Inactivation of *Cryptosporidium* oocysts and *Giardia* cysts by ultraviolet light in the presence of natural particulate matter

Kingsford Amoah, Stephen Craik, Daniel W. Smith and Miodrag Belosevic

ABSTRACT

Recent studies have shown that ultraviolet (UV) radiation reduces the infectivity of *Cryptosporidium parvum* oocysts and *Giardia* spp. cysts. The objective of this work was to further examine UV inactivation of *C. parvum* oocysts and *G. muris* cysts in the presence of suspended particulate matter. Naturally occurring particulate matter consisting of a heterogeneous mixture of biological and non-biological particles, mainly in the 5–25 μm size range, was concentrated from a lake. Different aliquots of this material were mixed with purified oocyst and cyst preparations in aqueous suspensions, and the suspensions were exposed to UV fluences of 5 and 40 mJ/cm^2 from a medium-pressure mercury arc lamp using a collimated beam apparatus. Parasite inactivation was determined using mouse infectivity assays. Addition of particulate matter was correlated with statistically significant reductions in *C. parvum* and *G. muris* inactivation of 0.8 log$_{10}$ and 0.4 log$_{10}$, respectively, even after the fluence was adjusted for increased absorbance due to the presence of the particles. The corresponding increases in turbidity were 0.3–20 NTU for *C. parvum* and 7.5–20 NTU for *G. muris*. The magnitude of the effect was a function of the final suspension turbidity but was independent of fluence. In this study, the effect of particles on parasite inactivation at turbidity values of less than 10 NTU was small and difficult to measure, and would likely be unimportant in practice. Although the mechanism for this reduction was not determined, the results suggest that the particulate matter present in natural surface waters may interact with oocysts and cysts and thereby result in a reduction in UV inactivation over and above that attributable to simple absorbance. The effect of turbidity on inactivation measured in this work may not necessarily apply generally since the characteristics of the particles that compose turbidity may differ considerably between water sources.

Key words | collimated beam, *Cryptosporidium*, disinfection, *Giardia*, particles, particulates, turbidity, ultraviolet

Kingsford Amoah
City of Lethbridge,
City Hall, 910 4th Avenue South,
Lethbridge,
Alberta T1J 0P6,
Canada

Stephen Craik (corresponding author)
Daniel W. Smith
Department of Civil and Environmental
    Engineering,
3-133 Markin/CNRL Natural Resources Engineering
    Facility,
University of Alberta,
Edmonton,
Alberta T6G 2W2,
Canada
Tel: +1 780 492 8293
Fax: +1 780 492 8289
E-mail: *scraik@civil.ualberta.ca*

Miodrag Belosevic
Department of Biological Sciences,
CW 330 Biological Sciences Bldg,
University of Alberta,
Edmonton,
Alberta T6G 2E9,
Canada

INTRODUCTION

Cryptosporidium parvum and *Giardia lamblia* are parasites of humans that have frequently been identified as the cause of waterborne diseases. A number of recent studies have reported that *Cryptosporidium parvum* oocysts (Bukhari *et al.* 1999; Clancy *et al.* 2000; Craik *et al.* 2001; Shin *et al.* 2001), *Giardia lamblia* cysts (Campbell & Wallis 2002; Linden *et al.* 2002; Mofidi *et al.* 2002) and *Giardia muris* cysts (Craik *et al.* 2000;

Mofidi *et al.* 2002; Hayes *et al.* 2003) are inactivated by relatively low exposures to ultraviolet (UV) radiation. In contrast to chemical disinfectants such as ozone and chlorine, UV radiation of drinking water appears to produce few byproducts of health concern (Malley *et al.* 1995). As a consequence, there is increasing interest in the application of UV radiation for providing additional public health protection against these parasites in drinking water treatment.

