

**EVALUATION OF  
MICROBIAL REMOVAL/INACTIVATION  
BY THE INNOWAVE 240<sup>®</sup>**

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## SUMMARY

The INNOWAVE 240 distillation system was evaluated for removal/inactivation of waterborne pathogenic microorganisms from water. The evaluation was divided into two parts. The first goal was to demonstrate the ability of the units to meet the U.S. Environmental Protection Agency's "Guide Standard and Protocol for Microbiological Water Purifiers." Following this protocol, three units were challenged several times with *Klebsiella terrigena*, poliovirus type 1, rotavirus SA-11, and *Cryptosporidium parvum* oocysts, using both general case and worst case test water, over a period of two weeks. The second part consisted of evaluating reduction/inactivation of the waterborne pathogens *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Vibrio cholera*, *Yersenia enterocolitica*, *Campylobacter jejuni*, hepatitis A virus, and adenovirus. In addition, a highly thermotolerant mycobacterium, *Mycobacterium fortuitum* was tested.

In all cases, the units exceeded the removal parameter levels of 99.9999% for bacteria, 99.99% for viruses and 99.9% for parasites according to the U.S. Environmental Protection Guide and Protocol for Testing Microbiological Water Purifiers.

## 1.0 INTRODUCTION

Development of in-line water treatment devices has evolved from consumer interest in improving and ensuring the quality of drinking water. The need also extends to the water quality of families or communities having individual home and small system water sources.

One major concern in water treatment is the requirement for removing disease-causing microorganisms from water for consumption, since it is recognized that infectious disease transmission by water is a significant public health concern. The majority of documented waterborne diseases in the United States are caused by infectious microorganisms (Craun, 1986). It is important that water treatment units or devices designed for the protection of human health be effective against pathogenic microorganisms and be capable of providing this capability over the designed operational life of the equipment.

To ensure the efficacy of microbiological water purifiers, a multidisciplinary task force was formed by the U.S. Environmental Protection Agency to develop a guide standard and protocol for testing such units. This Guide Standard and Protocol appeared in the Federal Register of May 26, 1986, and has been accepted on a provisional basis by the U.S. Environmental Protection Agency's Office of Drinking Water and Office of Pesticide Programs. This document recommends test and performance requirements for microbiological purifiers. While the document specifically deals with testing criteria for certain types of water treatment devices such as halogen disinfectants, ultraviolet light, ceramic filters, etc., its purpose was to serve as a guide

for all types of water treatment devices. The guide establishes that any microbiological water purifier be capable of removing or killing enteric bacteria, viruses, and protozoan parasites. Such units should be capable of reducing challenge levels of suggested microbial contaminants in each class of microorganism.

The units must demonstrate at least a 99.9999% removal of *Klebsiella terrigena*, a 99.99% removal of poliovirus and rotavirus, and a 99.9% removal of *Giardia*. The devices must also be capable of achieving these results under a realistic “worst case” water quality situation. In 1993, FIFRA Scientific Advisory Panel Antimicrobial subpanel, Office of Pesticides Programs, recommended the substitution of *Giardia* cysts by *Cryptosporidium* oocysts. *Cryptosporidium* oocysts (4-6  $\mu\text{m}$ ) are smaller than *Giardia* (8-12  $\mu\text{m}$ ) and more likely to pass through units which depend upon filtration for parasite removal. *Cryptosporidium* is extremely resistant to common water disinfectants (Korich et al, 1990) and has caused several large waterborne outbreaks in the United States and Europe in recent years (Smith and Rose, 1990). Thus any device capable of removing/inactivating *Cryptosporidium* should be able to eliminate *Giardia* cysts.

It is important that water treatment devices designed for the protection of human health be effective against pathogenic microorganisms and be capable of providing this capability over the designed operational life of the equipment in waters likely to be encountered in the United States. This is a necessary consideration for protection of the public's health by both the water industry and the government.

The INNOWAVE 240 system is a unique type of distillation-purification system, not currently covered in the Guide Standard testing protocol. However, the basic test

protocol was followed in the evaluation of these units to demonstrate their performance as a “Microbiological Water Purifier.” In addition, the units were challenged with specific waterborne pathogens and a thermotolerant *Mycobacterium* to further demonstrate the efficacy of the units.

