425 | 6 |
| | 16 - 20 | 0.118 | | 0.145 | 0.015 - 0.22 | 2 |
| | 21 - 25 | 0.201 | | 0.133 | 0.094 - 0.35 | 3 |
| | 26 - 30 | 0.035 | | | | 1 |
| Enterococci/ | 0 - 10 | 0.08 | 0.076 | 0.0628 | 0.012 - 0.21 | 9 |
| Fecal streptococci | 11 - 15 | 0.398 | 0.233 | 0.529 | 0.005 - 1.68 | 9 |
| | 16 - 20 | 0.395 | | 0.0495 | 0.36 - 0.43 | 2 |
| | 21 - 25 | 0.24 | | 0.183 | 0.029 - 0.36 | 3 |
| *Salmonella* spp. | 0 - 10 | 0.058 | 0.033 | 0.063 | 0.025 - 0.212 | 7 |
| | 11 - 15 | 0.811 | 0.452 | 0.961 | 0.088 - 3.01 | 7 |
| | 16 - 20 | 0.145 | | 0.005 | 0.14 - 0.15 | 2 |
| | 21 - 25 | 0.234 | 0.145 | 0.164 | 0.13 - 0.517 | 4 |
| *Shigella* spp. | 0 - 10 | 0.277 | | 0.105 | 0.17 - 0.42 | 3 |
| | 11 - 15 | 0.242 | 0.283 | 0.11 | 0.081 - 0.323 | 4 |
| | 20 - 25 | 0.19 | | 0.05 | 0.14 - 0.24 | 2 |
| *Clostridium perfringens* | 0 - 10 | 0.005 | | 0.00625 | 0 - 0.012 | 3 |
| | 11 - 15 | 0.016 | | 0.0156 | 0.005 - 0.027 | 2 |
| *Yersinia enterocolitica* | 0 - 10 | 0.023 | | 0.015 | 0.008 - 0.038 | 3 |
| | 15 - 25 | -0.137 | | 0.0802 | -0.16 | 3 |
| *Vibrio cholerae* | 11 | 0.3 | | | | 1 |
| *Aeromonas hydrophila* | 11 | 0 | | | | 1 |
| | 22 | 0.394 | 0.435 | 0.322 | 0.03 - 0.97 | 8 |
| *Cryptosporidium parvum* | 0 - 10 | 0.01 | 0.01 | 0 | 0.01 - 0.01 | 4 |
| | 11 - 15 | 0.0148 | 0.0145 | 0.00789 | 0.006 - 0.024 | 4 |

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Inactivation rate values as grouped in Table 18 for organism categories with sufficient data were used to construct graphs showing the mean inactivation (log/day) for each temperature group (Figures 1 and 2). Error bars on these graphs show the standard deviation for rate values in each temperature group, as shown in Table 18. These figures enable visual inspection of temperature trends for data from each group of organisms.

For coliform bacteria, including *E. coli*, *Klebsiella* spp., and non-specific total and fecal coliform results, inactivation rates from 8 studies were compared, in which the temperature ranged from 2 - 30º C (35.6 - 86° F), for a total of 26 rate values. From Table 16, the mean inactivation rate was 0.127 log/day, standard deviation 0.132, and the median value was 0.0.071 log/day. The middle 50% of values ranged from 0.033 – 0.203 log/day, and no strong outliers were observed. Overall data values ranged from –0.008 log/day, indicating an overall increase in bacteria concentrations, to 0.425 log/day. Thus, if not accounting for temperature effects, these rates would produce 1 log or 90% inactivation times of approximately 2 1/3 days to undeterminable. If only the middle 50% of values are considered, inactivation rates for coliform will give 90% inactivation times of 4.9 to 30.3 days.

The response of rates in relation to temperature ranges for coliform bacteria was not clear. From Table 18, the slowest mean rates are observed at higher temperatures, with the next slowest being at the lowest temperatures considered. Mean rates for each temperature grouping fluctuate with no clear trend such as increasing inactivation rates at higher temperatures. Figure 1 depicts this visually; coliform mean rates (light gray) fluctuate among the temperature groups but do not show a consistent trend. In addition, a scatterplot of inactivation rates from coliform bacteria studies was constructed (Figure 3). Once again,

temperature effects on coliform inactivation rates are not clear. When averaged by groups, the fastest rates fall in the 21 - 25° C grouping, but the mean rate declines by almost an order of magnitude for those observations between 26 - 30° C. This may be related to differences in experimental procedures and methods for rate calculation and reporting of data, compounded by few observations in these temperature ranges, or an indication of growth of the bacteria at higher temperatures. If inactivation rates for coliform bacteria are considered in light of whether the water was sterile or non-sterile, without regards to temperature, mean rates are 0.130 log/day in sterile water vs. 0.0784 log/day in non-sterile water. These averages also include inactivation rates determined from studies using sterile buffered saline. These can be calculated from data as shown in Appendix 1.

**Figure 1. Bacteria inactivation rates from reviewed studies, averaged by temperature group. Error bars show standard deviation about the mean for each temperature.**