The effectiveness of UV for disinfection may be influenced by the presence of particulate matter. Application of UV for drinking water disinfection, therefore, has traditionally been restricted mainly to water matrices in which the concentration of suspended particulate matter is low (i.e. < 1 NTU turbidity) such as groundwater or surface water pre-treated by filtration. Given the reported success of UV for inactivation of encysted protozoan parasites, UV disinfection is under consideration for treating non-traditional waters where the quality of the water to be treated is marginal and the concentration of suspended particulate matter is higher (e.g. poorly filtered or unfiltered surface water, municipal and agricultural industry wastewater). The nature of particle effects on microorganism inactivation by UV, however, is not entirely clear. For example, it was shown that some naturally occurring coliform bacteria contained within floc material present in secondary treated municipal wastewater are effectively shielded from UV exposure (Qualls *et al.* 1983; Loge *et al.* 1999; Emerick *et al.* 2000). On the other hand, others have reported that inactivation of MS2 coliphage spiked into different filtered drinking water was independent of turbidity, provided that the average UV fluence in the exposed suspensions was corrected for changes in absorbance (Batch *et al.* 2004). Similarly, UV inactivation of spiked MS2 coliphage was not significantly affected by the presence of artificially added clay particles (up to 12 NTU as turbidity) or algae (up to 42,000 cells/mL), again provided that the absorbance of the water was taken into account in the determination of fluence (Passantino *et al.* 2004). The effect of suspended solids on the UV inactivation characteristics of *Cryptosporidium parvum* and *Giardia* spp. parasites has received only limited attention in the literature. In most reported UV exposure studies, purified parasite preparations were suspended in either laboratory deionized water or filtered drinking water that contained little particulate matter. One study, however, reported successful UV inactivation of *C. parvum* oocysts added to filter backwash water from a drinking water treatment plant (Clancy *et al.* 2000).

The objective of this study was to measure the UV inactivation of *C. parvum* oocysts and *G. muris* cysts in the presence of naturally occurring particulate matter using a standard collimated beam exposure protocol. *G. muris*, a mouse-infecting species of the *Giardia* genus, has frequently

been used as a surrogate for the human pathogen *G. lamblia* in disinfection studies and has been previously shown to have UV resistance similar to that of *G. lamblia* (Mofidi *et al.* 2002).

MATERIALS AND METHODS

Cryptosporidium parvum methods

The methods for production, purification, concentration and viability determination by infectivity in neonatal CD-1 mice of *C. parvum* oocysts are described elsewhere (Gyürék *et al.* 1999; Neumann *et al.* 2000; Craik *et al.* 2001). Briefly, *C. parvum* oocysts originally from the Iowa strain were isolated from the feces of experimentally infected Holstein calves, purified by cesium chloride gradient centrifugation and stored at 4°C in antibiotics until used. After UV exposure experiments, oocysts were concentrated by centrifugation, counted by hemacytometer and administered to four-day-old CD-1 mice by gastric intubation. The proportion of mice positive for infection was determined 7 days following inoculation by examination of intestinal homogenates for oocysts using flow cytometry (Neumann *et al.* 2000). Inactivation was then interpreted using a logistic dose–response model of the following form:

$$\text{logit} = \beta_0 + \beta_1 \log_{10} d \qquad (1)$$

where d was the number of infectious oocysts in the inoculum to each mouse. The response logit was given by ln $[P/(1-P)]$, where P was the proportion of mice in a cohort that scored positive for infection based on the presence of oocysts in the intestinal homogenates. Inactivation was interpreted as the quantity $-\log_{10} d/d_0$, where d_0 was the total number of oocysts in the inoculum to each mouse determined by microscopic count and d was calculated from Equation (1).