## 2.0 MATERIALS AND METHODS

### 2.1 EPA Guide Standard Protocol Evaluation

The basic experimental design for evaluating the INNOWAVE 240 was based on the recommendation of the U.S. Environmental Protection Agency's Task Force Report on the Guide Standard and Protocol for Testing Microbiological Water Purifiers (Federal Register, May 26, 1986).

One set of tests followed the sampling plan outlined for ceramic candles/UV light systems as outlined in the Guide Standard. It was felt that this sampling plan best fits the INNOWAVE treatment system.

All INNOWAVE 240 systems were provided by INNOWAVE, 10250 Regency Circle, Suite 110, Omaha, NE and operated according to the manufacturer's instructions. The systems were challenged with the test microorganisms after different periods of operation with "general" and "worst" case water. Between challenges, general case water was dechlorinated (by passage of the tapwater through a column of activated carbon). University of Arizona tapwater was processed through the units.

Three INNOWAVE 240 systems were tested for the removal/inactivation of poliovirus, rotavirus, *Cryptosporidium* oocysts, and *Klebsiella terrigena* according to the USEPA's Guide Standard and Protocol. These systems were evaluated eight times in a period of 13 days according to the test plan in Table 1. A total of 104 gallons (393 liters) was processed by each unit during the test period.

**Table 1.**  
**TEST PLAN FOR POLIOVIRUS, ROTAVIRUS AND**  
**KLEBSIELLA TERRIGENA REMOVAL EVALUATION**

DAY	TEST PROCEDURE
1	Challenge
2	Aging
3	Challenge
4 to 5	Aging
6	Challenge
7	Challenge
8	Challenge
9	Challenge
10	Challenge
11 to 12	Aging
13	Challenge

**For days 1, 3, 6, and 7 challenges, General Case Water was used.**  
**For days 8, 9, 10, and 13 challenges, Worst Case Water was used.**

*Cryptosporidium* oocysts were noticed in the finished water after the challenge tests. Since it was not possible to assess viability by direct observation of the oocysts, another test was conducted in which the oocysts were collected and a stain used to assess viability. The sampling plan for this second test is shown in Table 2.

**TABLE 2.  
TEST PLAN FOR CRYPTOSPORIDIUM REMOVAL EVALUATION**

DAY	TEST PROCEDURE
1	Challenge
2	Aging
3	Challenge
4	Aging
5	Challenge
6	Aging
7	Challenge

**For days 1 and 3 challenges, General Case Water was used.  
For days 5 and 7 challenges, Worst Case Water was used.**

**2.2 Challenge Tests With Selected Waterborne Bacterial and Viral Pathogens**

The INNOWAVE 240 system was also challenged with the most important waterborne bacteria and hepatitis A virus (infectious hepatitis). Also included were a mycobacterium, *Mycobacterium fortuitum*, and adenovirus type 1, because of their resistance to inactivation by heat. The organisms were suspended in eight gallons (29.9L) of general case water and placed in the reservoir. Samples before and after processing through the unit were collected after processing 4, 15, and 29 liters through the unit.

## 2.3 WATER QUALITY PARAMETERS

### 2.3.1 General Test Water

Tap water provided at the University of Arizona was used for “general case” water. This water is obtained from deep wells located on the University of Arizona campus. It meets all of the requirements of “general case” water as defined in the USEPA Guide Standard. Its physical/chemical properties are listed in Table 3.

**Table 3.**  
**PHYSICAL/CHEMICAL PROPERTIES OF**  
**TAPWATER AT THE UNIVERSITY OF ARIZONA**

<b>Parameter</b>	<b>Value</b>
pH	7.5-7.8
Total Organic Carbon (TOC)	<1.0 mg/L
Turbidity	<1.0 NTU
Temperature	23-25 °C
Total Dissolved Solids (TDS)	200-300 mg/L

Before use the water was passed through an activated charcoal filter (Amway, Ada, MI) to remove any chlorine present. This water was passed through the systems between challenges.

### 2.3.2 Worst Case Test Water

The water was prepared by adding General Case Water to a 4-liter beaker and adding the necessary ingredients to obtain the desired water quality (Table 4).

