Potentials of Ozone to Control Cryptosporidium Oocysts in Swimming Pool Waters

Rip G. Rice, Ph.D.
RICE International Consulting Enterprises

Wolfram Hartwig, Ph.D.
ETS, Inc.

Gordon R. Finch, Ph.D
University of Alberta – Department of Civil Engineering

Knowledge that ozone is the strongest disinfectant and oxidizing agent available for controlling microorganisms in aqueous media is widespread and well understood. As such, ozone is quite capable of assuring the inactivation of cyst organisms, occasionally found in swimming pool waters, such as Giardia lamblia and Cryptosporidium parvum. However — to accomplish reliable inactivation of C. parvum and G. lamblia cysts, ozone must be present in sufficient concentrations in the waters containing these microorganisms and for sufficient periods of time so that the product of "C" (concentration of ozone in mg/L) times "T" (time of contact in minutes) is at least equal to the "CT" product specified by the U.S. EPA in the Surface Water Treatment Rule, promulgated in 1991 to ensure disinfection of municipal drinking water supplies.

These requirements to meet a specified "CT" value mean that ozone must be produced in the gas phase in sufficient concentration so that when applied to the pool water, a sufficient level of ozone will be present to measure and monitor. The higher the concentration of residual ozone dissolved in the water, the shorter will be the reaction time necessary to assure attainment of the given "CT" value for the specific cyst organism at the temperature of the water. This means that ozone generated by UV radiation cannot be effective for the inactivation of C. parvum oocysts (CT value of ca. 5 min-mg/L), since the levels of ozone in the gas phase generated by currently available UV equipment are so low as to preclude developing measurable ozone residuals in water for more than a few seconds in the immediate area of contact.

On the other hand, ozone generated by corona discharge techniques (in concentrations above 1-2% using dried air as the feed gas, and 3-5% using oxygen-enriched air feed gas) can produce significant levels of measurable residual ozone in water (several tenths of a mg/L). If these levels of residual ozone are held for the several minutes required by an appropriate "CT" value, C. parvum oocyst inactivation can be achieved readily.

The difficulties involved with the removal and inactivation of C. parvum from swimming pool waters with ozone, chlorine, and filtration will be discussed, and recommendations will be developed.

Proceedings of the 1st Annual Chemistry Symposium
National Spa and Pool Institute - November 1996
Pages 62–79

Introduction

The April 1993 outbreak of cryptosporidiosis in the drinking water of Milwaukee, WI focused national and international attention on this waterborne parasite. More than 400,000 people became ill and more than 100 died (mostly immuno–compromised individuals) (Mackenzie, *et al.* 1994). Between 1988 and 1993, five outbreaks of cryptosporidiosis were reported

from exposures in swimming pools (see Table 1; Gerba 1995). Three additional swimming pool–related outbreaks of cryptosporidiosis have been reported during 1996 (Gerba 1996). Consequently, it is important for all segments of the pool and spa industry to understand the nature of this microorganism, its life–cycle, how and why it infects humans, why it is so difficult to remove and/or destroy, but that it can be removed/destroyed, if present, by one or more water treatment management/treatment techniques. Most important to understand is what effects the use of ozone, generated by corona discharge or by ultraviolet radiation, has on this recalcitrant and pernicious microorganism.

These reported swimming pool cases usually are associated with fecal accidents in the pools, presumably from persons infected with *Cryptosporidium* oocysts. No spa–related outbreaks have been reported to date. Usually only outbreaks in which a local health department has made the effort to investigate are reported. There is no requirement in the United States that such outbreaks be studied or reported; thus, the actual number of outbreaks undoubtedly is far greater. Therefore, the reported outbreaks represent only the tip of an iceberg in the true number of cryptosporidiosis cases associated with swimming pools.

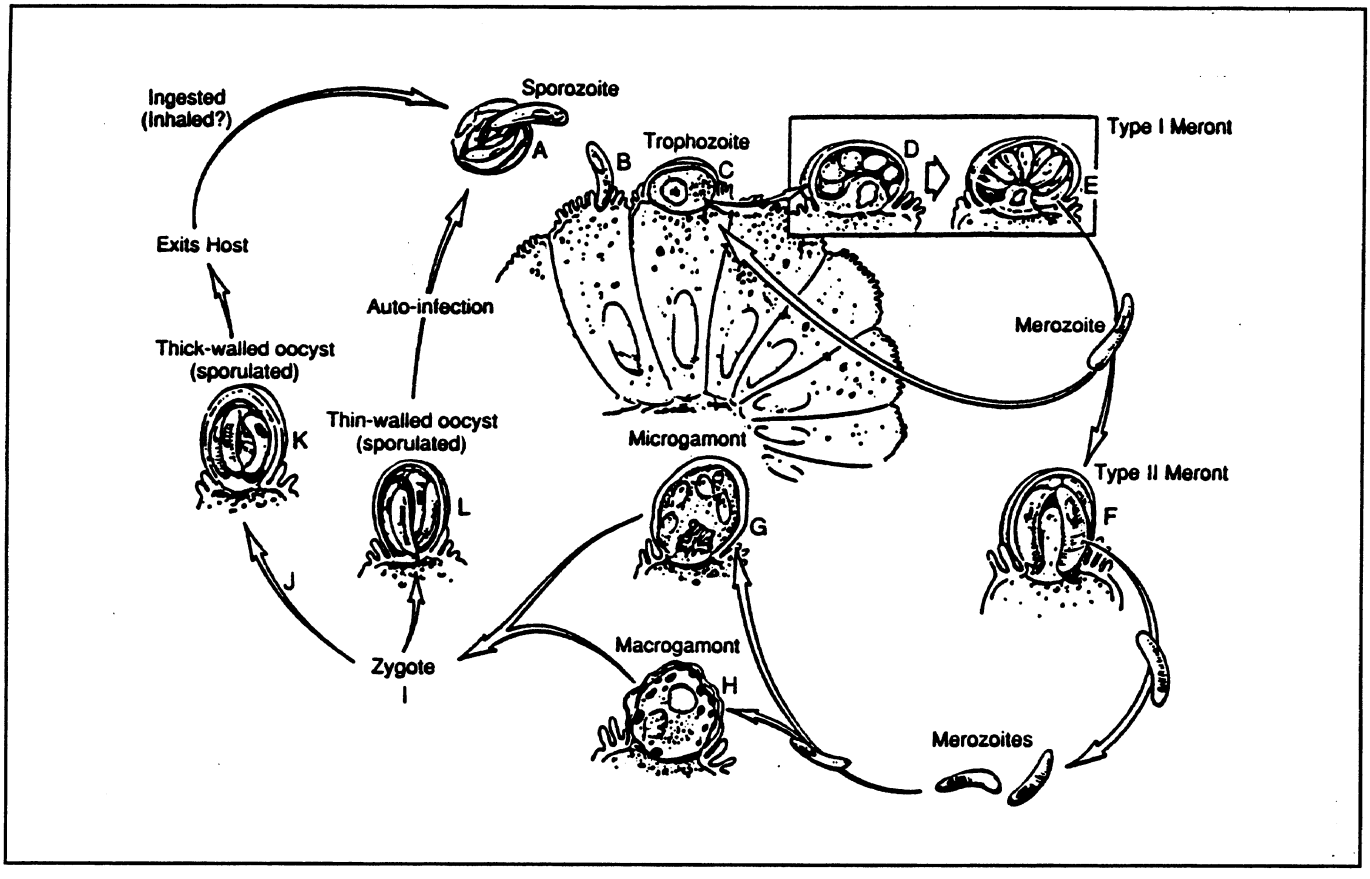
It is the purpose of this communication to describe the basic characteristics of the microorganism (*Cryptosporidium parvum*) and to relate these to the various water treatment techniques currently used in the treatment of pool waters. Special emphasis will be placed on the use of ozone, primarily because this is the most effective disinfectant/oxidant for coping with *Cryptosporidium* oocysts, although ozone's use is the least well understood in this role.

Cryptosporidium parvum (C. parvum)

Cryptosporidium has become recognized as a frequent cause of waterborne disease in humans (Dawson *et al.* 1993). Cryptosporidiosis outbreaks in surface water supplies have been documented in the United States and Great Britain (Gallaher *et al.* 1989; Grimason *et al.* 1990; Hayes *et al.* 1989; Richardson *et al.* 1991; Rose 1990; Rush *et al.* 1990), and it has been speculated that many other cases of waterborne outbreaks of gastroenteritis may have been caused by *Cryptosporidium* (Rose 1990). Outbreaks have been

Year/Location/ Source/No. Of People Affected	Probable Cause	Incubation Period	Symptoms	Disinfection Rate
1988/Los Angeles County (Sorvillo *et al.* 1988; 1992)/60	Fecal accident. Filtration rate < 30% of normal	5 days, median	Watery diarrhea (80% of cases) and fever (50%)	Adequate chlorine: 2 mg/L
1988/Doncaster, UK (Galbraith, 1989; Joce *et al.* 1991)/79	Fecal accident; + defective sewage disposal system	3 weeks	Severe prolonged diarrhea	Crypto oocysts found in beginners' pool
1990/Vancouver, BC (Bell *et al.* 1983)/87	Inadequate chlorination	12 days	Abdominal cramping, watery diarrhea, fatigue, nausea, weight loss	Available chlorine residual had fallen below recommended 0.5 mg/L level
1993/Dane County, WI (80 mi west of Milwaukee) (Bongard *et al.* 1994)/37	Possible fecal contamination	2 weeks	Watery diarrhea (94%), stomach cramps (93%), vomiting (53%)	Lab confirmed Crypto infection in 9-person cluster
1992/Lane County, OR (McAnulty *et al.* 1994)/55	Probably exposure to contaminated wave pool water	6–25 days	Diarrhea (98%), cramping abdominal pain (79%), vomiting (52%), low grade fever (50%)	Not specified

Table 1 – Swimming Pool Cryptosporidiosys Outbreaks (Gerba 1995)



1. Excystation (emergence of the sporozoites from the oocysts) in the intestine of the host,
2. Replication within the host,
3. Gamete formation,
4. Fertilization,
5. Oocyst wall formation, and
6. Sporozoite formation.