To establish the infectivity of the oocyst batch used in this study, three dose–response experiments were carried out at the beginning, the middle and at the end of the experimental period. In each experiment, four cohorts of 10 neonatal CD-1 mice were administered 50, 100, 200 and 400 non-UV-exposed oocysts per mouse. The results of the dose–response experiments are presented in Figure 1. Values of the dose–response parameters, β_0 and β_1 in Equation (1), determined by maximum likelihood
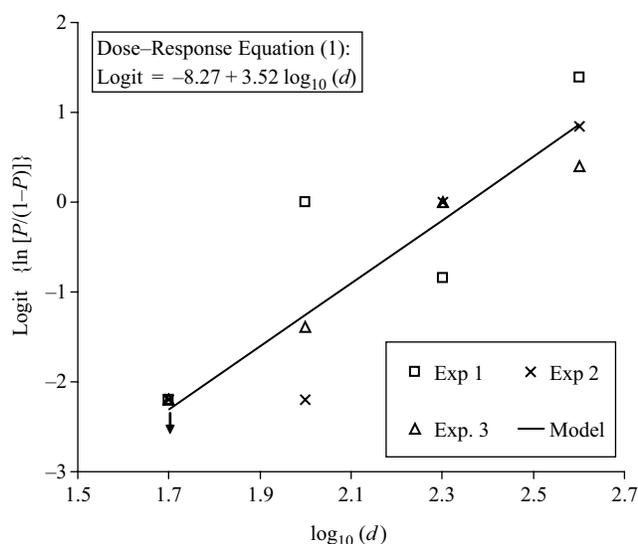
Figure 1 │ Results of three dose–response experiments for the *C. parvum* oocyst batch used in UV exposure experiments, and the computed dose–response model. Note: each datum represents infectivity determined in 10 mice. The arrow indicates that the result for one of the three experiments conducted at the dose of 50 oocysts per mouse was below detection (no mice were positive for infection).

regression (Gyürék *et al.* 1999), were -8.3 (-8.7, -7.9) and 3.5 (3.3, 3.7), respectively. The values in brackets are upper and lower 95% confidence limits. The computed ID_{50} (227 oocysts) was higher but comparable to that of oocysts used in a previous UV exposure study (53–113 oocysts) (Craik *et al.* 2001). Inactivation values reported in UV exposure experiments were calculated based on the infectivity results for those cohorts in which $0 < P < 1$. For trials in which $0 < P < 1$ for more than one cohort, an arithmetic average was reported. If none of the mice were infected (e.g. $P = 0$) in any of the cohorts, inactivation was computed assuming $P = 1$ in the largest inoculum cohort and the result was reported as greater than ($>$). Cohorts of sentinel mice were housed with the experimental mice but were not administered oocysts. These mice were consistently negative for infection.

Giardia muris methods

Methods for production, purification, concentration and viability determination of *G. muris* cysts in adult C3H/HeN mice were adapted from a previous study (Craik *et al.* 2000) with some modification. Briefly, *G. muris* cysts from a strain originally isolated by Roberts-Thomson *et al.* (1976) were

maintained in the laboratory by passage through CD-1 or C3H/HeN mice. Cysts for UV exposure experiments were isolated and purified from the feces of infected mice by flotation of emulsified feces on 1 M sucrose. In this study, cyst preparations were further purified using Percoll-sucrose density gradient centrifugation as described by Nieminski & Ongerth (1995). Purified cysts were stored at 4°C and were used in experiments within 72 hours of preparation. Cysts from experiments were concentrated by centrifugation, counted by hemacytometer and administered to cohorts of 5 adult male C3H/HeN mice using gastric intubation. The feces from each mouse were collected for 2 h each day following inoculation, were purified with 1 M sucrose flotation and were examined microscopically for the presence of cysts. The latent period (*LP*) of infection before the onset of cyst production in the feces was determined. The number of infectious cysts in the oral inoculum, d, is related to the latent period (*LP*) according to (Craik *et al.* 2000)

$$\log(d) = \alpha_0 + \alpha_1 \log(LP) \tag{2}$$

The infectivity characteristics of prepared cysts were determined in dose–response experiments in which 3 cohorts of five mice each were administered doses of 50, 5,000 and 50,000 freshly purified cysts per mouse. The dose–response characteristics of cysts purified using sucrose flotation followed by Percoll-sucrose gradient density gradient centrifugation and cysts that were purified using sucrose flotation are presented in Figure 2. The method of purification appeared to have little effect on cyst infectivity. Therefore, the results of the two dose–response experiments were combined and the values of the parameters $\alpha_0 = -11.5$ (-13.8, -9.1) and $\alpha_1 = 9.5$(8.15, 10.6) in Equation (2) were computed by least-squares regression. The values in brackets are the computed upper and lower 95% confidence limits. The cysts used in this study were slightly more infectious than those used in a previous study (Craik *et al.* 2000).