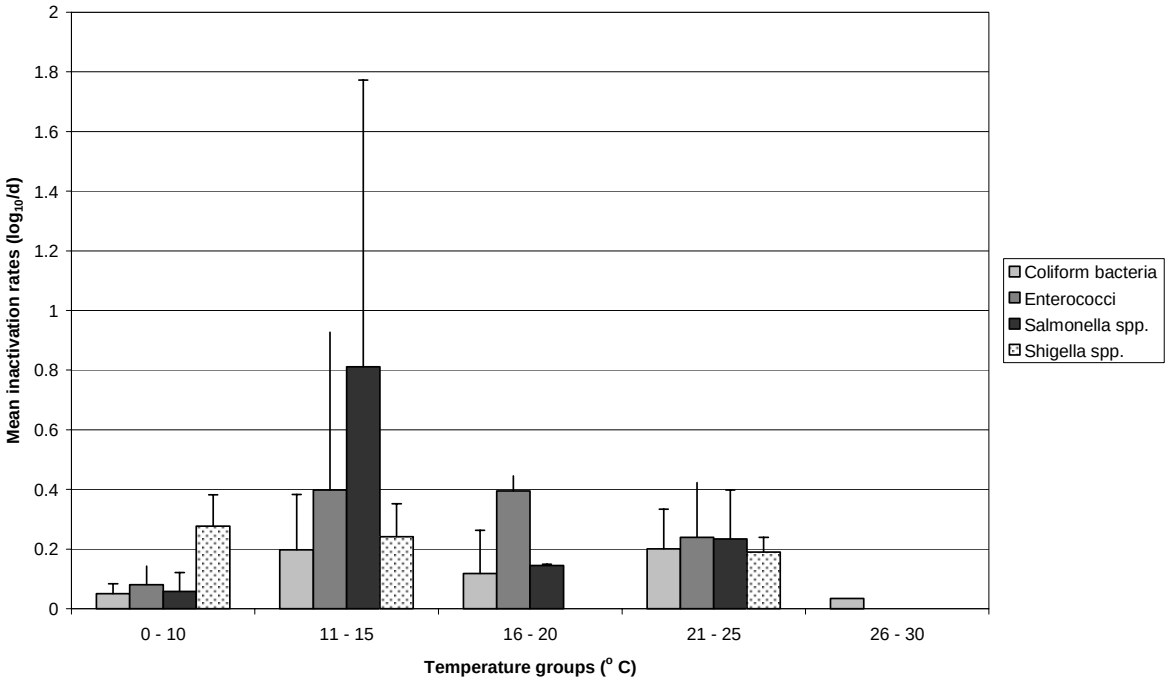


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**Figure 2. Virus inactivation rates from reviewed studies, averaged by temperature group. Error bars show standard deviation about the mean for each temperature.**

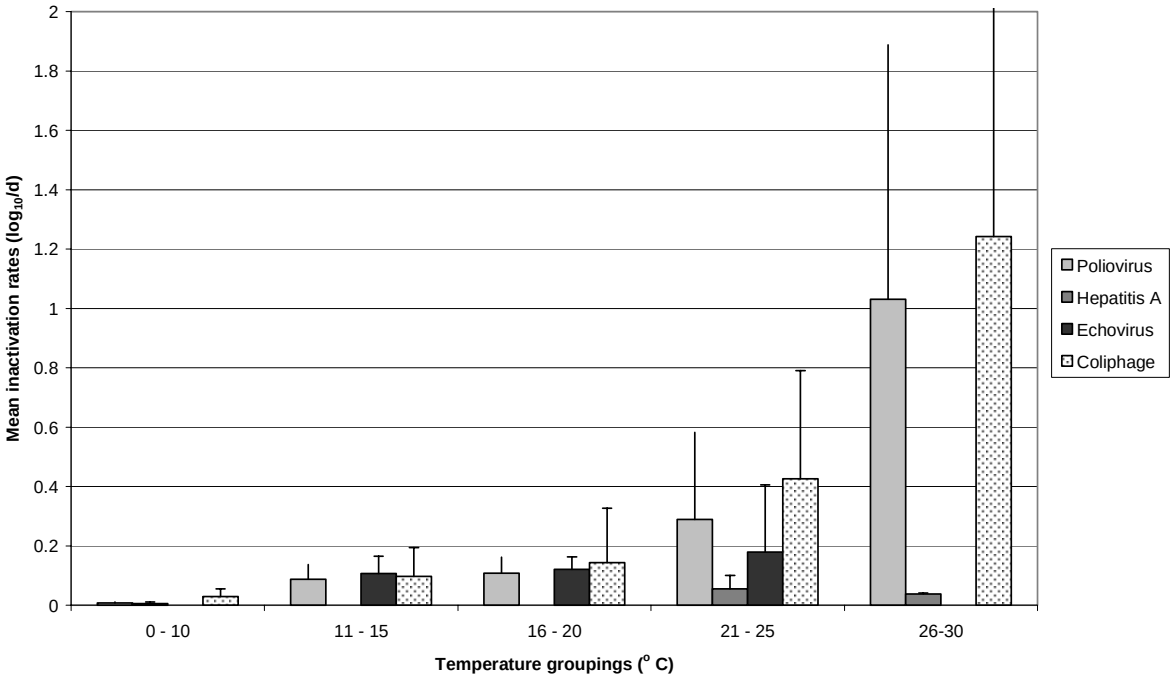


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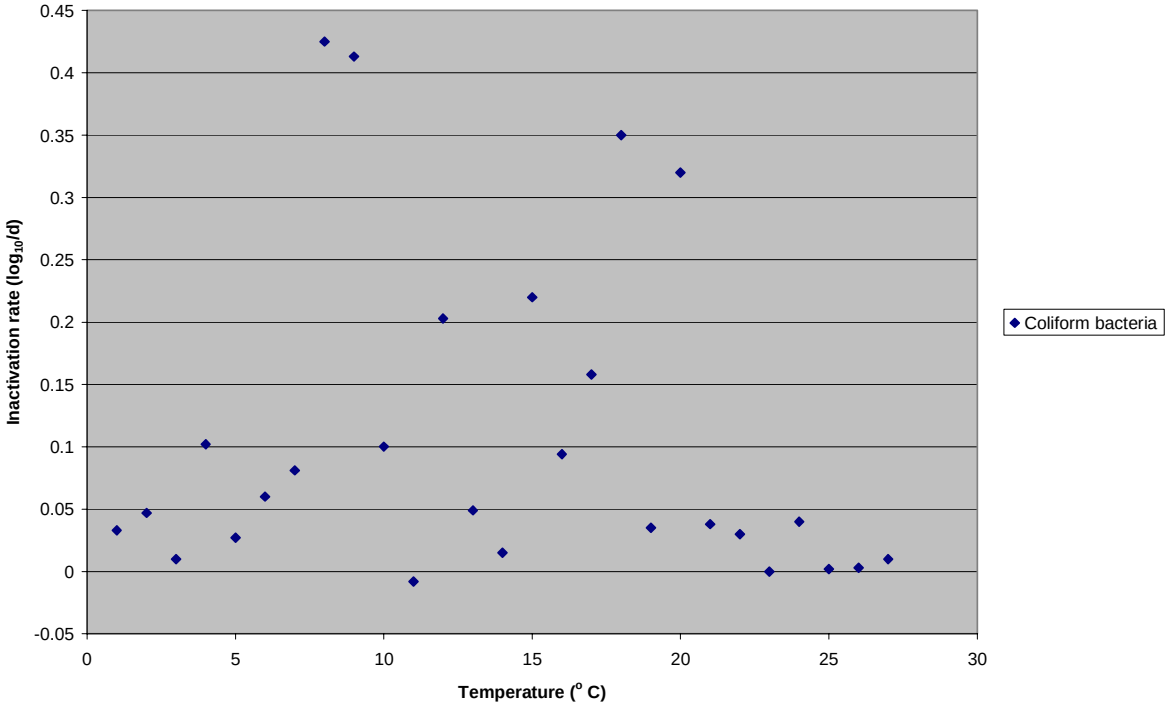
**Figure 3. Scatterplot of coliform bacteria inactivation rates vs. temperature.**