Figure 1 – Life cycle of *Cryptosporidium* spp. in warm-blooded animals [from Finch *et al.* 1994. Reprinted from W.L. Current and B.L. Blagburn 1990].

associated with water supplies that have been contaminated by sewage or are from watersheds under intensive agricultural usage, particularly if large dairy cow herds are present. Another common factor in the outbreaks of waterborne cryptosporidiosis are municipal water plants that meet the regulatory water quality standards, but are operated at less than optimum conditions.

The biology of *Cryptosporidium* spp. has been well documented in review articles (Current 1987; Fayer and Ungar 1986). Its life cycle can be summarized by six events (see Figure 1).

Of most interest to the pool and spa water industry is the oocyst, which occurs in two forms, one a thin–walled form that is autoinfective within the host and is not believed to survive outside of the host. The other is a thick–walled oocyst that is capable of surviving for several weeks in the environment and is the main means for transmission of the parasite (Current 1987). The oocyst is approximately 5 μm (microns) in diameter, but can vary from this size and can be elongated depending on the species (Figure 2). It has been observed that oocysts are capable of passing through membrane filters greater than 1 μm in pore size (Dawson *et al.* 1993). This means that filters normally employed in swimming pool and spa water treatment (which do not remove particulates of 1 μm size), cannot be relied upon to remove *Cryptosporidium* oocysts. Thus disinfection must remain the ultimate barrier to these microorganisms.

Isolation and Viability Issues

Cryptosporidium oocysts are thick–walled particles which contain multiple sporozoites. Of funda-

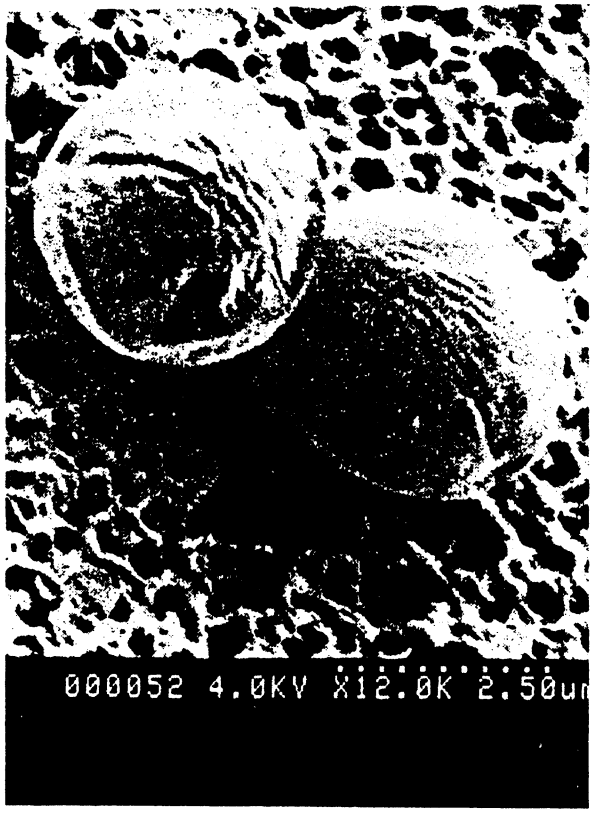


Figure 2 – Scanning electron micrograph of *C. parvum* oocysts, magnified 12,000 times (Finch *et al.* 1994)

mental importance is the fact that the thick–walled particle, *per se*, cannot cause infection unless the contained sporozoite is alive. When the thick wall is pierced, or opens inside its host, the sporozoites are released. If they are alive, then infection can occur — but if all are dead, there can be no infection.

These fundamental oocyst characteristics lead to the following two major conclusions:

1. Simply isolating one or more *Cryptosporidium* oocysts does not mean *a priori* that the contained sporozoites are alive.
2. The primary question is how to tell whether the sporozoites contained in oocysts that have been isolated are alive — e.g., capable of causing infection.

Of course, this is a simplistic argument. There are other parameters significant to the subject as well. One of these important secondary considerations is whether it is possible to isolate all *Cryptosporidium* oocysts. Current consensus opinion is that only a fraction of *Cryptosporidium* oocysts actually present in a water sample can be isolated (Finch *et al.* 1994). Even if all oocysts actually isolated turn out to contain dead sporozoites, this in no way guarantees that sporozoites present in the oocysts that are not isolated also are dead.

The current isolation procedure involves filtration of several hundred liters of water through special 1 μm filters. The filters then are sent to a laboratory where they are cut open and extracted. In addition to *Cryptosporidium* oocysts, these filters contain many different types of organic and inorganic particles such as bacteria, fungi, algae, etc. After extraction, the sample is concentrated, then examined microscopically to search for *Cryptosporidium* oocysts. Special fluorescent stains have been developed which attach to oocysts, but these are not totally specific. Sometimes these fluorescent stains become attached to the other plants and animals naturally found in water and thus can produce false–positive identifications.

Even if this procedure were 100% accurate for ***identification*** of *Cryptosporidium* oocysts, there is still the problem of determining whether the contained sporozoites are viable (e.g., whether they are alive and are capable of causing infection). Currently there are no rapid methods available for determining the viability of oocysts found in environmental samples. ***Therefore, the efficacy of water treatment must be measured by setting process operating conditions such that oocysts will not survive.*** The operating conditions developed to date are determined in water disinfection research laboratories where large numbers of oocysts are exposed to disinfectants and then recovered from the water and subjected to viability assays such as *in vitro* excystation, vital stains, or animal infectivity. For example, *in vitro* excystation requires 50,000 oocysts for analysis. Contrast this with the numbers found in natural waters or tap waters, e.g., less than one oocyst per 100 liters in very clean water to a few thousand per hundred liters of sewage. Swimming pool waters containing fecal deposits fall somewhere in between.

Work in research laboratories has suggested that animal infectivity assays are superior to *in vitro* excystation or vital stains when assaying chemically disinfected water (Finch *et al.* 1993; 1995). Excystation and vital stains were found to grossly underestimate the inactivation of oocysts when compared with infectivity.

Treatability of *Cryptosporidium parvum*

From the above considerations of oocyst properties and characteristics, as well as the issues of infectivity and its difficulty of measurement, it is clear

that one appropriate treatment technique might involve filtration through membranes or other media that can assure retention of **all** oocyst particulates, e.g., 1 μm. Another approach is to heat the oocysts to warm temperatures, such as found in a domestic hot water tank. It has been reported that infectivity is lost after two minutes at 64.2°C (Fayer, 1994). This latter approach, although appropriate for treating potable water supplies at the point of use, is not practical for swimming pools which operate between 28 and 30°C.

A third approach is the application of a disinfectant that either can pass through the thick outer shell of the oocyst and kill the sporozoite, or can oxidatively disrupt the outer oocyst shell, thus exposing the naked sporozoite to the oxidizing action of the added reagent. Ozone has been shown to be very effective for control of *C. parvum* (Finch *et al.* 1993, 1994, 1995; Peeters *et al.* 1989). This will be the major topic for the balance of this paper. With respect to ozone treatment for control of *C. parvum*, it is critical for the practitioner to realize the dimensions of the ozonation parameters which are involved — e.g.:

- ozone solubility in water
- factors which affect ozone solubility
- the necessity to hold a measurable residual of ozone (in mg/L) over a specific period of time (in minutes) to satisfy a defined "C x T" product (to be described below)

The C x T Concept (U.S. EPA, 1989)

The Surface Water Treatment Rule (SWTR) promulgated by the U.S. EPA in 1991 requires that drinking water utilities must demonstrate that 99.9% (3–logs) of *Giardia* cysts and 99.99% (4–logs) of enteric viruses are removed/inactivated by the treatment process employed. However, to demonstrate that such inactivations by disinfection are attained consistently, some method must be available which allows instantaneous confirmation of the degree of disinfection attained — not the currently available after–the–fact microorganism counting procedures. To answer this need, EPA has adopted the "CT" concept, in which the term "C" is the concentration of a particular disinfectant (expressed in mg/L) and "T" is the time of contact with that disinfectant in the water to be treated (expressed in minutes). Disinfecting residual ozone concentrations usually can be measured on–line or very shortly thereafter, and ozone contact time can be (a) fixed by design of the ozone contact chamber and (b) varied by changing the water flow rates.

In the Guidance Manual accompanying the SWTR (Malcolm Pirnie Inc. 1991), tables of "CT" values are presented for attaining specified numbers of logs of inactivation of *Giardia* cysts and viruses with ozone, chlorine, chlorine dioxide and monochloramine. As long as the product of "C" times "T" equals the specified value, it does not matter how that value is obtained. That is to say, for any particular value of disinfectant concentration "C", the time of contact "T" can be adjusted, and *vice versa*. Of course, contact times for waters contained in pools and spas can be varied only by increasing or decreasing the size(s) of water containers — which may pose some engineering impracticalities — or by changing flow rates, which would change turnover rate(s). The simpler variable to increase or decrease in pools and spas is the concentration of disinfectant.