Experimental water suspensions

For all experiments, parasite preparations were suspended in samples of water collected from Lake Okanagan, BC, Canada, at the City of Kelowna. Samples were collected
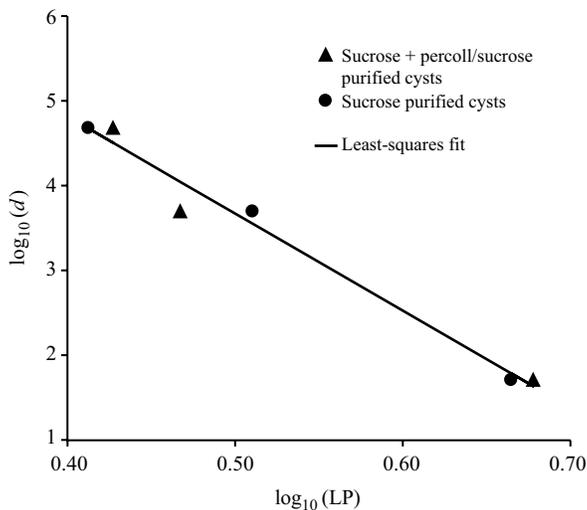
Figure 2 | Results of dose–response experiments in adult male C3H/HeN mice with *G. muris* cysts. (LP = latent period of infection in days as defined by Equation (2)).

Figure 3 | Particle size distribution of the suspended particulate matter concentrated from Lake Okanagan, BC (before dilution and parasite addition. The distribution does not include the contribution of the prepared parasites.).

from a 20 m depth during average summer time weather conditions and were not treated. The turbidity in the lake water samples was less than 0.5 NTU. To simulate higher particle concentration and turbidity, approximately 1000 L of the lake water was filtered through a 1 μm nominal pore size polypropylene yarn wound cartridge filter. The filter was cut lengthwise and the sections were hand washed in pH 7, 0.05 M phosphate buffered water to remove the captured particulate matter. The particulate matter was then concentrated by centrifugation (600 × *g* for 10 min at 4°C). The concentrated particulate matter was characterized by gravimetric analysis according to Standard Methods (Eaton *et al.* 1995) and by particle size distribution measurements using a Potable Water Sensor (L & H Environmental Inc., Roseburg, OR). The concentrated particulate matter was 67% volatile and was composed mainly of particles between 5–25 μm in size, although larger particles were present (Figure 3). Aliquots of this concentrated particulate matter were added to samples of the lake water to provide the desired suspension turbidity. Parasite preparations were then added to the lake water samples and the suspensions were stirred gently at laboratory temperature (approx. 22°C) for a minimum of 1 h prior to UV exposure experiments. The turbidity of each parasite suspension was measured using a calibrated 2001P Turbidimeter (Hach Co., Loveland, CO).
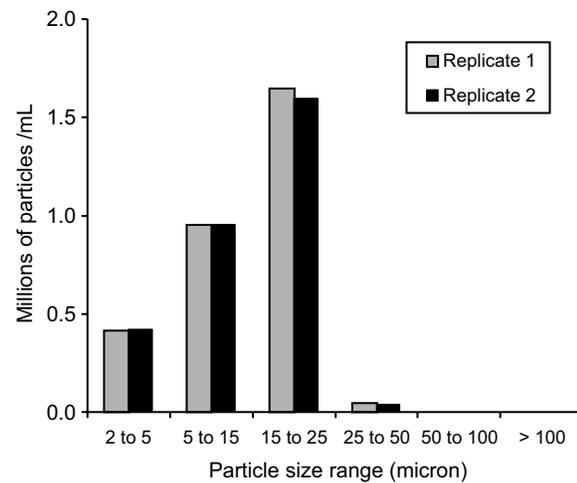
Collimated beam UV exposures