**Table 4.**  
**PHYSICAL/CHEMICAL PROPERTIES OF WORST CASE WATER**

Parameter	Value
pH	9.0+/-0.2
Total Organic Carbon	10 mg/L
Turbidity	30 NTU
Temperature	4 °C +/- 1°C
Total Dissolved Solids	1,500 mg/L

The reagents suggested in the Guide Standard were used to adjust water quality for all test waters used for microbial testing (Table 3).

**Table 5.**  
**Reagents Used to Adjust Water Quality**

Parameter	Reagent
pH	1N NaOH or 1N HCl
Total Organic Carbon	Humic Acids (Aldrich Chemical Co. Milwaukee, WI Cat# H1, 675-2)
Turbidity	AC Fine Test Dust (AC Spark Plug Division, GM. Flint, MI Cat#1543094)
Total Dissolved Solids	Sea Salts (Sigma Chemical Co., St. Louis, MO)

## **2.4 CHALLENGE PROCEDURE**

The INNOWAVE 240 system produces up to 8 gallons of water in a 24-hour period. Raw, untreated water is stored in the raw water reservoir and moves through a pre-softener for mineral removal. Using the process of distillation, the water is purified in the boiling chamber. Steam rises and passes through a finned condensing coil. A fan cools the steam in the condensing coil. The water then passes through a carbon filter to enhance the taste. Systems were aged by connecting them to the tapwater supply at the University of Arizona and used daily for two weeks before the start of the test.

For each microbial challenge, 3 liters of challenge water containing the test microorganisms were added to the raw water storage tank (100 ml of challenge water were collected for influent) and one liter sample (effluent) was collected after 3 hours from the product water tank (this is the approximate period after one gallon of product water is produced). All microbial challenges and aging of the system were carried out as explained previously.

## **2.5 MICROBIAL TEST METHODS**

### **2.5.1 Bacteria**

The test organisms used for assessment of removal/inactivation of bacteria were *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Vibrio cholera*, and *Yersenia enterocolitica*, kindly provided by Emily Pejovich from the bacterial culture collection of the Department of Microbiology, University of Arizona. Also used for the evaluation were *Klebsiella terrigena* (ATCC-33257) and *Campylobacter jejuni*

provided by Dr. Lynn Jones from the Department of Veterinary Science, University of Arizona.

All bacteria with the exception of *Campylobacter* and *Yersenia* were prepared by overnight growth in Tryptic Soy Broth (Difco, Detroit, MI) to obtain the organism in the stationary growth phase (Asburg, 1983). The organisms were collected by centrifugation (in a Beckman Floor centrifuge, Model J2-21, Palo Alto, CA) and resuspended three times in the test water.

*Campylobacter jejuni* was grown on CVA agar and *Yersina entocolitica* on CIN agar. The selective media on which the bacteria were assayed from the water samples is shown in Table 6.

**Table 6.**  
**BACTERIA AND MEDIA USED FOR BACTERIAL ANALYSIS**

<b>BACTERIA</b>	<b>MEDIA</b>
<i>Escherichia coli</i>	m-endo Agar LES (BBL, Cockeysville, MD)
<i>Shigella dysenteriae</i>	SS Agar (DIFCO, Detroit, MI)
<i>Salmonella typhimurium</i>	Hektoen Enteric Agar (DIFCO, Detroit, MI)
<i>Yersinia enterocolitica</i>	Yersinia Selective Agar (CIN) (Microbio, Tempe, AZ)
<i>Campylobacter jejuni</i>	Campylobacter CVA Agar (Microbio, Tempe, AZ)
<i>Vibrio cholera</i>	Thiosulfate-citrate-bile salts-sucrose Agar (BBL, Cockeysville, MD)
<i>Klebsiella terrigena</i>	M---endo LES (BBL, Cockeysville, MD)
<i>Mycobacterium fortuitum</i>	Trypticase Soy Agar-TSA (DIFCO, Detroit, MI) (BBL, Cockeysville, MD)

Assays were conducted by the membrane filtration or spread plate method on the specific media for each bacteria (Table 6). Appropriate dilutions of influent samples were made in sterile Tris-buffered saline (Trisma Base, Sigma Chemical, St. Louis, MO). A 10-ml sample of undiluted effluent was also assayed. All assays were in triplicate according to Standard Methods (APHA, 1992).