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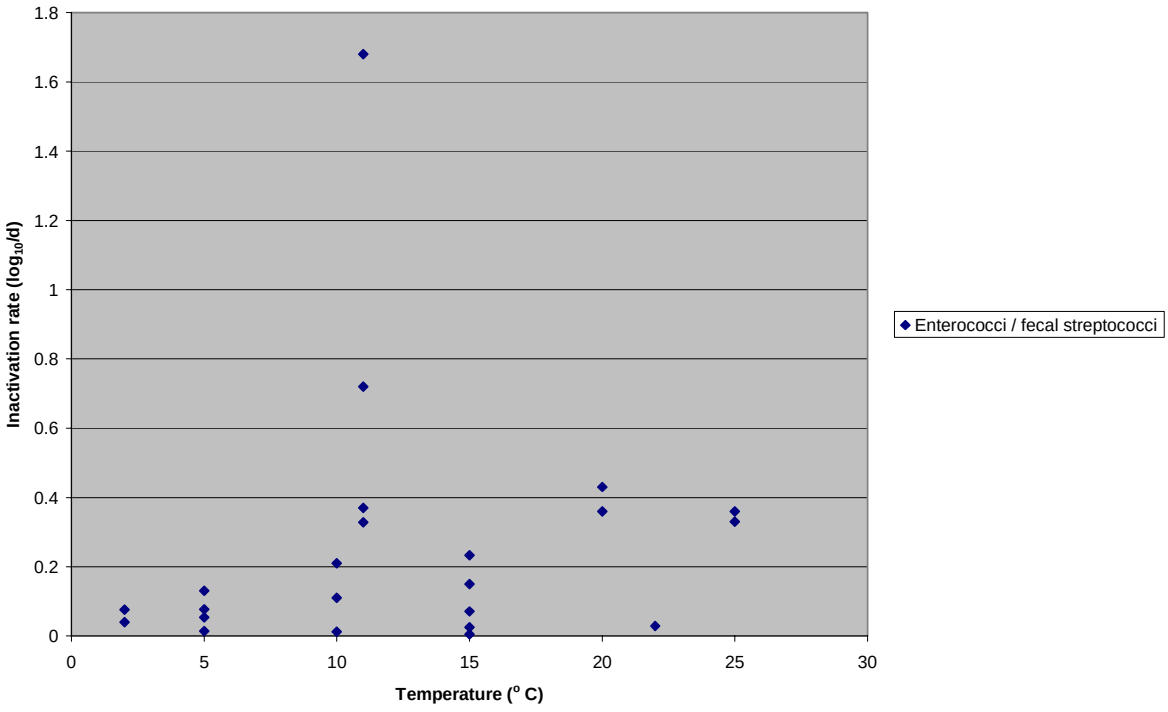
733 Six studies included evaluations of enterococci and/or fecal streptococci for a total of
734 25 observations compiled into summary statistics (Table 16). Temperatures in these studies
735 ranged from 2 to 25° C (36 - 77° F). Some studies employed isolated species such as
736 *Enterococcus faecalis*, *Streptococcus equines*, or *Streptococcus bovis* while most values were
737 derived from studies using mixed populations of these bacteria. The mean inactivation rate
738 was 0.243 log/day, standard deviation of 0.34, and a median value of 0.130 log/day. The
739 range of all inactivation rate values was 0.005 - 1.68 log/day, while interquartile boundaries
740 were 0.035 - 0.345 log/day, with one strong outlier in the data (maximum rate observed of
741 1.68 log/day). Times for 90% inactivation given these ranges of inactivation rates would be
742 0.6 days to 200 days for all data and 2.9 to 28.6 days when considering the middle 50% of
743 rate values. Values separated into temperature groups are shown in Table 18. Wide ranges

of values were reported, with three out of four temperature groups having ranges of greater than an order of magnitude. Averaged inactivation rates from these groups show that above 10° C (50° F), there is little variation among the means at least (Figure 1). As with coliform bacteria, a consistent temperature effect on inactivation rates is not apparent from compiled observations. A scatterplot of temperature vs. inactivation rates for enterococci was also constructed (Figure 4). If experiments performed in sterile water (n=20), including buffered saline, are compared to those in non-sterile water (n=5), mean inactivation rates are 0.284 log/day vs. 0.0768 log/day in non-sterile experiments.