Procedures for conducting UV exposures and for determining UV fluence are described in detail elsewhere (Craik *et al.* 2001). Briefly, 20 mL aliquots of the experimental parasite suspensions were added to 150 mL open top glass beakers (50 mm inside diameter) and these were exposed to controlled UV fluence levels from a 1 kW medium-pressure UV lamp using a collimated beam apparatus (Calgon Carbon Co., Pittsburgh, PA). In all experiments, the depth of the suspensions was 0.85 mm and the suspensions were continuously mixed during the UV exposure period using a 10 mm long × 3 mm diameter magnetic stir bar (Fisher Scientific, Nepean, Ont.). Exposure time was controlled by means of a pneumatically operated shutter located beneath the lamp. The fluence rate at the surface and center of the suspensions was measured with a calibrated radiometer (IL1400 with a SED240 detector, International Light Instruments, Newburyport, MA). The center point fluence rate reading was corrected for radial variation, reflection at the air–water interface, spectral output of the lamp and spectral response of the sensor between 200–300 nm. The absorbance spectrum between 200–300 nm of the suspension (after addition of concentrated particulate and parasite preparations) was measured prior to each UV exposure using a HP3452A diode-array spectrophotometer (Hewlett Packard, Wilmington, DE) and a 10 mm quartz cell.

The samples were not filtered prior to this measurement. The depth-averaged fluence rate in the suspension was calculated using the Beer–Lambert law. The average germicidal fluence rate, E_g, was determined by weighting the fluence at each wavelength against the absorbance spectrum of DNA between 200–300 nm. Average germicidal fluence, H_g, was determined by multiplying the average germicidal irradiance by the exposure time, t ($H_g = E_g t$). The influence of reflectance off the inside walls of the beaker on the fluence rate at the liquid surface was investigated by potassium iodide–iodate actinometry and using a low-pressure mercury arc lamp in the collimated beam apparatus. There was little difference between the fluence rate generated measured using the beaker and the fluence rate measured using a 55 mm Petri dish. In preliminary experiments (data not shown), inactivation of *C. parvum* in the 150 mL beakers was found to be comparable to inactivation in the 55 mm ID Petri dishes used in previous studies (Craik *et al.* 2001) for the same calculated UV fluence. The use of a 150 mL beaker with well-mixed samples, therefore, had little impact on the experimental results.

For low turbidity experiments with *G. muris*, preparations containing between 2×10^6 and 4×10^6 enumerated cysts were prepared in 20 mL aliquots of untreated lake water and were exposed to UV radiation using a procedure similar to that described previously (Craik *et al.* 2000). Following UV exposure, the 20 mL suspension was centrifuged to concentrate the cysts for infectivity analysis. Addition of the purified cyst preparation to the 20 mL suspension resulted in an increase in turbidity of the lake water suspension from < 0.5 NTU to greater than 7 NTU. For the high turbidity experiments with *G. muris*, and for all experiments with *C. parvum*, the exposure procedure was modified to reduce the influence of parasite addition on the suspension turbidity as follows. Between 2×10^6 and 4×10^6 prepared cysts or 3×10^7 mL prepared oocysts were added to 1 L of a lake water–particle suspension. The suspension turbidity remained below 0.5 NTU after parasite addition. Fifty 20 mL aliquots of the suspension were then exposed to the specified UV fluence one after the other. Following UV exposure, the 20 mL aliquots were combined and the composite suspension was concentrated by centrifugation in preparation for infectivity analysis. Microscopic examination of the concentrated parasite suspensions revealed a heterogeneous mixture of biological and non-biological particles (Figure 4).