## **2.5.2 Viruses**

The test viruses were poliovirus type 1 (LSC) and rotavirus strain SA-11. All stocks were grown by the method described in Smith and Gerba (1982). Hepatitis A virus (Strain HM 175) was obtained from Dr. Mark Sobsey and grown in the Fhrk-4 cell line. Adenovirus type 2 was grown in the CaCO<sub>2</sub> cell line.

Poliovirus type 1 and rotavirus SA-11 were assayed simultaneously by the plaque assay method on the MA-104 cell line using procedure described by Smith and Gerba (1982). Dilutions if necessary were conducted using Tris-buffered saline (Sigma Chemical Co., St. Louis, MO). Hepatitis A virus and adenovirus type 2 was assayed by the TCID<sub>50</sub> method. All assays were done in triplicate.

## **2.5.2 *Cryptosporidium***

Oocysts were obtained from the feces of infected calves (Pleasant Hill Farms, Troy, ID) and then purified by discontinuous sucrose gradient. Samples for parasite assays were examined using two methods. Influent samples were assayed using the hemocytometer method (Guidance Manual, 1990) and the effluent samples were done by the Propidium Iodide method (Campbell et al, 1992).

Influent samples of 20 mL were centrifuged in a IEC Clinical Centrifuge (Nedham Hts, MA) at 400 xg for 15 minutes to pellet oocysts. The supernatant was aspirated to 1 mL above the pellet. After resuspension of the pellet in PBS buffer, the oocysts were counted using SPolite Hemocytometer (Baxter Healthcare Corp., McGraw Park, IL) using a phase contrast microscope (BH-2 Olympus, Japan) at 400x magnification. At

least 12 chamber aliquots were counted for each sample according to the procedure outlined in the Guidance Manual (USEPA, 1990). An average of all readings was done and multiplied with the conversion factor of  $1.0 \times 10^4$ . Total number of oocysts were divided by 20 to determine the number of oocysts per mL of sample.

For the water processed through the unit, 400 mL of the sample was centrifuged at 2800 rpm with the help of a Beckman GS-6 table top centrifuge (Beckman, Palo Alto, CA). The pellet was then transferred to a microcentrifuge and centrifuged with the aid of a MicroSpin 24S microcentrifuge (Sorvall Instruments, Wilmington, DE) at 20% for 3 minutes. The supernatant was aspirated and the pellet resuspended with 100  $\mu$ L of 0.1 M HCl. The resuspended pellet was then incubated in a waterbath for one hour at 37°C. After incubation the sample was washed twice with 1 mL of Hank's balanced salt solution (GIBCO, Grand Island, NY) to achieve a neutral pH. After each wash the sample was aspirated to the pellet. Once the pH was neutral (red color), the sample was then centrifuged and aspirated to the pellet. Finally, 10  $\mu$ L of Propidium Iodide (Sigma, St. Louis, MO) were added to the sample and incubated for 30 minutes at 37°C. Samples were observed under a Differential Interference Contrast (DIC)/Ultraviolet light equipped microscope (BH-2, Olympus, Japan). An 8- $\mu$ L volume was placed on a drop well slide and observed under DIC (1000x magnification). Ultraviolet microscopy was used to detect viability of oocysts. Fluorescence inside the oocyst was counted as dead and those that did not fluoresce in the interior were considered live.

### 3.0 RESULTS AND DISCUSSION

The results of the microbial removal studies are shown in Tables 7 through 10. These results show that the units can achieve percent removals of 99.9999% for bacteria, 99.99% for viruses, and 99.9% for *Cryptosporidium*. In most cases, the removal exceeded those required in the EPA Guide Standard and Protocol for Microbiological Water Purifiers.

In summary, the INNOWAVE 240 system can meet the microbial removals as required by the U.S. Environmental Protection Agency's Guide Standard and Protocol for Testing Microbiological Water Purifiers. In addition, it was proven capable of removing all of the major waterborne disease causing bacteria from water. Hepatitis A virus, one of the most heat resistant enteric viruses, was also removed.