**Figure 4. Scatterplot of enterococci inactivation rates vs. temperature**




*Salmonella* species bacteria were used in 5 studies reviewed here, with 20 observations being used to summarize inactivation rates. Temperatures for *Salmonella* experiments ranged from 2° to 25° C. Summary descriptive statistics, as shown in Table 16,

are a mean inactivation rate of 0.366 log/day, a median rate of 0.135 log/day, and standard deviation of 0.681. The minimum rate was 0.025 log/day and maximum was 3.01 log/day. Quartile boundary values were 0.039 and 0.414 log/day, with the maximum value of 3.01 log/day being the only strong outlier. If *Salmonella* rates are divided into temperature groups (Table 18 and Figure 1), the mean rate at temperatures of 10° C or less was lower than mean rates at higher temperatures, with the highest mean rate at 11-15° C. However, this grouping also contains the outlier value of 3.01 log/day, and thus considering the median value of 0.452 log/day for this group may be more appropriate. In either case, however, a consistent increase in inactivation rates with increasing temperature was not apparent (Figure 1, black bars). Little difference was observed between mean inactivation rates from sterile (0.370 log/day) and non-sterile (0.324 log/day) conditions as well. Based on all *Salmonella* rates, 90% inactivation times range from less than 1 day to 40 days, but rates found in the middle 50% of values produce a range of 90% inactivation times of 2.4 to 25.6 days.

Other bacterial groups were evaluated in fewer reviewed studies, with *Shigella* spp. (n=9 observations), *Clostridium perfringens* (n=5 observations), *Yersinia enterocolitica* (n=6 observations), and *Aeromonas hydrophila* (n=9 observations) all being employed in two studies each. Means, medians, and standard deviations for each group are listed in Table 16, but quartile ranges and outliers were not calculated. *Shigella* spp. mean and median inactivation rates were both 0.24 log/day with a range of 0.081 to 0.42 log/day. This range of inactivation rates leads to 90% inactivation periods of 2.4 to 12.3 days, similar to the $T_{90}$ values for the middle 50% of rates for *Salmonella* spp. bacteria. *Clostridium perfringens* mean inactivation rate was 0.009 log/day, ranging from 0 to 0.027 log/day. Inactivation rates for *Yersinia enterocolitica* based on two studies show the bacteria can grow in microcosm

studies, as indicated by the negative mean inactivation rate of –0.057 log/day. Both studies on this organism employed filter sterilized water, with growth occurring in studies at or above 15° C (59º F) only. Also, the study in which growth of these bacteria was observed (Evison, 1988), was performed using fresh surface water rather than ground water. *Aeromonas hydrophila* was also evaluated in two separate studies, with mean and median inactivation rates being 0.35 and 0.37 log/day respectively. However, observed rates ranged from no decline to 0.970 log/day.

*Cryptosporidium parvum* survival in water was evaluated by three studies reviewed here, but it was only possible to estimate quantitative inactivation rates from two of those. The mean inactivation rate in studies performed at 5 – 15° C was 0.0116 log/day, and the median value was 0.01 log/day. Estimated or reported inactivation rates ranged from 0.005 – 0.024 log/day.

When considering virus survival studies, more data were available for poliovirus and coliphage than for any of the bacterial groups (Table 17). Some type of coliphage were evaluated in 10 studies reviewed here, for a total of 72 observations. Poliovirus were included in 7 studies for 41 observations (once again, not including sterile buffered saline or sterile de-ionized water conditions). The temperature ranges covered by data for both viruses was 4 - 30° C (39 - 86° F). Summary statistics for coliphage are a mean inactivation rate of 0.251 log/day, median of 0.079 log/day, and standard deviation of 0.457 log/day. Similar numbers for poliovirus studies were observed, notably a median inactivation rate of 0.081 log/day, and a mean rate of 0.203 log/day. Means are skewed towards the high end of both data sets, with both mean values near the top of the respective middle 50% of observations

from each set (Q3). In addition, 4 strong outliers were observed from the coliphage data and 5 from the poliovirus data. Thus given that median values are more resistant to the effect of outliers, the similarity of median inactivation rates from the two virus data sets is noteworthy, supporting the effectiveness of coliphage as an indicator of enterovirus survival. For echovirus, which were evaluated in 2 reviewed studies for a data set of 15 observations, the median inactivation rate was also 0.079 log/day while the mean rate was 0.138 log/day. One strong outlier for echovirus was also observed. Hepatitis A viruses were also evaluated in two studies with 10 observations from environmental water. Mean inactivation rate for hepatitis A was 0.044 log/day and the median was 0.036 log/day, with a standard deviation of 0.0.04 log/day. The maximum rate observed at 0.140 log/day was the single strong outlier.

The range of reported and estimated inactivation rates for polioviruses, echoviruses, hepatitis A and coliphage and the interquartile boundary rate values can be converted to estimated times for 90% inactivation. For coliphage, the range of all values produced $T_{90}$ estimates of 0.4 days to undeterminable, while the middle 50% of rates gave a $T_{90}$ range of 4.1 to 31.3 days. Poliovirus rates converted to 0.6 to 200 days for all rates, and 5.8 to 21.3 days when considering only the middle 50% of inactivation rates, which is similar to the range of days to 90% inactivation as for coliphage. Echovirus inactivation rates produced $T_{90}$ times of 1.6 to 19.6 days for all rates and 5.7 to 17.5 days for the middle 50% of rates. Finally, hepatitis A rates gave $T_{90}$ ranges of 7.1 to 1000 days for all values and 27.8 to 66.7 days for the middle 50% of values. Thus hepatitis A appear to decline at an overall slower rate than the other viruses examined when considering a large body of data spanning a large temperature range.

Considering the data grouped by temperature as in Table 18, however, a more apparent effect of temperature can be observed with the viruses than for bacteria. When looking at the temperature groupings for poliovirus and coliphage, a consistent increase was observed in both the median and mean inactivation rates for each temperature category as temperature increased. Bar graphs of mean inactivation rates for each temperature group (Figure 2) also show increasing inactivation with increasing temperature. Scatterplots of the reported and estimated rates for coliphage and poliovirus against temperature also seem to reveal generally greater inactivation rates at higher temperatures (Figures 5 and 6).