Experimental design and statistical analysis

The experimental turbidity targets were 7.5 and 20 NTU for *G. muris* and 0.25, 5, 10 and 20 NTU for *C. parvum*. The UV
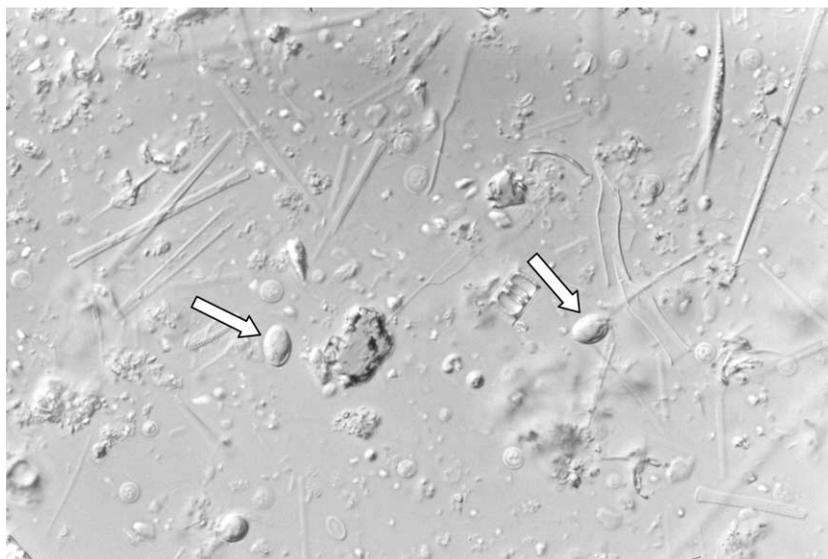

Figure 4 | Photograph of a 20 NTU suspension of prepared *Giardia muris* cysts and suspended particulate matter viewed on a hemacytometer slide under 400 × light microscopy after concentration by centrifugation. (The arrows indicate putative *Giardia muris* cysts.).

fluence targets were 5 and 40 mJ/cm^2. These were achieved by accounting for the measured UV absorbance of the suspension and adjusting the exposure time appropriately. Each UV fluence–turbidity combination was carried out in triplicate for each parasite. Experiments were randomized and replicate trials were carried out on different experimental days. For each parasite, a linear equation of the following form was regressed to the combined results from all UV exposure trials using least-squares criteria:

$$Inactivation = a_0 + a_1 \times (UV \ \ Fluence) + a_2 \times (Turbidity) \quad (3)$$
$$+ a_{12} \times (UV \ \ Fluence \times Turbidity)$$

Least-squares regression and analysis of variance (ANOVA) computations were carried out using the Analysis ToolPak in Microsoft™ Excel 2000.

RESULTS AND DISCUSSION

Inactivation in the positive controls

Inactivation measured in the non-UV-exposed positive control trials as a function of suspension turbidity is presented in Figure 5. The overall mean inactivation in the *C. parvum* controls (-0.1 log$_{10}$) was not statistically different from zero at the 95% confidence level ($p = 0.06$, two-sided *t*-test) and the observed variation (-0.8 log$_{10}$ to 0.5 log$_{10}$) is typical of the neonatal CD-1 mouse assay (Craik *et al.* 2001). Mean *G. muris* inactivation (0.6 log$_{10}$) in
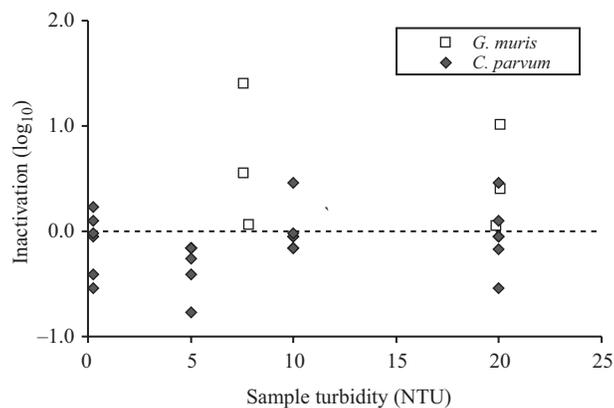