EPA's Surface Water Treatment Rule did not consider *Cryptosporidium parvum* when it was promulgated – consequently, there were no "CT" values specified to cope with this microorganism in this rule. Since passage of the SWTR, however, there has been considerable study of *Cryptosporidium* and its ability (or not) to be inactivated by chlorine, ozone, monochloramine, and chlorine dioxide. This work is reviewed and presented in detail by Finch *et al.* (1994). It is now well understood that to inactivate 99.9% (3–logs) of *Cryptosporidium* oocysts with chlorine at pH 7.5 at 25°C requires a "CT" value of >2,500 min–mg/L. With monochloramine, the "CT" value is even higher. With ozone the "CT" value is on the order of 5 min–mg/L (depending upon temperature), a much more readily attainable number, particularly with respect to pool water treatment (Gerba 1995).

Ozone Inactivation of *C. parvum*

Past Studies (as reviewed by Finch *et al.* 1994) – Studies of ozone inactivation of ***Cryptosporidium*** oocysts have been reported by Korich *et al.* (1990); Langlais *et al.* (1990); Parker *et al.* (1993); and Peeters *et al.* (1989). Many of these studies used animal infectivity as a measure of the degree of inactivation. Korich *et al.* (1990) used neonatal BALB/c mice and *C. parvum*.

Peeters *et al.* (1989) used Swiss OF1 mice and *C. parvum*, and Langlais *et al.* (1990) used immune–suppressed male Sprague–Dawley rats and *C. baileyi*. One report (Parker *et al.* 1993) did not use animal infectivity but used vital dye exclusion to assess oocyst viability. Another study (Ransome *et al.* 1993) used excystation during a series of tests designed to screen the efficacy of water disinfectants for inactivation of *C. parvum*.

The reported efforts to achieve 99 percent inactivation of oocysts are summarized in Table 2. Compared with the requirements for *G. lamblia* (Wickramanayake *et al.* 1984), it appears that *Cryptosporidium* requires 14 to 58 times more effort at 25°C (close to temperatures of pools and spas) than that required for *G. lamblia*.

Species	Ozone residual, mg/L	Contact time, minutes	Temp., °C	Conventional CT for ≥ 99% inactivation, min–mg/L	References
C. parvum	0.77	6	Room	4.6	Peeters *et al.* 1989
	0.51	8		4	
C. parvum	1.0	5 and 10	25	5–10	Korich *et al.* 1980
C. baileyi	0.6 and 0.8	4	25	2.4–3.2	Langlais *et al.* 1990
C. parvum	0.44	6	20	2.6	Perrine *et al.* 1990
C. parvum	3	6	20	18	Parker *et al.* 1993
	5	2		10	
G. lamblia	0.11–0.48	0.94–5	5	0.53	Wickramanayake
	0.03–0.15	1.06–5.5	25	0.17	*et al.* 1984

Table 2 – Summary of reported ozonation requirements for inactivation of *Cryptosporidium* spp. oocysts compared with published requirements for *Giardia lamblia* (Finch *et al.* 1994)

Recent Studies of Ozone Inactivation of *Cryptosporidium parvum* – The objectives of a study sponsored by the American Water Works Association Research Foundation were to compare the *in vitro* excystation of *C. parvum* oocysts with infectivity in neonatal CD–1 mice to determine viability after disinfection, to determine the ozonation requirements at room temperature and at 7°C in a controlled laboratory water, and to determine the kinetics of inactivation of *C. parvum* with ozone (Finch *et al.* 1994). Of most importance to pool water treatment specialists are the following findings:

1. The *in vitro* excystation technique was found to underestimate the oocyst inactivation when compared with the dose–response model using neonatal CD–1 mice. This means that the simple CT values reported by researchers using *in vitro* excystation (Ransome *et al.* 1993; Sundermann *et al.* 1987) may be misleading, partly because of the shortcomings of the excystation procedure. It appears that use of the animal infectivity model remains the best choice for determining *C. parvum* inactivation by chemical means.

2. Figure 3 is an electron micrograph of *C. parvum* oocysts treated with ozone after exposure to concentrations sufficient to inactivate the oocysts by 4–log units (Figure 3A) with control specimens. The shell of the oocyst becomes gossamer–like (Figure 3A) relative to the control oocysts (Figure 3B). Ozone appears to act on the oocyst surface in much the same manner as it acts on *Giardia* cysts.

3. Simple CT values of about 3.5 and 7 mg–min/L were required for 99% inactivation (2–logs) by ozone at 22°C and 7°C, respectively. For 99.9% inactivation (3–logs), simple CT values of about 5 and 10 mg–min/L were required at 22°C and 7°C, respectively. It is clear that higher water temperatures require lower CT values for ozone inactivation of *C. parvum*, because colder water temperatures decrease the inactivation rate.

   From the standpoint of ozone treatment of swimming pool water (at 26–28°C), the CT value for 99% (2–logs) inactivation of *C. parvum* oocysts, although not yet determined and reported, should be approximately 2–3 mg–min/L, and for 99.9% (3–logs) inactivation, the corresponding CT value should be approximately 3–4 mg–min/L.

4. Applying the more rigorous kinetic approach to the data, the maximum–likelihood estimates for the parameters k, n, and m in the Hom model (Equation [2] – see Hom, 1972) were obtained using animal infectivity data. At 7°C the resulting equation was:

$$\log \frac{N}{N_0} = -29 C_{avg}^{0.68} T^{0.95}$$

   At 22°C the resulting equation was:

$$\log \frac{N}{N_0} = 0.82 C_{avg}^{0.68} T^{0.64}$$

   The disinfection response surface derived from Equation [6] is illustrated in Figure 4. The required contact time for a predetermined integrated ozone residual to achieve 99 and 99.9

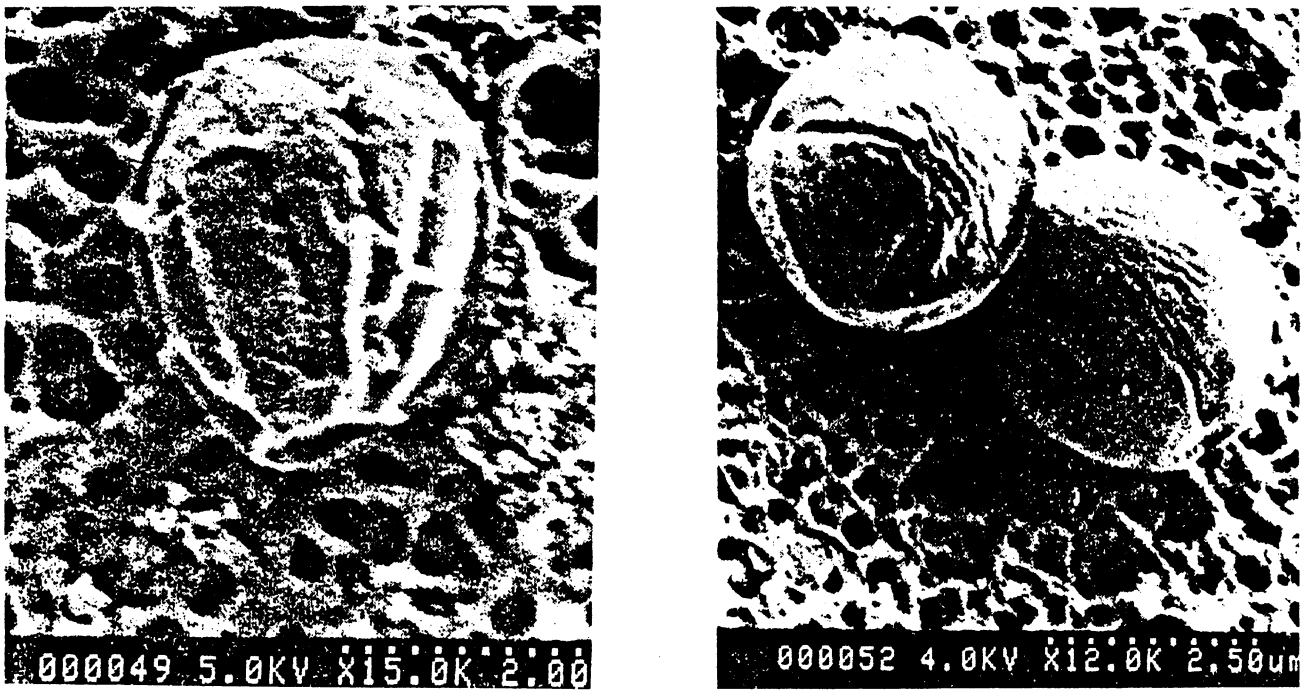


Figure 3 – (A) Scanning electron micrograph of *C. parvum* oocysts following ozonation (magnified 15,000 times) compared with (B) a control (magnified 12,000 times) (Finch *et al.* 1994).