**Figure 5: Coliphage inactivation rates with respect to temperature.**

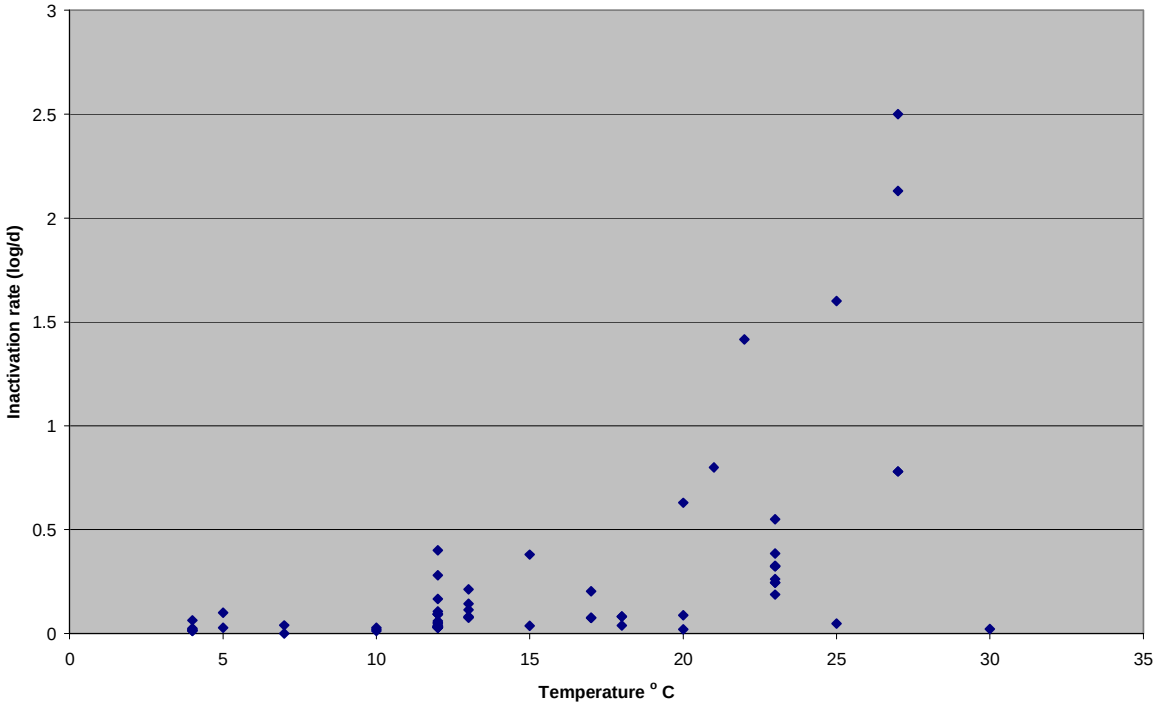
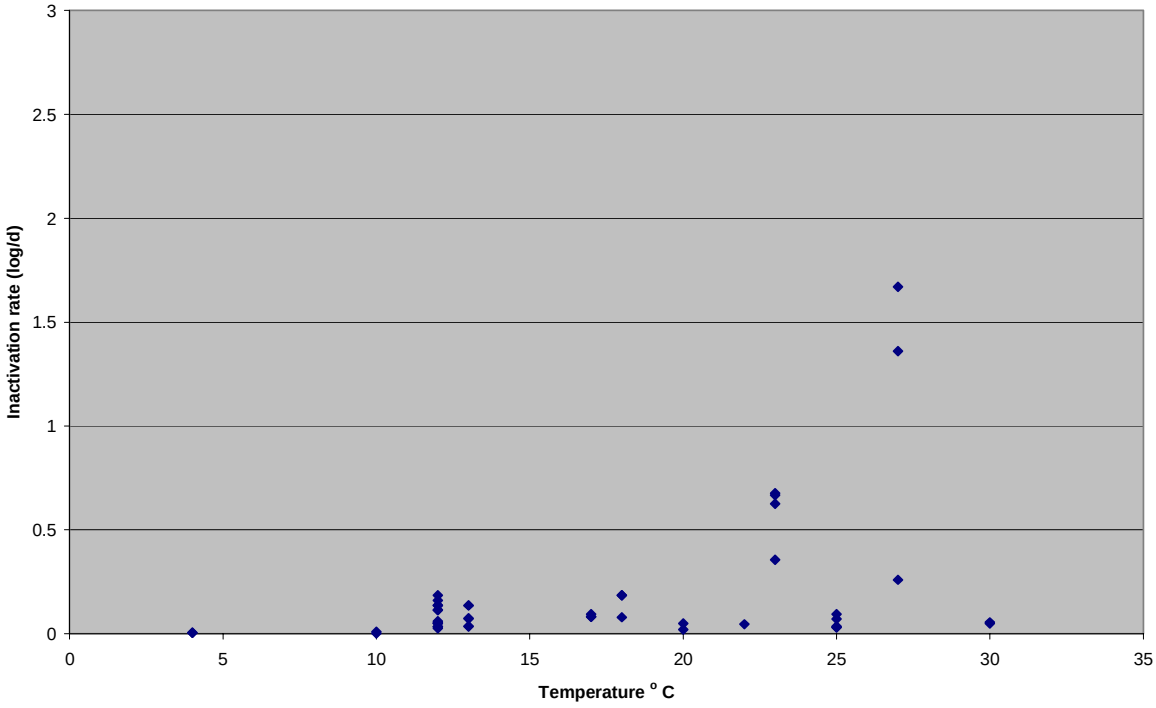


**Figure 6. Poliovirus inactivation rates with respect to temperature.**




Hepatitis A and echovirus mean inactivation rates also increase at higher temperatures, with mean inactivation being faster for hepatitis A above 20° C (68º F) than below 10° C (50º F), while echovirus inactivation increases slightly between the three temperature brackets as shown in Table 18. Figure 2 also shows the greater inactivation rates for echovirus and hepatitis A at temperatures above 10 ° C (50° F). However, the increase is not as dramatic as for poliovirus and coliphage. One point, although, is that the total number of rate values represented by the means for echovirus and hepatitis A are much fewer than for poliovirus 1 and the coliphage. Thus the significance of these inactivation rates being much slower than poliovirus or coliphage, which are more commonly used as indicators of virus presence, is uncertain for ground water. Clearly, a closer look should be taken at the persistence of hepatitis A and echovirus under these conditions to determine if indeed they are more persistent than indicator phage or enteroviruses such as polio 1.