Since a few oocysts were noted in the product water in the initial challenge testing of the units, a vital dye was used to assess that viability (Campbell et al., 1992). The carryover of the oocysts into the product water may have been due to frothing of the water during boiling of the worst case water. Worst case water contains 30 mg/L of organic matter as humic acid. The results demonstrated that none of the observed oocysts were viable. This is not surprising since *Cryptosporidium* oocysts are known to be very sensitive to heat inactivation. In addition, the very heat resistant hepatitis A, adenovirus type 4, and *Mycobacterium fortuitum* virus were not detected in the product water despite very high challenge levels.

## REFERENCES

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**TABLE 7.  
BACTERIAL REMOVAL/INACTIVATION RESULTS  
(CFU/mL)**

<b>BACTERIA</b>	<b>CHALLENGE</b>	<b>PRODUCT WATER</b>	<b>PERCENT REDUCTION</b>
<i>Escherichia coli</i>	1.02X10 <sup>9</sup>	<33	>99.999997
<i>Salmonella thyphimurium</i>	3.20X10 <sup>9</sup>	<33	>99.999999
<i>Shigella dysenteriae</i>	2.30X10 <sup>9</sup>	<33	>99.999999
<i>Campylobacter jejuni</i>	1.00X10 <sup>8</sup>	<33	>99.99997
<i>Yersinia enterocolitica</i>	1.20X10 <sup>8</sup>	<33	>99.999973
<i>Vibrio cholera</i>	1.28X10 <sup>8</sup>	<33	>99.999974
<i>Mycobacterium fortuitum</i>	1.5X10 <sup>10</sup>	<33	>99.999991
<i>Hepatitis A virus*</i>			
<i>Adenovirus type 2*</i>	1.55X10 <sup>9*</sup>	<166	>99.99999

**CFU = colony forming units**

**\*TCID<sub>50</sub> For adenovirus type 2, 30 mL of product water was assayed**

**TABLE 8.**  
**REMOVAL/INACTIVATION OF *KLEBSIELLA TERRIGENA***  
**(CFU/100mL)**

<b>DAY</b>	<b>WATER TYPE</b>	<b>CHALLENGE</b>	<b>UNIT 1 PW</b>	<b>UNIT 2 PW</b>	<b>UNIT 3 PW</b>	<b>GEOMETRIC AVERAGE</b>	<b>PERCENT REDUCTION</b>
1	General	1.68x10 <sup>10</sup>	<33	<33	<33	<33	>99.999999
2	General	9.57x10 <sup>9</sup>	<33	<33	<33	<33	>99.999999
6	General	1.27x10 <sup>10</sup>	<33	<33	<33	<33	>99.999999
7	General	1.01x10 <sup>10</sup>	<33	<33	<33	<33	>99.999999
8	Worst	1.27x10 <sup>9</sup>	<33	<33	<33	<33	>99.999999
9	Worst	1.24x10 <sup>10</sup>	<33	<33	<33	<33	>99.999999
10	Worst	8.67x10 <sup>9</sup>	<33	<33	<33	<33	>99.999999
13	Worst	7.1x10 <sup>9</sup>	<33	<33	<33	<33	>99.999999

**PW = product water**

**CFU = colony forming unit**

**TABLE 9.**  
**REMOVAL/INACTIVATION OF POLIOVIRUS TYPE 1**  
**AND ROTAVIRUS SA-11**  
**(PFU/LITER)**

<b>DAY</b>	<b>WATER TYPE</b>	<b>CHALLENGE</b>	<b>UNIT 1 PW</b>	<b>UNIT 2 PW</b>	<b>UNIT 3 PW</b>	<b>GEOMETRIC AVERAGE</b>	<b>PERCENT REDUCTION</b>
1	General	7.56x10 <sup>7</sup>	<111	<111	<111	<111	>99.9998
2	General	8.00x10 <sup>6</sup>	<111	<111	<111	<111	>99.998
6	General	3.10x10 <sup>7</sup>	<111	<111	<111	<111	>99.9996
7	General	6.67x10 <sup>6</sup>	<111	<111	<111	<111	>99.998
8	Worst	1.40x10 <sup>6</sup>	<111	<111	<111	<111	>99.992
9	Worst	5.33x10 <sup>6</sup>	<111	<111	<111	<111	>99.997
10	Worst	5.67x10 <sup>6</sup>	<111	<111	<111	<111	>99.998
13	Worst	1.00x10 <sup>6</sup>	<111	<111	<111	<111	>99.99