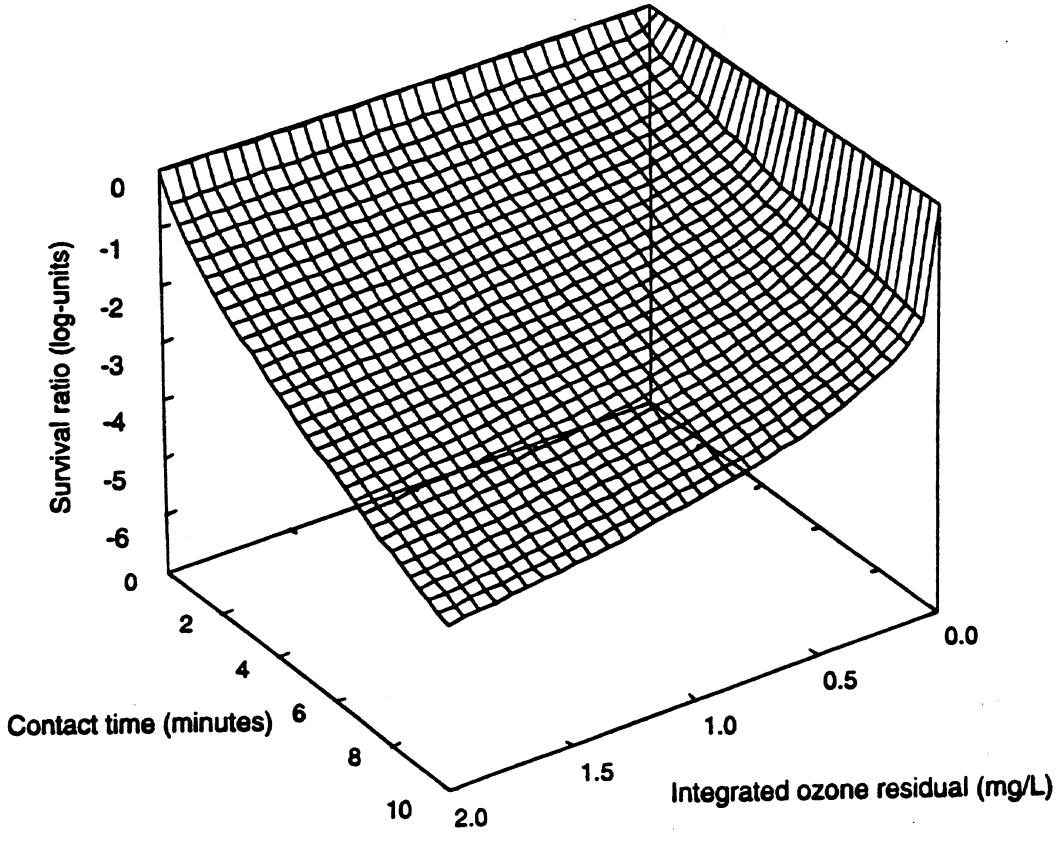


Figure 4 – Theoretical response surface for *C. parvum* inactivation by ozone using a Hom–type kinetic model at 22°C (Finch *et al.* 1994).

percent inactivation of *C. parvum* at 7°C and 22°C is summarized in Tables 3 and 4. For 22°C, the model results in CT values of 2.4 and 3.7 mg–min/L for 99 and 99.9 percent inactivation, respectively. The corresponding 7°C values are 6.9 and 10.3 mg–min/L for 99 and 99.9 percent inactivation, respectively, indicating approximately three times greater exposure to ozone than at 22°C to attain the same levels of disinfection.

5. Preliminary interpretation of the results of this study suggests that the concentration of ozone may be of reduced importance when compared with contact time, ***provided that sufficient ozone is present in the water*** (emphasis added). The operating target for ozonation of water to inactivate *Cryptosporidium* oocysts depends on the water quality and temperature. However, for C_{avg} (average concentration) to be calculated, it is implied that an ozone residual must be measured at the beginning and end of the contact time. Ozone residual measurement methods are such that less than 0.01 mg/L cannot be determined reliably in practice.

   As contact time increases, the ozone dose may need to be increased to produce the desired C_{avg} shown in Tables 3 and 4 for the target inactivation of *C. parvum*. This is because ozone decay is a function of time — in high quality water it may take 20 minutes for half of the ozone to disappear, but in poor quality water (such as recirculating swimming pool waters) it takes only about a minute to lose all of the ozone. Nevertheless, if no decay occurs during the contact time, then the starting and ending ozone residual is the same as C_{avg}.

6. Tables 3 and 4 illustrate the differences in the design conditions for ozone contact time and integrated ozone when compared with the simple CT values for the same ozonation conditions. For example, in Table 3, an integrated ozone residual of 0.25 mg/L requires a contact time of 6.6 minutes for 99 percent inactivation (simple CT of 1.7 mg–min/L). If the integrated ozone residual is doubled to 0.50 mg/L, the required contact time drops to 5.2 minutes for 99 percent inactivation (simple CT of 2.6 mg–min/L). Note that the simple CT value has increased. This example illustrates the nonlinearity of the model.

Table 5 summarizes the predicted required contact times for 99 and 99.9 percent inactivation of *G. muris* and *C. parvum* for identical integrated ozone residuals at 22°C. Since swimming pools operate about 5°C higher, it is logical to expect that the corresponding CT values for both microorganisms will be lower, perhaps about 2.0

Integrated ozone residual* mg/L	Required contact time, minutes		Conventional linear CT, min–mg/L		Nonlinear C^nT^m n = 0.68 m = 0.95	Nonlinear C^nT^m n = 0.23 m = 0.64
	7°C	22°C	7°C	22°C	7°C	22°C
0.25	20.6	6.6	5.1	1.7	6.9	2.4
0.50	12.5	5.2	6.3	2.6	6.9	2.4
0.75	9.4	4.5	7.0	3.3	6.9	2.4
1.00	7.6	4.0	7.6	4.0	6.9	2.4
1.25	6.5	3.7	8.1	4.6	6.9	2.4
1.50	5.7	3.5	8.6	5.2	6.9	2.4
1.75	5.1	3.3	8.9	5.8	6.9	2.4
2.00	4.6	3.1	9.3	9.3	6.9	2.4
2.25	4.3	3.0	9.6	6.8	6.9	2.4
2.50	4.0	2.9	9.9	7.2	6.9	2.4
2.75	3.7	2.8	10.2	7.7	6.9	2.4
3.00	3.5	2.7	10.4	8.1	6.9	2.4

* The integrated ozone residual is the average ozone residual over the contact time duration.

Table 3 – Summary of ozonation design criteria required to achieve 99% inactivation of *Cryptosporidium* oocysts predicted from a Hom–type model (Finch *et al.* 1994)

Integrated ozone residual* mg/L	Required contact time, minutes		Conventional linear CT, min–mg/L		Nonlinear C^nT^m	
					n = 0.68 m = 0.95	n = 0.23 m = 0.64
	7°C	22°C	7°C	22°C	7°C	22°C
0.25	31.6	12.5	7.9	3.1	10.3	3.7
0.50	19.2	9.7	9.6	4.9	10.3	3.7
0.75	14.4	8.4	10.8	6.3	10.3	3.7
1.00	11.7	7.6	11.7	7.6	10.3	3.7
1.25	10.0	7.0	12.5	8.8	10.3	3.7
1.50	8.8	6.6	13.1	9.8	10.3	3.7
1.75	7.8	6.2	13.7	10.9	10.3	3.7
2.00	7.1	5.9	14.2	11.8	10.3	3.7
2.25	6.5	5.7	14.7	12.8	10.3	3.7
2.50	6.1	5.5	15.2	13.6	10.3	3.7
2.75	5.7	5.3	15.6	14.5	10.3	3.7
3.00	5.3	5.1	16.0	15.3	10.3	3.7

* The integrated ozone residual is the average ozone residual over the contact time duration.

Table 4 – Summary of ozonation design criteria required to achieve 99.9% inactivation of *Cryptosporidium* oocysts predicted from a Hom–type model (Finch *et al.* 1994)

for *G. muris* and 3.0–3.2 mg/L–min for *C. parvum* (for 3–logs of inactivation).

Recently, Finch *et al.* (1995) compared the inactivation of *Cryptosporidium parvum* oocysts by chlorine and ozone. Initially, each disinfectant was used alone, then each disinfectant treatment was followed by monochloramine addition, under conditions which might be found at a municipal water treatment plant, and which might exist in recirculating swimming pool or spa waters treated with ozone followed by chlorine. Whereas chlorine and ozone each used alone provided the expected results (e.g., little short term inactivation by chlorine, much better inactivation with ozone), both disinfectants followed by chloramine addition gave much higher levels of oocyst inactivation. Data are shown in Figures 5 and 6. The authors theorize that the oocyst shell probably is affected by ozone and chlorine, allowing penetration of monochloramine to the sporozoite, which then apparently is inactivated rapidly.

Most pools are operated to attempt to minimize the concentration of monochloramine, because of its volatility and presence in pool hall atmospheres. Nevertheless, pools which use ozone followed by chlorine will form some monochloramine when bathers are added to the chlorine–containing pool waters. These studies by Finch *et al.* (1994; 1995) show that

Organism	Required contact time (minutes)		C^nT^m product	
	99.9 percent inactivation	99.9 percent inactivation	99.9 percent inactivation	99.9 percent inactivation
G. lamblia	1.2	6.4	0.63	0.96
C. parvum	5.2	9.7	2.4	3.7

Note: The integrated ozone residual is the average ozone residual over the contact time duration.