Figure 5 | Inactivation ($-\log_{10} d/d_0$) of *Giardia muris* cysts and *Cryptosporidium parvum* oocysts in non-UV-exposed positive control experiments as a function of suspension turbidity.

the controls, on the other hand, was statistically different from zero at the 95% confidence level ($p = 0.01$, two-sided *t*-test) and the variation (0.05–1.4 log$_{10}$) was somewhat larger than expected. Unlike the *C. parvum* experiments, in which a single oocyst batch was used, three different batches were used in the *G. muris* experiments. The highest inactivation levels (1.0 and 1.4 log$_{10}$) were associated with experimental cyst batch 2, which suggests that much of the variation observed in the *G. muris* controls may have been due to lower infectivity of this batch of cysts. Although the control results for batch 2 cysts were unusual, there was no statistically valid reason for rejecting the results associated with this batch. Most importantly, there was no statistical relationship between suspension turbidity and measured cyst or oocyst inactivation ($p = 0.73$ for *G. muris* and $p = 0.22$ for *C. parvum*). This finding implies that the presence of particulate matter in the samples by itself (without UV exposure) had no apparent effect on the measurement of parasite infectivity. The control results were not subtracted from the inactivation results in the UV exposure experiments.

UV exposure conditions

The UV exposure conditions for the *G. muris* and *C. parvum* experiments are summarized in Tables 1 and 2, respectively. Suspension turbidity was used as an indirect and relative measure of particulate matter concentration in the UV-exposed parasite suspensions. In the low turbidity experiments with *G. muris* the single-exposure protocol was used and added cyst concentration was 1×10^5 to 2×10^5 mL^{-1}. Most of the measured suspension turbidity in these trials (approx. 7.5 NTU) was due to the cysts themselves or to residual fecal debris present in the cyst preparations. In the high turbidity experiments with *G. muris*, the added cyst concentration was fifty times lower (2×10^3 to 4×10^3 mL^{-1}) and most of the measured turbidity (approx. 20 NTU) in these suspensions was due to the added natural particulate matter. Since the *G. muris* cyst preparations were rich in dissolved organic and colloidal material and absorbed strongly in the UV region, the characteristics and absorbing properties of the water used in the low and high turbidity experiments were different. As a result, the fluence rates were actually lower and the exposure times longer in the low

Table 1 | Results of UV exposure experiments with *Giardia muris* cysts

Trial	Cyst batch	Cyst conc. ($\times 10^{-4}$ mL^{-1})	Turbidity (NTU)	E_g (mW/cm^2)	t (s)	H_g (mJ/cm^2)	d_0	LP (d)	d	$-\log d/d_0$
1	1	10–20[a]	7.8	0.17	28.1	4.9	50,800	4.5	75	2.8
2	1	0.2–0.4[b]	19.3	0.22	22.0	4.9	51,300	4.6	73	2.8
3	1	10–20[a]	7.8	0.18	227	39.9	50,900	5.6	6	3.9
4	1	0.2–0.4[b]	20.0	0.23	177	40.0	51,300	6.0	3	4.2
5	2	10–20[a]	7.0	0.19	27.0	5.0	51,000	4.9	31	3.2
6	2	0.2–0.4[b]	21.3	0.21	24.0	5.0	50,300	4.4	115	2.6
7	2	10–20[a]	7.5	0.19	215	39.9	55,100	>7	<1[c]	>4.7
8	2	0.2–0.4[b]	20.7	0.21	191	39.8	50,100	5.6	7	3.9
9	3	10–20[a]	7.5	0.33	15.0	4.9	53,200	4.8	36	3.2
10	3	0.2–0.4[b]	20.5	0.41	12.0	4.9	50,200	4.1	214	2.4
11	3	10–20[a]	7.5	0.34	117	39.9	26,700	6.6	1	4.4
12	3	0.2–0.4[b]	20.2	0.43	93.0	39.9	51,300	6.0	3	4.3

[a]Trials conducted with higher cyst concentration had greater background absorbance.
[b]Trials conducted with lower cyst concentration had lower background absorbance.
[c]Less than the practical detection limit of the assay (1 cyst per mouse).

turbidity trials compared to the high turbidity trials (e.g. compare trials 1 and 2 in Table 1).