Inactivation rates as in Appendix 1 were separated into sterile vs. non-sterile water to determine if a consistent trend regarding this was observed from these viral studies. For coliphage, mean inactivation rates were 0.278 log/day in sterile water (n=32) and 0.205 log/day in non-sterile water (n=46). Poliovirus means were 0.196 log/day in sterile water (n=19) and 0.177 log/day (n=28) in non-sterile water, while hepatitis A mean rates were 0.0228 log/day in sterile water (n=6) and 0.0455 log/day in non-sterile (n=8) water. For echovirus studies, only three observations were recorded for sterile water, but the mean rates were 0.058 log/day (n=3) and 0.148 log/day in non-sterile water (n=13). Given these comparisons, there does not appear to be an obvious effect when considering sterile against non-sterile water for all rates reported or estimated for each virus group. Poliovirus and coliphage rates were slightly slower in non-sterile water, while the opposite was true of hepatitis A and echovirus.

Part of the purpose for this review was to analyze a large body of published data to elucidate possible trends in inactivation rates in response to environmental variables. When considering the compiled data as discussed above, few consistent trends are readily apparent. For the bacteria, temperature does not produce a definite trend such as more rapid inactivation of all organisms at higher temperatures. For some viruses such as polioviruses and coliphage, temperature effects were apparent. Likewise, the effect of indigenous microbiota (as in considering sterilized vs. raw water) does not appear to result in consistently faster inactivation rates. However, it is important to consider that the data as summarized in Appendix 1 is derived from many independently-performed experiments. The data from these reports were originally presented in disparate ways, and the number of

observations for a given condition may not have been large enough to draw conclusions. It may be significant that the organisms for which the largest number of observations were reviewed, coliphage and poliovirus, were also those with more apparent trends. In addition, many variables may come into play in bench-scale experiments, particularly the source and handling of organisms. For instance, some studies involved populations of bacteria or viruses derived from natural sources such as wastewater or animal feces, while others utilized pure strains maintained in laboratory conditions for many generations. Treatment of organisms prior to seeding survival experiments also varied, such as propagation and purification procedures. Evaluating the impact of these protocol variations among many studies is difficult. However, some individual studies evaluated the impact of various parameters within more or less controlled conditions and the findings of these types of studies may reveal more on possible trends. Table 19 summarizes trends regarding inactivation rates for various organisms that were observed in several studies reviewed here.

**Table 19. Description of trends for inactivation rates observed in independent studies**

| Organism | Affect on inactivation rate | Conditions contrasted | Reference |
|---|---|---|---|
| MS-2 (coliphage) | increased | w / increasing temp. | Yates '85 |
| MS-2 (coliphage) | increased | w / increasing temp. | Yates & Gerba '85 |
| MS-2 (coliphage) | increased | w / increasing temp. | Yates '90 |
| poliovirus 1 | increased | w / increasing temp. | Yates '85 |
| poliovirus 1 | increased | w / increasing temp. | Yates & Gerba '85 |
| poliovirus 1 | increased | w / increasing temp. | Yates '90 |
| poliovirus 1 | increased | at 25 vs 5 C | Sobsey '86 |
| poliovirus 1 | increased | w / increasing temp. | Nasser '99 |
| echovirus | increased | at 25 vs 5 C | Sobsey '86 |
| hepatitis A | increased | at 25 vs 5 C | Sobsey '86 |
| hepatitis A | increased | w / increasing temp. | Nasser '99 |
| Cryptosporidium | increased | at 15 vs 5 C | Medema '97 |
| Enterococcus faecium | decreased | at 15 vs 5 C | Medema '97 |
| MS-2 (coliphage) | increased | w / increasing Ca hardness | Yates '85 |
| Cryptosporidium | increased | in non-sterile vs sterile | Medema '97 |
| MS-2 (coliphage) | decreased | in non-sterile vs sterile | Alvarez '00 |
| poliovirus 1 | decreased | in non-sterile vs sterile | Alvarez '00 |
| E. coli | increased | in non-sterile vs sterile | Medema '97 |
| poliovirus 1 | increased | in non-sterile vs sterile | Sobsey '86 |
| echovirus | increased | in non-sterile vs sterile | Sobsey '86 |
| hepatitis A | increased | in non-sterile vs sterile | Sobsey '86 |
| Aeromonas hydrophila | increased | in non-sterile vs sterile | Kersters '96 |

As was observed by comparing inactivation rates compiled from many studies to the impact of temperature, several investigators observed that virus inactivation increases with increasing temperature, while similar consistent trends for bacteria were not observed. This is not coincidental, of course, since the data that were used to depict trends as in Figures 2 and 5 and 6 for poliovirus and coliphage were derived largely from these studies. Several studies also described an increase in inactivation rates in non-sterile vs. sterile water sources, however, the opposite was also observed in some cases. Regarding the effect of salinity or TDS, no studies reviewed here demonstrated an impact of salinity in the fresh water range. Unfortunately, a large proportion of reviewed studies did not include TDS as a reported parameter, which made analysis of TDS effects using data from many studies, as was done with temperature, difficult.

While these results of individual survival studies do reveal some significant impacts of environmental parameters (while others do not), comparisons of inactivation rates from many studies in this review also reveal some interesting points. For one, the median value for inactivation rates for coliphage, poliovirus, echovirus, and coliform bacteria are almost identical (Tables 16 and 17). In addition, the Q1 values (lower boundary of the middle 50% of data points) of data sets for coliform bacteria, enterococci, *Salmonella* spp., coliphage, poliovirus, and echovirus are all similar, ranging from 0.032 to 0.057 log/day inactivation. $T_{90}$ times corresponding to this range are 17.5 to 31.3 days to 90% inactivation. Naturally, these inactivation rates were derived in many different ways from numerous studies at different temperatures and other conditions, and 25% of the calculated or approximated rates for each organism are below this. However, their similarity does seem quite striking and considering this observation represents 212 data points, it may indicate that better agreement for inactivation rates may be obtained if larger data sets are considered, serving to dilute the impact of differences in experimental procedure. It should also be noted however, that 11 out of the 13 groups of organisms for which rates were compared show ranges of values representing over an order of magnitude. Lack of consistency in experimental protocols and the calculation and reporting of data likely plays a significant role in these differences, along with other unexamined environmental parameters. An additional point of concern is the extrapolation of experimental results from bench-scale studies to *in-situ* behavior of these many types of organisms. Of the studies reviewed here, only part of one evaluated *in-situ* decline of indicator organisms. Recalling the results from Bitton *et al.* (Table 8), inactivation rates of total coliform, fecal coliform, and fecal streptococci (enterococci) were approximately 0.02 - 0.03 log inactivation per day. These values are at the slow side of the

middle 50% of observed rates reviewed here for their respective organism type. However, these rates still extrapolate to $T_{90}$ inactivation times in a reasonable time frame, on the order of 30 to 50 days.