Table 5 – Comparison of predicted required contact times at an integrated ozone residual of 0.5 mg/L for inactivation of 99% and 99.9% of *G. lamblia* and *C. parvum* at 22°C in phosphate buffer (Finch *et al.* 1994)

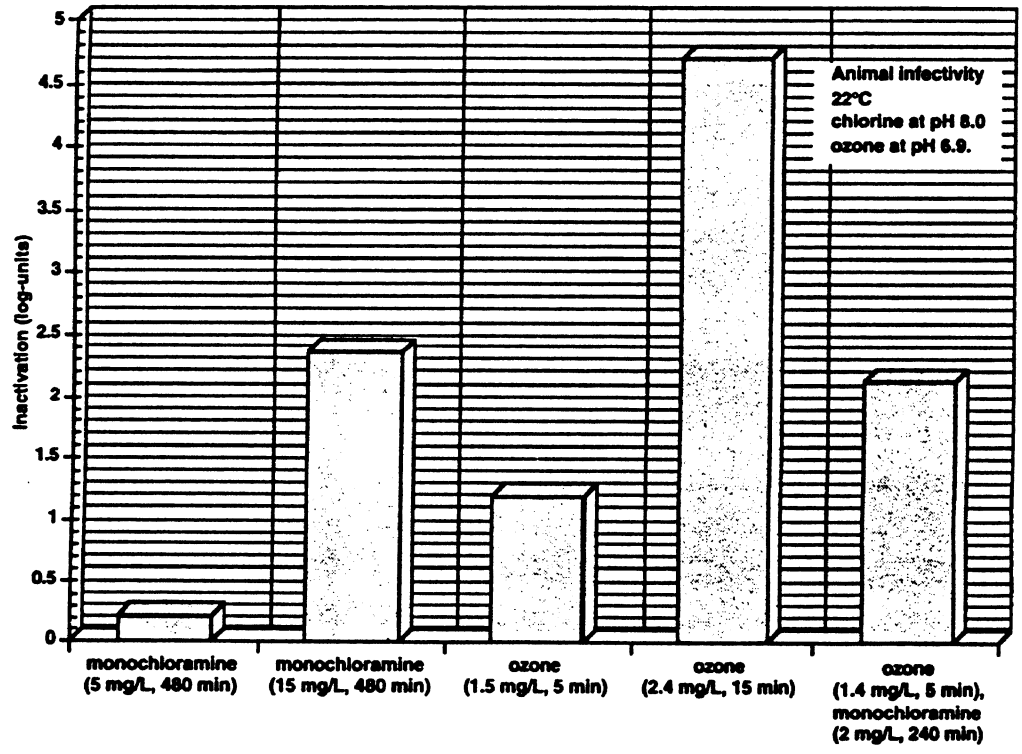


Figure 5 – Effectiveness of monochloramine, ozone, and ozone + $ClNH_2$ for inactivation of *C. parvum* oocysts (Finch *et al.* 1995).

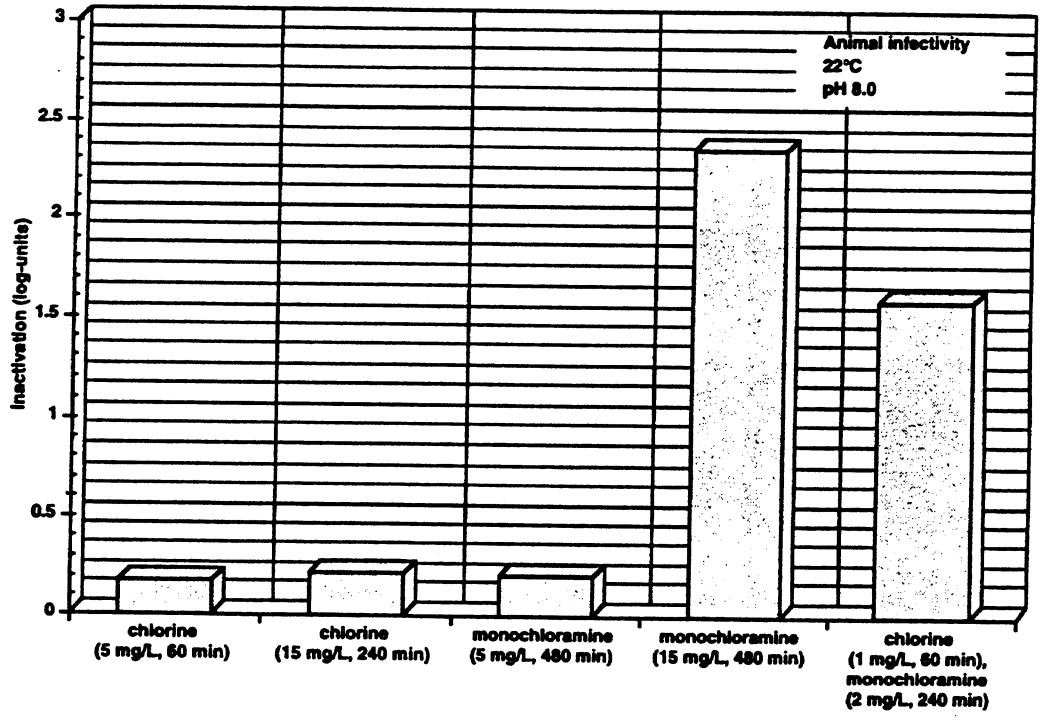


Figure 6 – Effectiveness of free chlorine, monochloramine and chlorine + $ClNH_2$ for inactivation of *C. parvum* oocysts (Finch *et al.* 1995).

monochloramine following ozonation actually is a help in controlling the viability of *Cryptosporidium* oocysts.

Implications for Swimming Pool and Spa Water Treatment

Swimming pools in North America usually are treated with chlorine or bromine chemicals. Only in the past 15–20 years has the use of ozone become prevalent, but always coupled with chlorine or bromine. Most pools currently using ozone have installed ultraviolet (UV) devices to generate ozone rather than to use corona discharge techniques. With respect to inactivation of *C. parvum* oocysts (or any other microorganism for that matter), generation of ozone by UV radiation techniques produces only small amounts of ozone, and those at very low concentrations. Rice (1995) has compared the equilibrium solubilities of ozone in water at 5°, 25° and 30°C as a function of the concentration of ozone in the gas phase (Table 6). This table is important to the pool water treatment practitioner because ozone is only partially soluble in water — and the solubility of a partially soluble gas in water is directly proportional to its partial pressure (e.g., concentration) in the gas phase, as expressed by Henry's law:

$$Y = H \cdot X$$

in which Y is the concentration (solubility) of ozone in the aqueous phase, X is the partial pressure of ozone in the gas phase, and H is the Henry's law constant, which can vary with temperature, pH, ionic strength, and other water quality parameters.

Consideration of Henry's law leads to the obvious conclusion that the higher the gas–phase concentration of ozone, the greater will be the solubility of ozone in the pool or spa water. The reciprocal statement also is true: the lower the concentration of ozone in the gas phase, the lower will be the concentration of ozone in the pool or spa water. It should be clear from data in Table 6 that when ozone is generated by UV radiation, much less ozone is available in the water for either oxidation or disinfection than when generated by corona discharge.

Strictly speaking, the data in Table 6 represent the maximum amounts of ozone that can be dissolved in water at equilibrium, which implies time and quiescence. However, in treating pool waters, ozone gas normally is applied continuously and mixes rapidly and continuously with the water being treated. This means that equilibrium conditions never can be attained, or even closely approximated, because contact time is brief and quiescence is impossible. Therefore the "practical" ozone solubilities in pools will be lower than those shown in Table 6, a result which favors the use of ozone generated by corona discharge even more. For example, for CD–generated ozone (1% by weight) at 30°C, the maximum practical level of ozone that can be attained is on the order of only 0.4–0.5 mg/L. This means that to attain a "CT" value of 5 mg/L–min necessary to provide ~2–logs of inactivation of *Cryptosporidium parvum* oocysts will require 10 minutes or more of contacting time. Stated in other words, if pool water is treated with ozone generated by UV_{185} (0.1% by weight) as compared with corona discharge (1% by weight), the contact time required for UV–generated ozone must be at least 10 times that required for CD–generated ozone to attain the same CT value.

Expected Impacts Of Ozonation During Pool/Spa Water Treatment

There is little question that maintaining a residual ozone concentration of 0.4 mg/L over a minimum period of time, e.g., four minutes, provides a considerable amount of bacterial, viral, and *Giardia* and *Cryptosporidium* cyst disinfection. In the terminology of EPA's Surface Water Treatment Rule,

Temperature	Gas Phase Ozone Concentration (% by weight)					
	0.001% $UV_{254\ nm}$	0.1% $UV_{185\ nm}$	1% CD	1.5% CD	2% CD	3% CD
	Equilibrium Ozone Water Solubility (mg/L)					
5°C	0.007	0.74	7.39	11.09	14.79	22.18
25°C	0.004	0.35	3.53	5.29	7.05	10.58
30°C	0.003	0.27	2.70	4.04	5.39	8.09

Table 6 – Equilibrium solubilities of ozone in water (in mg/L) as generated by UV radiation and corona discharge techniques (Rice 1995, adapted from Stover *et al.* 1986)

0.4 mg/L of dissolved ozone residual held over 4 minutes provides a CT value of 1.6 mg–min/L. While this CT value is quite sufficient to provide 3–4 logs of inactivation of *Giardia* cysts and 5–7 logs of virus inactivation, much less disinfection is provided for destruction/inactivation of *Cryptosporidium* oocysts. The most recent studies by Finch *et al.* (1994; 1995) show that an average ozone residual of 0.4 mg/L for 6 and 10 minutes is necessary for 99% (2–logs) and 99.9% (3–logs) inactivation of *Cryptosporidium parvum*, respectively, at 22°C. Approximately double the contact time is necessary at 7°C, at the same ozone residual levels. The relationship is complicated by the fact that ozone persists longer at colder temperatures than at warmer temperatures.