**PFU = plaque forming unit**  
**PW = product water**

**TABLE 10.**  
**REMOVAL/INACTIVATION OF *CRYPTOSPORIDIUM PARVUM***  
**(OOCYST/LITER)**

<b>DAY</b>	<b>WATER TYPE</b>	<b>CHALLENGE</b>	<b>UNIT 1 PW</b>	<b>UNIT 2 PW</b>	<b>UNIT 3 PW</b>	<b>GEOMETRIC AVERAGE</b>	<b>PERCENT REDUCTION</b>
1	General	2.00x10 <sup>6</sup>	<920	<920	<920	<920	>99.95
3	General	1.61x10 <sup>6</sup>	<920	<920	<920	<920	>99.94
5	Worst	1.04x10 <sup>6</sup>	<920	<920	<920	<920	>99.91
7	Worst	9.11x10 <sup>5</sup>	<920	<920	<920	<920	>99.89

Influent samples were obtained using the Hemocytometer Method.

Effluent samples were obtained using the Propidium Iodide Method. Only live oocysts were use for percent reduction calculations.

PW = product water

## APPENDIX 1

### Sample Calculations

Calculator: Casio Fx-300s

### Geometric Mean

$$\log X_g = \frac{\sum (\log X_i)}{n}$$

$$X_g = \text{antilog} (\log X_g)$$

Example: Table 8, test number 8 for viral challenges

$$\begin{aligned}\log X_g &= \frac{\sum[(\log 111)+(\log 111)+(\log 111)]}{3} \\ &= \frac{2.045323+2.045323+2.045323}{3} \\ X_g &= \text{antilog} (2.045323)=111\end{aligned}$$

### Percent Reduction

Geometric average x 100 = A

A is the n divided by Challenge concentration = B

B then is subtracted from 100 = Percent Reduction

Example: Table 8, test number 8 for viral challenge

$$111 \times 100 = 11,110$$

$$11,100 \div 1.00 \times 10^6 = 0.0111$$

$$0.0111 - 100 = 99.989$$

## Appendix 1

### Thermal Destruction of Waterborne and Water Based Pathogens

The thermal destruction of microorganisms has been studied in great detail in the food industry because of the importance of this process in killing food borne spoilage bacteria and pathogenic microorganisms. However, less well studied is the thermal destruction of waterborne and waterbased pathogens. Waterborne pathogens are those which are transmitted from one human or animal to another by the water route e.g. *Salmonella*, enteric viruses. Waterbased pathogens are those organisms which grow in the water, i.e. *Legionella*, blue-green algae. A list of the major waterborne and waterbased pathogens is shown in Table 1. While boiling water is thought to kill all waterborne pathogens, few studies have been done to determine how long they can survive in boiling water. An extensive review of the literature was conducted to determine temperatures at which these pathogens would be inactivated in water, i.e. time for 99.9% or more inactivation or kill in minutes. The review suggested that most waterborne pathogens die within a few minutes at 70°C or above (Tables 2-4). However, the thermal inactivation for many new waterborne pathogens has not been studied (i.e., astroviruses). Generally enteric viruses appear to be more thermally resistant than waterborne protozoan parasites and bacteria. The cysts and oocysts of protozoan parasites are very thermal sensitive and the waterborne and water based pathogenic bacteria do not form spores.

Many water quality factors may influence the thermal resistance of microorganisms. These are listed in Table 5. The greatest thermal resistance of bacteria is usually seen in the range of pH 6-8, which is the range of most tap waters.