Considerable data have been presented in this literature review. In order to summarize it in what may be a more useful manner, particularly for Florida ground water, the following tables were constructed. Since many of the studies reviewed here evaluated inactivation at temperatures that were largely below those found in aquifers in Florida, the compiled inactivation rates for several organisms were re-grouped into fewer temperature ranges to summarize the corresponding days for 90% inactivation into more appropriate temperature groups. These temperature groups are: under 15º C (< 59º F), which would not normally be expected in Florida ground water, 15 - 20º C (59 - 68º F), which may be encountered in Florida ground water, particularly in winter months, and temperatures over 20º C (> 68º F), which would encompass likely summer month temperatures, especially from injected or recharged surface water. Inactivation rates were not sub-grouped above 20º C, even though this may have been even more descriptive of ground water temperatures in Florida during summer months, due to lack of data from reviewed studies at temperatures above 25º C (77º F). Tables 20 and 21 show days to achieve 90% inactivation in these temperature categories, as were extrapolated from compiled inactivation rates from reviewed studies. For each organism and temperature group, the range of corresponding $T_{90}$ times and the mean are listed, determined from ranges and mean inactivation rates for that temperature group (i.e. mean $T_{90}$ are from the mean inactivation rates from each group, not from averaging all corresponding $T_{90}$ from that temperature group). Only environmental water

946 sources were considered, buffered saline and de-ionized water were omitted. However, data
947 from both sterilized and non-sterilized natural water experiments were grouped together.
948
949 **Table 20. Days to achieve 90% inactivation of selected viral microorganisms at three**
950 **temperature ranges**
951

| Organism | Temp Range $^{o}$ C ($^{o}$ F) | Range of $T_{90\%}$ (d) | Mean $T_{90\%}$ (d) |
|---|---|---|---|
| Poliovirus 1 | < 15 ( < 59) | 4.8 - 200 | 11.7 |
| | 15 - 20 (59 - 68) | 5.4 - 20 | 9.3 |
| | > 20 ( > 68) | 0.6 - 31.3 | 2.1 |
| Hepatitis A | < 15 ( < 59) | 100 - 1000 | 182 |
| | 15 - 20 (59 - 68) | ** | 66.7 |
| | > 20 ( > 68) | 7.1 - 31.3 | 17.1 |
| Echoviruses | < 15 ( < 59) | 5.8 - 19.6 | 9.4 |
| | 15 - 20 (59 - 68) | 6.6 - 11.0 | 8.3 |
| | > 20 ( > 68) | 1.6 - 17.5 | 5.6 |
| Coliphage | < 15 ( < 59) | 2.5 - undefined | 12.8 |
| | 15 - 20 (59 - 68) | 1.6 - 50 | 6.4 |
| | > 20 ( > 68) | 0.4 - 45.4 | 1.4 |

952 ** Only one value in that temperature range
953
954
955 **Table 21. Days to achieve 90% inactivation of selected bacterial microorganisms at**
956 **three temperature ranges.**
957

| Organism | Temp Range $^{o}$ C ($^{o}$ F) | Range of $T_{90\%}$ (d) | Mean $T_{90\%}$ (d) |
|---|---|---|---|
| Coliform Bacteria | < 15 ( < 59) | 2.4 - 100 | 6.6 |
| | 15 - 20 (59 - 68) | 4.5 - undefined | 10.4 |
| | > 20 ( > 68) | 2.9 - 28.6 | 6.3 |
| Enterococci / fecal | < 15 ( < 59) | 0.6 - 83.3 | 3.5 |
| streptococci | 15 - 20 (59 - 68) | 2.3 - 200 | 5.5 |
| | > 20 ( > 68) | 2.8 - 34.5 | 4.2 |
| Salmonella | < 15 ( < 59) | 0.3 - 40 | 2 |
| | 15 - 20 (59 - 68) | 6.7 - 11.4 | 8.4 |
| | > 20 ( > 68) | 1.9 - 7.7 | 4.3 |
| Shigella | < 15 ( < 59) | 2.4 - 5.9 | 3.5 |
| | 15 - 20 (59 - 68) | 7.1 - 12.3 | 9 |
| | > 20 ( > 68) | ** | 4.2 |

958 ** Only one value in that temperature range
959

By looking at inactivation times in this way, some trends are still readily apparent. For all viruses in Table 20, $T_{90}$ from mean rates show the most rapid inactivation at temperatures above 20º C. Also, inactivation rates from hepatitis A virus are somewhat longer than those from coliphage, poliovirus 1 and echoviruses. One important point to make is that the number of rate values for hepatitis A were fewer than poliovirus 1 and coliphage, and most came from Sobsey, *et al* (1986), which involved experiments conducted using soil/water mixtures. Nonetheless, as stated previously, perhaps the adequacy of viral indicators like coliphage for predicting hepatitis A presence in ground water needs further study. For the four bacterial groups in Table 21, all have the longest $T_{90}$ in water at 15 - 20º C. Also, it should be noted that for all bacterial groups and viruses except for hepatitis A virus, mean $T_{90}$ values for temperature groups 15 - 20º C and > 20º C are equal to or less than 10 days. Also, for bacteria, the upper bound of the indicator $T_{90}$ values, shown by coliform and enterococci, are greater than the upper $T_{90}$ ranges for the potentially pathogenic bacterial groups *Shigella* and *Salmonella*.