These CT considerations for ozone generated by corona discharge techniques mean that **all** of the pool water must be treated with the appropriate amount of ozone for such time as is commensurate with obtaining the desired CT value. The logical extension of this fact is that slip–stream or side stream ozonation systems, in which only 10–25% of the pool water is treated with the necessary amount of ozone, even when generated by corona discharge techniques, probably will not be able to control *C. parvum*, except in that portion of the water actually passing through the side–stream ozonation apparatus.

A word should be said at this point about systems which employ ozone in combination with bromide ion. In the United States, the source of bromide ion usually is bromo–chloro–dimethylhydantoin (BCDMH) rather than sodium bromide. The reaction of bromide ion with ozone is very fast ($t_{0.5}$ < 10 seconds – Haag and Hoigné 1984). Consequently, if the bromide ion level is allowed to increase to excessively high levels (>> 15 mg/L) nearly all, if not all, of the ozone added will react with bromide ion, leaving little if any ozone residual to cope with *C. parvum* (Barlow 1993). However, as long as the bromide ion is maintained at about 15 mg/L, CD–generated ozone will be present in the ozone reactor to ensure disinfection of *C. parvum*, provided that sufficient reaction time also is provided.

Impacts of Filtration

It has been pointed out earlier that the *Cryptosporidium* oocyst, although approximately 5 μm in diameter, can elongate and pass through filtration media that retain particles down to 1 μm in size. Consequently, only filters that retain particles less than 1 μm in size can remove *Cryptosporidium* oocysts with certainty. Unfortunately, it is not normally practical to utilize 1 μm filters in swimming pool water circulation systems.

Modern pool filter systems employ one of three available filtration methods to remove solid contaminants from the water:

- high rate sand filters,
- diatomaceous earth (DE) filters, and
- cartridge filters.

Only the first two of these are used commonly in public pools. Cartridge filters generally are installed almost exclusively at private pools. Older filter types, such as rapid sand filters with much lower filter rates, are rare, and are not being installed in new facilities.

High rate sand filters usually operate at filter rates up to 15 gpm/ft², although some states permit up to 20 gpm/ft². The media depth, excluding support gravel for the underdrain, usually is in the range of 24 to 30 inches. Note that U.S. sand filters normally have only 50% to 63% of the depth specified for pressure filters in the German pool standard (DIN 19,643) at comparable filter rates (maximum 30 m³/m²/h = m/h 12.3 gpm/ft² for single medium high rate sand filters, 50 m/h 20.5 gpm/ft² for multi–media filters).

DE filters are available in several different configurations, depending on the placement of the filter pump (vacuum vs. pressure filter), the method of applying the DE (continuous slurry feed vs. intermittent coating, the degree of automation, etc.). All DE filters operate at much lower filter rates, usually 2 gpm/ft² or less. There is a minimum filtration rate due to the fact that the water pressure must support the DE on the filter leaves.

Common to all filters is the tenet that a dirty filter works better than a clean one. While a dirty filter will show an increased pressure differential between influent and effluent, it also will be more efficient in removing contaminants from the water flowing through it. Therefore, one can only give **a range for the particle size removed** by a certain filter type. Other considerations that must be included in an evaluation include the age of the filter media, especially important for sand filters, where the gradual rounding of the sand grains leads to a loss of filtration efficacy; the average size of the sand; the actual filtration rate; duration and frequency of the backwash; the use of additional treatment chemicals such as polyaluminum chloride as flocculant; the injection of ozone before or after the filter; etc.

The lower limits for the size of particles retained by each type of filter vary by a wide degree. One manufacturer states: "DE traps particles 1 to 5 microns and sand normally filters anything over 25 microns in size (Reemay 1993).

These numbers are ridiculed in a book on pool maintenance, in which the author claims that "sand filters strain particles down to about 60 microns", while "DE filters strain particles down to about 7 microns." (Tamminen 1995). Considering that the average size of the *Cryptosporidium* oocyst is 4–6 microns (Gerba 1995), and that this organism is able to change its shape from spherical to an elongated

elliptical form, it is clear that no sand filter will be guaranteed effective in removing *Cryptosporidium* oocysts from pool water. DE filters may retain some oocysts, but they, too, do not ***guarantee*** the removal efficiency required for safe operation. Even if filtration were a possible oocyst removal method, new questions arise about the proper, safe removal and disposal of the contaminated DE, or the discharge of the sand filter backwash effluent.

The conclusion from these references is that no current swimming pool filtration system can be relied upon to remove *Cryptosporidium* oocysts from pool and spa waters, and that inactivation through chemical oxidation/disinfection rather than filtration must be the more effective way to proceed.

However, in a diatomaceous earth filtration experiment conducted with surface water at a drinking water treatment plant at Shingletown PA, diatomaceous earth filtration achieved more than 3–logs (99.9%) of *Cryptosporidium* oocyst removal at an influent oocyst level of 4.4–11.1 x 10^5 oocysts/100 gal without alum or cationic polymer addition (Schuler and Ghosh 1990). The grade of diatomaceous earth used was either B or C (grade C being the finer). Addition of cationic polymer also did not improve the oocyst removal compared to diatomaceous earth grade C. However, addition of alum increased the removal of oocysts to more than 5–logs (99.999%). A diatomaceous earth precoat of 1 kg/m^2 was used for all filter runs. The filter was operated with an hydraulic loading rate of 4.88 m/h. No other studies have been found in the literature with regard to the effectiveness of diatomaceous earth filtration in removal of *Cryptosporidium* oocysts.

What to do if *Cryptosporidium* contamination is suspected and CD Ozone is not available

Since most of the reported swimming pool cryptosporidiosis events have been associated with fecal accidents, the pool professional must assume that ***any*** fecal accident is a potential source of *Cryptosporidium* oocysts. If CD ozone addition has been properly designed into the water treatment system and has been operating to provide the appropriate CT value to ***all*** of the water, there is not much to worry about, other than to remove the debris deposited. On the other hand, if the pool water treatment system has been relying on ozone generated by UV ozone or chlorine or bromine, or combinations thereof, the threat of cryptosporidiosis is real. Given the exceptionally high CT value for chlorine (> 2,500 mg/L–min), the only recourse is to follow the lead of several state health department recommendations as exemplified by the following excerpts from the state of Wisconsin's "*Cryptosporidium* Fact Sheet for Swimming Pool Operators" (Wisconsin, undated):

> "Cryptosporidium is a coccidian protozoan found mainly in fecal contaminated environments. One of these environments can be swimming pools. The organism is transmitted through a fecal–oral route, and resides in the intestinal tract. The infective dose can be very low; as few as 10 organisms have been demonstrated to cause illness in animals. The illness caused by *Cryptosporidium* has an incubation period of 1 to 12 days with an average of about 7 days. The most common sign or symptom of illness is diarrhea, which is usually profuse and watery and often accompanied by abdominal cramping. Malaise, fever, loss of appetite, nausea, and vomiting also can occur.
>
> Oocysts, the infectious stage of the organism, appear in the stool at the onset of symptoms and can continue to be excreted in the stool for several weeks after the symptoms resolve. Outside the body they may be infective for 2–6 months in a moist environment.
>
> The oocyst stage is highly resistant to halogen (chlorine/bromine) disinfection. It can withstand relatively high levels of hypochlorous acid for a long period of time. This is a concern in pools where the primary protection against disease transmission is the halogen disinfection system.
>
> Because of the size of the oocyst (2–4 microns in size), they can pass through a sand filter or most cartridge filters. A diatomaceous earth filter can capture most of the oocysts. However, even with good removal it may take as long as 2.5 days to remove the majority of the oocysts from a pool (assuming a 6 hour turnover and good capture).
>
> Once the pool is contaminated, the oocyst resistance to halogens and the difficulty of removing the cysts by filtration can result in pools which are contaminated for lengthy periods of time. Pool operators can reduce the risk of initial contamination by using common sense operating practices.
>
> The following recommendations for training and operation are suggested ....:
>
> **Pool Disinfection After Fecal Accidents, and When There is Suspected Contamination**
>
> Our best current recommendation for handling fecal accidents is to treat any accident involving unformed stool as a possible *Cryptosporidium* contamination and disinfect

accordingly.

The following steps need to be taken if a pool is either suspected of or is known to be contaminated with *Cryptosporidium*:

1. Close the pool and notify the local public health authorities.
2. Add chlorine to raise the disinfectant residual to 50 ppm (mg/L). Stabilize the pH to 7.2 to 7.8 so the chlorine is effective. (Remember high levels of chlorine can cause a purple interference color when using phenol red to test for pH. If this happens, neutralize the sample with a small amount of sodium thiosulfate.) Run the recirculation equipment for 12 hours with the high level of chlorine.
3. Clean and brush down the walls of the pool, the skimmers housings, and skimmer baskets.
4. Backwash the filter thoroughly. If this is a whirlpool, drain the pool at this time.
5. Disinfect the filter.

Sand – add a gallon of chlorine bleach (sodium hypochlorite) directly into the filter and let stand for 4–6 hours (more may be needed with filters over 36" diameter). Backwash again.

Cartridge – remove the cartridge and clean the filter casing thoroughly with a 200 ppm (mg/L) solution of chlorine bleach (sodium hypochlorite). Allow to stand for several hours. Clean the cartridge thoroughly and soak in a 200 ppm (mg/L) solution of bleach. Rinse and allow to dry completely.

DE Filters – Clean the D.E. off the filters, dispose of the D.E., and soak the tank and septums in a 100 ppm (mg/L) solution of bleach.