The presence of soluble organic matter generally increases the thermal survival of bacteria and viruses. For example, the amino acid cystine can greatly enhance the thermal stability of enteroviruses at high temperatures (Pohjanpelto, 1961). However, the effects of dissolved solids and suspended solids has received the greatest amount of attention on virus survival at high temperatures. In tapwater at 50°C, 75% of the initial liter of rotavirus SA-11 can be inactivated (Estes et al., 1979), whereas in the presence of 2M magnesium sulfate, no inactivation of the virus takes place. Suspended aquatic sediments (i.e., turbidity) can also greatly enhance the survival of enteroviruses in water (Liew and Gerba, 1980). At 50°C 99.99% inactivation of echovirus type 1 occurs in water vs. only 90% when sediment solids are present. Unfortunately, an extensive literature review did not reveal the influence of these factors at boiling temperatures.

In summary, little information exists on the survival of water borne and water based pathogens in water at high temperatures. The review suggests that most pathogens should be killed within a few minutes above 70°C, although thermal inactivation under a wide variety of water quality conditions has not been studied. Enteric viruses appear to be the most thermally resistant of the water borne pathogens. Hepatitis A virus and *Mycobacterium* spp. appear to be the most thermally resistant water pathogens and their inactivation in the INNOWAVE unit was evaluated in high salt, high organic, turbid water quality conditions since these conditions would most likely have the greatest thermal resistance of these organisms.

**Table 1.**  
**BACTERIA, PARASITES, AND VIRUSES**  
**FOUND AS WATERBORNE PATHOGENS**

<b>BACTERIA</b>	<b>PARASITES</b>	<b>VIRUSES</b>
Campylobacter spp.	Entamoeba histolytica	Polio virus
Escherichia coli	Giardia lamblia	Echovirus
Legionella spp.	Cryptosporidium parvum	Coxsackie virus A and B
Mycobacterium spp.	Ascaris lumbricoides	Hepatitis virus A and E
Pseudomonas aeruginosa		Rotavirus
Salmonella spp.		Reovirus
Shigella spp.		Adenovirus
Vibrio cholera		Norwalk virus
Yersinia enterocolitica		Astrovirus
Aeromonas hydrophila		

**TABLE 2.  
THERMAL DEATH TIMES OF WATERBORNE PATHOGENIC BACTERIA**

<b>BACTERIUM</b>	<b>TEMPERATURE(°C)/TIME (MIN)</b>	<b>REFERENCE</b>
Aeromonas hydrophila	50/3*	Gordon et al., 1992
Campylobacter spp.	75/1	Bandres et al., 1988
Escherichia coli	65/1	Bandres et al., 1988
Legionella	66/0.45*	Sanden et al., 1989
Mycobacterium spp. M. avium	70/2 70/2.3*	Robbecke and Buchhotz 1992
Salmonella spp.	65/1	Bandres et al., 1988
Shigella spp.	65/1	Bandres et al., 1988
Vibrio cholera	55/1*	Roberts and Gilbert, 1979
Yersinia enterocolitica	60/30	Frazier and Westhoff, 1988

\*Time for 90% inactivation of microorganism.

**TABLE 3.  
THERMAL DEATH TIMES OF WATERBORNE PARASITES**

<b>PARASITE</b>	<b>TEMPERATURE(°C)/TIME (MIN)</b>	<b>REFERENCE</b>
Cryptosporidium parvum	72.4/1	Fayer, 1994
Giardia lamblia	50*/1	Cerva, 1955
Entamoeba histolytica	60/1	Chang, 1943

\*Time for 90% inactivation of microorganism.

**Table 4.**  
**THERMAL DEATH TIMES OF WATERBORNE VIRUSES**

<b>VIRUS</b>	<b>TEMPERATURE (°C)/TIME (MIN)</b>	<b>REFERENCE</b>
Poliovirus	60/25	Larkin and Fasolitis, 1979
Adenovirus	60/20	Mahnel, 1977
Coronavirus	55/2	Laude, 1981
Hepatitis A virus	70/10	Siegl et al., 1984
Reovirus	60/20	Mahnel, 1977
Rotavirus	50/30	Estes et al., 1979

**Table 5.**  
**FACTORS CONTROLLING THE THERMAL INACTIVATION  
OF PATHOGENIC MICROORGANISMS IN WATER**

pH
Suspended Solids
Salt Concentration (Dissolved solids)
Type and Concentration of Organic Matter

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