In conclusion, studies following consistent experimental procedures need to be performed to hopefully reduce variability among investigators' findings. Standards for performing bench scale survival studies should include protocols for the propagation and preparation of seeded organisms, and should include controls such as ATCC strains of MS-2 and *E. coli* to preclude differences in the organisms themselves. In addition, more field studies are needed. While the introduction of potentially harmful microorganisms into the environment is generally opposed, innovative studies of ground water contamination by natural sources could prove helpful. If the proper safeguards could be ensured, controlled

field studies involving seeded non-pathogenic microorganisms could prove even more beneficial if the results of such studies are expressed in quantitative terms and are published in peer-reviewed literature to enable wide dissemination of this information.

## References


Alley, W. M., T. E. Reilly and O. L. Franke (1999). Sustainability of Ground-Water Resources, U.S. Geological Survey Circular 1186. Denver, U.S. Geological Survey.

Alvarez, M. E., M. Aguilar, A. Fountain, N. Gonzalez, O. Rascon and D. Saenz (2000). "Inactivation of MS-2 phage and poliovirus in groundwater." Canadian Journal of Microbiology **46**(2): 159-165.

Bitton, G., S. R. Farrah, R. H. Ruskin, J. Butner and Y. J. Chou (1983). "Survival of Pathogenic and Indicator Organisms in Groundwater." Ground Water **21**(4): 405-410.

Bitton, G., O. C. Pancorbo and S. R. Farrah (1983). "Effect of hydrostatic pressure on poliovirus survival in ground water." Ground Water **21**(6): 756-758.

Dowd, S. E. and S. D. Pillai (1997). "Survival and transport of selected bacterial pathogens and indicator viruses under sandy aquifer conditions." Journal of Environmental Science and Health Part a- Environmental Science and Engineering & Toxic and Hazardous Substance Control **32**(8): 2245-2258.

Drew, R. (2001). Aquifer Storage and Recovery – UIC Class V Wells: DEP's Perspective. Orlando, FL.

1005 Evison, L. M. (1988). “Comparative Studies on the Survival of Indicator Organisms and
1006 Pathogens in Fresh and Sea-Water.” Water Science and Technology **20**(11-12): 309-
1007 315.
1008 Filip, Z., D. Kaddumulindwa and G. Milde (1988). “Survival of Some Pathogenic and
1009 Facultative Pathogenic Bacteria in Groundwater.” Water Science and Technology
1010 **20**(3): 227-231.
1011 Freire-Santos, F., A. M. Oteiza-Lopez, C. A. Vergara-Castiblanco and M. E. Ares-Mazas
1012 (1999). “Effect of salinity, temperature and storage time on mouse experimental
1013 infection by Cryptosporidium parvum.” Veterinary Parasitology **87**(1): 1-7.
1014 Hurst, C. J. (1988). “Effect of Environmental Variables on Enteric Virus Survival in Surface
1015 Fresh-Waters.” Water Science and Technology **20**(11-12): 473-476.
1016 Kersters, I., G. Huys, H. VanDuffel, M. Vancanneyt, K. Kersters and W. Verstraete (1996).
1017 “Survival potential of Aeromonas hydrophila in freshwaters and nutrient-poor waters
1018 in comparison with other bacteria.” Journal of Applied Bacteriology **80**(3): 266-276.
1019 Keswick, B. H., C. P. Gerba, S. L. Secor and I. Cech (1982). “Survival of Enteric Viruses
1020 and Indicator Bacteria in Groundwater.” Journal of Environmental Science and
1021 Health Part a- Environmental Science and Engineering & Toxic and Hazardous
1022 Substance Control **17**(6): 903-912.
1023 McFeters, G. A., Bissonne.Gk, J. J. Jezeski, C. A. Thomson and D. G. Stuart (1974).
1024 “Comparative Survival of Indicator Bacteria and Enteric Pathogens in Well Water.”
1025 Applied Microbiology **27**(5): 823-829.
1026 Medema, G. J., M. Bahar and F. M. Schets (1997). “Survival of Cryptosporidium parvum,
1027 Escherichia coli, faecal enterococci and Clostridium perfringens in river water:

Influence of temperature and autochthonous microorganisms." Water Science and Technology **35**(11-12): 249-252.

Nasser, A. M. and S. D. Oman (1999). "Quantitative assessment of the inactivation of pathogenic and indicator viruses in natural water sources." Water Research **33**(7): 1748-1752.

Pyne, R. D. G. (2002). "Aquifer Storage Recovery Wells: The Path Ahead." Florida Water Resources Journal: 19-27.

Robertson, L. J., A. T. Campbell and H. V. Smith (1992). "Survival of Cryptosporidium-Parvum Oocysts under Various Environmental Pressures." Applied and Environmental Microbiology **58**(11): 3494-3500.

Sobsey, M. D., P. A. Shields, F. H. Hauchman, R. L. Hazard and L. W. Caton (1986). "Survival and Transport of Hepatitis a Virus in Soils, Groundwater and Waste-Water." Water Science and Technology **18**(10): 97-106.

Yahya, M. T., L. Galsomies, C. P. Gerba and R. C. Bales (1993). "Survival of Bacteriophages Ms-2 and Prd-1 in Ground-Water." Water Science and Technology **27**(3-4): 409-412.

Yates, M. V. and C. P. Gerba (1985). "Factors Controlling the Survival of Viruses in Groundwater." Water Science and Technology **17**(4-5): 681-687.

Yates, M. V., C. P. Gerba and L. M. Kelley (1985). "Virus Persistence in Groundwater." Applied and Environmental Microbiology **49**(4): 778-781.

Yates, M. V., L. D. Stetzenbach, C. P. Gerba and N. A. Sinclair (1990). "The Effect of Indigenous Bacteria on Virus Survival in Ground- Water." Journal of Environmental

Science and Health Part a- Environmental Science and Engineering & Toxic and Hazardous Substance Control **25**(1): 81-100.

**Appendix 1**

**Appendix 2**