6. Restart the recirculation system and neutralize the chlorine slowly back to normal or fill, if a whirlpool.
7. Balance the water and reopen.
8. Monitor the disinfectant levels carefully.

Additional assistance can be obtained by calling your local health department. For more specific information on this procedure, please call the Environmental Sanitation Unit at (608) 266–2835."

It is clear that in the event of an Accidental Fecal Release (= AFR – Gerba, 1996) there will be a great deal of lost time of use for the pool so anointed. That time and aggravation should be balanced with the costs of installing a properly designed corona discharge ozonation system — one that is capable of avoiding these problems as well as providing additional water quality benefits as well without lost down–time.

It should also be clear at this point that close attention to good pool housekeeping principles may be the lowest cost procedure to follow. Those classes of people who may be given to providing AFRs (e.g., young, diapered children, incontinent elderly, etc.), as well as animals (birds, ducks, geese, dogs, etc.), should be kept from contact with pools. Open outdoor pools should consider installation of covers when pools are not in use. In particular, those individuals with diarrhea (which might indicate that they are crypto–infected already) should be educated to avoid entering pools in the first place.

Hot whirlpools and spas use volumes of water which may be too small for economic consideration of CD–generated ozone in quantities and for reaction times sufficient to guarantee inactivation of *C. parvum*. In such instances, it will be less costly simply to dump the fecal–contaminated water suspected of containing oocysts, disinfect the lines and equipment, and refill the whirlpool or spa.

Positions of the Centers for Disease Control (CDC)

In a document released on June 16, 1995 (CDC, 1995), the CDC discusses cryptosporidiosis in immuno–compromised individuals and recommends specific measures for such persons to handle their drinking water to ensure the absence of viable *Cryptosporidium* oocysts. This report confirms that boiling water before use or filtration through 1 μm absolute filters (including reverse osmosis units) are the two surest ways of killing (distillation) or removing (filtration) *Cryptosporidium* oocysts. The CDC stands silent on the impacts of ozonation, because of insufficient studies available (in their opinion) to quantify the amounts of oocyst inactivation related to ozone residuals and contact times.

Summary and Conclusions

1. *Cryptosporidium* is a "new" microorganism in the sense that it has only recently been identified as a cause of waterborne disease, e.g., cryptosporidiosis. Immuno–compromised individuals have died as a result of contracting this disease (100 people in Milwaukee).
2. Most of the reported instances of cryptosporidiosis

contracted in swimming pools stem from fecal accidents in the pools, primarily in juvenile pools. Therefore, any fecal accident in a pool or spa (particularly a watery fecal accident) must be viewed as a potential *Cryptosporidium* event.

3. The *Cryptosporidium* oocyst is about 5 μm in diameter; however, it has the capability to elongate, becoming elliptical in shape, and can pass through filters which remove particles down to 1 μm. Only filters capable of removing particle sizes 1 μm and below can be counted upon to remove oocysts with certainty (CDC position). These types of filters are not used today in commercial swimming pools/spas. Thus oxidation/disinfection may be the sole approach capable of controlling *C. parvum* oocysts with certainty.
4. Disinfection should be designed to inactivate *Cryptosporidium* oocysts according to accepted CT principles, tailored along the lines of current U.S. EPA drinking water disinfection recommendations.
5. Chlorine as the sole disinfectant has little short term effect on *Cryptosporidium* oocysts (CT = > 2,500 min–mg/L at 25°C and pH 7.5 for 2–logs of inactivation and > 5,000 min–mg/L for 3–logs of inactivation.
6. There is no information available on the efficacy of free bromine against *Cryptosporidium* oocysts. However there is every reason to expect that it will be no more effective than free chlorine. On the other hand, the ratio of $HOBr/BrO^-$ over the pH range 7.2–7.8 (optimum for pools) is considerably higher than the ratio of $HOCl/ClO^-$ over the same pH range. That fact, plus the greater bactericidal propensities of hypobromite ion over hypochlorite ion, *might* mean that free bromine could show somewhat greater effectiveness than chlorine against *C. parvum*.
7. Ozone is quite capable of providing several logs of inactivation of *Cryptosporidium* oocysts in pool waters. However, a controlled residual of 0.2 to 0.4 mg/L is required to be held over 15 to 5 minutes, respectively at 22°C to assure 2–logs of inactivation. Because the rates of disinfection increase with increasing temperature, somewhat less stringent conditions will be required at 27–30°C, the temperatures of normal pool operation. Definitive studies are needed to pinpoint the specific ozone CT values to inactivate 2– and 3–logs of *Cryptosporidium* oocysts at temperatures of pools and spas and in real–world pool and spa waters.
8. If ozone is relied upon to control *C. parvum* oocysts, it will be necessary to monitor residual ozone during ozone reaction (contacting). At the present state of analytical technology, this cannot be accomplished by means of ORP (oxidation–reduction potential). Thus, specific ozone residual analyses must be conducted in order to know the levels of dissolved ozone actually present in the water being treated at any time with certainty. Only with accurate residual ozone analyses can the true CT value being attained at any instant of time be determined with confidence.
9. These CT considerations also mean that the ozonation system must be designed so as to treat ***all*** pool waters, and not just portions (e.g., no side–stream or slip–stream ozonation). This critical conclusion argues for installation of ozonation systems according to the precepts of the well–established German DIN Standard 19,643 involving ozone, but with either ozone dosage or ozone reaction time (or both) properly adjusted to assure the necessary number of logs of inactivation of *Cryptosporidium parvum* being attained continuously.
10. For those pools using ozone and bromide ion (with bromo–chloro–dimethylhydantoin as the source of bromide ion), attention must be paid to the chemistries involved. Current recommendations by suppliers of the ozone/BCDMH system are to install just sufficient CD–ozone to generate free bromine. This amount of ozone is insufficient to assure oxidation of organic pool contaminants and develop a measurable residual of ozone so as to provide a CT value within the ozone reaction chamber. With currently supplied ozone/BCDMH systems, the level of bromide ion in the water is allowed to increase considerably beyond the optimum concentration of 15 mg/L. The higher the concentration of bromide ion present, the faster is the rate of ozone oxidation of bromide ion, concomitantly increasing destruction of ozone added. The combination of small amounts of added CD–ozone and excessive bromide leads to less residual ozone being available to cope with microorganisms.
11. With pools using ozone and sodium bromide as the source of bromide ion, concentrations of ozone in water sufficient for inactivation of *C. parvum* can be attained, provided that sufficient CD–ozone is installed, the concentration of bromide ion is maintained at approximately 15 mg/L, and ***all*** of the water is treated with ozone.
12. Because ozone generated by UV radiation is present in very much lower gas phase concentrations than when generated by corona discharge, very little ozone will be present in waters treated by these devices. **Clearly, UV devices generating ozone cannot develop dissolved residual ozone levels sufficiently high, and for sufficient lengths of time to satisfy the currently accepted CT values necessary to ensure *Cryptosporidium* oocyst inactivation.**

13. For those pools and spas that do not use ozone generated by corona discharge and applied in amounts so as to attain the appropriate ozone CT values, the procedure currently recommended by several state public health departments to cope with fecal accidents suspected to contain *Cryptosporidium* oocysts involves:

    a. Closing the pool
    b. Raising chlorine residual to 50 mg/L and stabilizing pH at 7.2–7.8
    c. Recirculating 12 hours with this high chlorine level
    d. Cleaning and brushing pool walls and all equipment exposed to the contamination
    e. Backwashing the filter thoroughly
    f. Disinfecting the filter (sand filters – 4–6 hours with chlorine; cartridge filters – soak several hours in 200 mg/L chlorine bleach; DE filters – dispose of the DE and soak tank and septa in 100 mg/L chlorine bleach)

14. **Under no circumstances should reliance be placed on devices that generate ozone by ultraviolet radiation to inactivate *Cryptosporidium* oocysts.** Pools that currently use these devices should follow the recommended procedures listed above in the event of a fecal accident suspected of containing *Cryptosporidium* oocysts.
15. Even though ozone generated by corona discharge can be designed and operated to control *C. parvum* in pools, its use adds considerably to the overall pool water treatment costs, even though other water quality benefits are obtained at the same time while reducing the amounts of chemicals added and extending times to pool blowdown. Consequently, all pools should institute and enforce housekeeping programs designed to discourage entry into the pools of people who have diarrhea and/or who are prone to accidental fecal releases (AFRs).
16. Hot whirlpools presented with accidental fecal releases should simply discharge the contaminated water, clean and disinfect the whirlpool and associated equipment, and refill.

References

Barlow, P., 1993, "Treatment of Swimming Pools with Ozone and Bromine", in *Ozone in Water and Wastewater Treatment, Vol. 2, Proc. Eleventh Ozone World Congress, San Francisco, CA* (Stamford, CT: Intl. Ozone Assoc., Pan American Group), pp. S–12–1 to S–12–23.

Bell, A., R. Guasparini, D. Meeds, R. G. Mathias, and J. D. Farley, 1993, "A Swimming Pool–Associated Outbreak of Cryptosporidiosis in British Columbia", *Can. J. Public Health* 84:334–337.

Bongard, J., R. Savage, R. Darn, H. Bostrum, J. Kazmierciak, S. Keifer, H. Anderson, and J. P. Davis, 1994, "*Cryptosporidium* Infections Associated with Swimming Pools – Dane County, Wisconsin", *J. Am. Med. Assoc.* 272:914.

CDC, 1995 (U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, Atlanta, GA, USA), "Assessing the Public Health Threat Associated with Waterborne Cryptosporidiosis: Report of a Workshop", *Morbidity and Mortality Weekly Report,* 44(RR–6), June 16, 1995.

Current, W. L., 1987, "*Cryptosporidium*: Its Biology and Potential for Environmental Transmission", CRC Critical Reviews in Environmental Control, 17(1):21–51.

Current, W. L., and B. L. Blagburn, 1990, *Coccidiosis of Man and Domestic Animals,* (Boca Raton, FL: CRC Press, Inc.).

Dawson, D. J., M. Maddocks, J. Roberts, and J. S. Vidler, 1993, "Evaluation of Recovery of *Cryptosporidium parvum* oocysts Using Membrane Filtration", *Letters in Applied Microbiology* 17(6):276–279.

Fayer, R., and B. L. P. Ungar, 1986, "*Cryptosporidium* spp. and Cryptosporidiosis", *Microbiological Reviews,* 50(4):458–483.

Fayer, R., 1994, "Effect of High Temperature on Infectivity of *Cryptosporidium parvum* Oocysts in Water", *Applied and Environmental Microbiology* 60(8):2732–2735.

Finch, G. R., E. K. Black, L. Gyürék, and M. Belosevic, 1993, "Ozone Inactivation of *Cryptosporidium parvum* in Demand–Free Phosphate Buffer Determined by *in vitro* Excystation and Animal Infectivity", *Applied and Environmental Microbiology* 59(12):4203–4210.

Finch, G. R., E. K. Black, L. Gyürék, and M. Belosevic, 1994, *Ozone Disinfection of Giardia and Cryptosporidium,* (Denver, CO: Am. Water Works Assoc., Research Foundation).

Finch, G. R., E. K. Black, and L. Gyürék, 1995, "Ozone and Chlorine Inactivation of *Cryptosporidium*", in *Proc. Water Quality Technology Conf., Nov. 6–10, 1994, San Francisco, CA* (Denver, CO: Am. Water Works Assoc.).

Galbraith, N. S., 1989, "Cryptosporidiosis: Another Source", *British Medical Journal* 298:276–277.

Gallaher, M. M., J. L. Herndon, L. J. Nims, C. R. Sterling, D. J. Grabowski, and H. F. Hull, 1989, "Cryptosporidiosis and Surface Water", *Am. J. Public Health,* 79(1):39–42.

Gerba, C. P., and P. Gerba, 1995. "Outbreaks Caused by *Giardia* and *Cryptosporidium* Associated with Swimming Pools", *J. Swimming Pool and Spa Industry* 1(3):9–18.

Gerba, C. P., 1996, during presentation entitled, "Virus–Associated Outbreaks in Swimming Pools". NSPI Annual Meeting, Phoenix, AZ, Nov. 20.

Grimason, A. M., H. V. Smith, P. G. Smith, M. E. Jackson, and R. W. A. Girdwood, 1990, "Waterborne Cryptosporidiosis and Environmental Health", *Environmental Health,* 98(9):228–233.

Hayes, E. B., T. D. Matte, T. R. O'Brien, T. W. McKinley, G. S. Logsdon, J. B. Rose, B. L. P. Ungar, D. M. Word, P. F. Pinsky, M. L. Cummings, M. A. Wilson, E. G. Long, E. S. Hurwitz, and D. D. Juranek, 1989, "Large Community Outbreak of Cryptosporidiosis due to Contamination of a Filtered Public Water Supply", *New England J. Medicine,* 320(21):1372–1376.

Hom, L. W., 1972, "Kinetics of Chlorine Disinfection in an Ecosystem". *J. Sanitary Engineering Div., Proc. Am. Soc. Civil Engineering,* 98(SA1):183–193.

Joce, R. E., J. Bruce, D. Kiely, N. D. Noah, W. B. Dempster, R. Stalker, P. Gumsley, P. H. Chapman, P. Norman, J. Watkins, H. V. Smith, T. J. Price, and D. Watts, 1991, "An Outbreak of Cryptosporidiosis Associated with a Swimming Pool", *Epidemiology and Infectious Disease* 107:496–508.

Korich, D. G., J. R. Mead, M. S. Madore, N. A. Sinclair, and C. R. Sterling, 1990, "Effects of Ozone, Chlorine Dioxide, Chlorine, and Monochloramine on *Cryptosporidium parvum* Oocyst Viability", *Applied and Environmental Microbiology,* 56(5):1423–1428.

Langlais, B., D. Perrine, J. C. Joret, and J. P. Chenu, 1990, "The C.T. Value Concept for Evaluation of Disinfection Process Efficiency; Particular Case of Ozonation for Inactivation of Some Protozoans: Free Living Amoeba and *Cryptosporidium*," in *New Developments: Ozone in Water and Wastewater Treatment; Proc. Intl. Ozone Assoc. Spring Conference, March 27–29, 1990, Shreveport, LA,* (Stamford, CT: Intl. Ozone Assoc., Pan American Group.

MacKenzie, W. R., N. J. Hoxie, M. E. Proctor, M. S. Gradus, K. A. Blair, D. E. Peterson, J. J. Kazmierczak, D. G. Addiss, K. R. Fox, J. B. Rose, and J. P. Davis, "A Massive Outbreak in Milwaukee of *Cryptosporidium* Infection Transmitted Through the Public Water Supply", *New England J. of Medicine* 331:161–167.

Malcolm Pirnie Inc. and HDR Engineering Inc., 1991, *Guidance Manual for Compliance With the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources,* (Denver, CO: Am. Water Works Assoc.).

McAnulty, J. M., D. W. Fleming, and A. H. Gonzalez, 1994, "A Community–Wide Outbreak of *Cryptosporidium* Associated With a Wave Pool", *J. Am. Medical Assoc.* 272:1597–1600.

Parker, J. F. W., G. F. Greaves, and H. V. Smith, 1993, "The Effect of Ozone on the Viability of *Cryptosporidium parvum* Oocysts and a Comparison of Experimental Methods", *Water Science and Technology,* 27(3–4):93–96.

Peeters, J. E., E. A. Mazas, W. J. Masschelein, I. V. Martinez de Maturana, and E. Debacker, 1989, "Effect of Disinfection of Drinking Water With Ozone or Chlorine Dioxide on Survival of *Cryptosporidium parvum* Oocysts", *Applied and Environmental Microbiology,* 55(6):1519–1522.

Perrine, D., P. Georges, and B. Langlais, 1990, "Efficacité de l'Ozonation des Eaux sur l'Inactivation des Oocystes de *Cryptosporidium*" (Efficacity of ozonation of waters on the inactivation of *Cryptosporidium* Oocysts), *Bull. Academic and National Medicine* 174(6):845–850.

Ransome, M. E., T. N. Whitmore, and E. G. Carrington, 1993, "Effect of Disinfectants on the Viability of *Cryptosporidium parvum* Oocysts", *Water Supply,* 11:75–89.

Reemay Pool and Spa Filtration Media: A Guide to Pool and Spa Cartridge Filters (Manufacturer's Brochure) (1993), p. 4.

Rice, R. G., 1995, "Chemistries of Ozone for Municipal Pool and Spa Water Treatment — Facts and Fallacies", *J. Swimming Pool and Spa Industry* 1(1):25–44.

Richardson, A. J., R. A. Frankenberg, A. C. Buck, J. B. Selkon, J. S. Colbourne, J. W. Parsons, and R. T. Mayon–White, 1991, "An Outbreak of Waterborne Cryptosporidiosis in Swindon and Oxfordshire", *Epidemiology and Infections,* 107(3):485–495.

Rose, J. B., 1990, "Occurrence and Control of *Cryptosporidium* in Drinking Water", in *Drinking Water Microbiology,* G. A. McFeters, Editor, (New York, NY: Springer–Verlag).

Schuler, P. F. and M. M. Ghosh, 1990, "Diatomaceous Earth Filtration of Cysts and Other Particulates Using Chemical Additives", *J. Am. Water Works Assoc.* 82(12):67–75.

Sorvillo, F. J., K. Fujoka, B. Nahlen, P. Tormey, R. Kebabjian, and L. Mascola, 1992, "Swimming–Associated Cryptosporidiosis", *Am. J. Public Health* 82:742–744.

Stover, E. L., C. N. Haas, K. L. Rakness, and O. K. Scheible, 1986, *Design Manual – Municipal Wastewater Disinfection,* U.S. EPA Report # EPA/625/1–86–021 (Cincinnati, OH: U.S. EPA, Water

Engineering Research Laboratory, 1986), p. 100.

Sundermann, C. A., D. S. Lindsay, and B. L. Blagburn, 1987, "Evaluation of Disinfectants for Ability to Kill Avian *Cryptosporidium* Oocysts", *Companion Animal Practice,* 2(11):36–39.

Tamminen, T., *The Professional Pool Maintenance Manual,* TAB Books (1995), p. 126.

U.S. EPA, 1989, "Drinking Water; National Primary Drinking Water Regulations; Filtration, Disinfection; Turbidity, *Giardia lamblia,* Viruses, *Legionella,* and Heterotrophic Bacteria; Final Rule", Federal Register, 54(124):27486–27541.

Wickramanayake, G. B., and O. J. Sproul, 1988, "Ozone Concentration and Temperature Effects on Disinfection Kinetics", *Ozone: Science and Engineering,* 10(2):123–135.

WISCONSIN, State of, Undated, "*Cryptosporidium* Fact Sheet for Swimming Pool Operators", State of Wisconsin, Dept. Of Health and Social Services, Division of Health, 1 West Wilson Street, P.O. Box 309, Madison, WI 53701–0309.