The added oocyst concentration was constant at 3×10^4 mL^{-1} for all of the *C. parvum* trials. Addition of oocyst preparations resulted in very little increase in either turbidity or UV absorbance of the suspension and the suspension turbidity was due mainly to the added particulate matter. Addition of particulate matter resulted in only a marginal increase in the UV absorbance of the suspensions and a small decrease in the average UV fluence rate. For example, the average fluence rate in the *C. parvum* experiments decreased by only 17% (from 0.36 mW/cm^2 to 0.30 mW/cm^2) when the turbidity was increased from an average of 0.28 NTU to an average of 20.1 NTU (Table 2). It should be noted that these measurements are based on conventional spectrophotometry and do not take into account the possibility that some fraction of the incident UV radiation was scattered by particles. These measurements

may tend to underestimate the true fluence rate when particles are present.

Parasite inactivation

Infectivity results corresponding to the UV exposure trials are presented in Tables 1 and 2 for *G. muris* and *C. parvum*, respectively. In eleven of the twelve *G. muris* experiments, cysts were eventually detected in the feces of all five mice in the cohort and a latent period was determined (Table 1). For trial 7, infections were detected in only 3 out of 5 mice after nine days post-inoculation and the latent period was poorly defined. For this trial, the latent period was assumed to be greater than 7 days and the inactivation result reported as >4.7 log$_{10}$. In six of the eleven remaining trials it was necessary to extrapolate the dose–response model (Equation (2)) to $d < 10$ cysts in order to quantify inactivation. Interestingly, inactivation in the UV

Table 2 | Results of UV exposure experiments with *Cryptosporidium parvum* oocysts

Trial	Turbidity (NTU)	E_g (mW/cm^2)	t (s)	H_g (mJ/cm^2)	d_0 ($\times 10^{-4}$) Cohort 1	Cohort 2	Cohort 3	P Cohort 1	Cohort 2	Cohort 3	$-\log d/d_0$
1	0.24	0.41	12.5	5.1	1	10	100	0/5	0/5	3/5	3.5
2	4.9	0.38	13.0	5.0	1	10	100	0/5	2/5	5/5	2.8
3	0.28	0.39	102.0	40.1	1	10	100	0/5	0/5	0/5	>4.0
4	5.1	0.37	108.0	40.2	1	10	100	0/5	0/5	2/5	3.8
5	9.9	0.36	14.0	5.0	1	10	100	0/5	2/5	5/5	2.8
6	19.6	0.33	15.0	5.0	1	10	100	0/5	3/5	5/5	2.5
7	9.6	0.35	115.0	40.1	1	10	100	0/5	0/5	5/5	3.5
8	20.4	0.32	125.0	40.0	1	10	100	0/5	3/5	5/5	2.5
9	0.20	0.36	14.0	5.1	0.5	5	50	0/5	0/5	0/5	>3.8
10	5.6	0.33	15.0	5.0	1	10	100	0/5	0/5	3/5	3.5
11	0.28	0.36	111.0	40.0	1	10	100	0/5	0/5	1/5	4.0
12	4.8	0.34	117.0	40.0	1	10	100	0/5	0/5	1/5	4.0
13	9.8	0.33	15.0	4.9	1	10	100	0/5	1/5	4/5	3.5
14	19.9	0.30	16.0	4.8	0.5	5	50	0/5	2/5	5/5	2.5
15	9.9	0.31	130.0	40.0	0.5	5	50	0/5	0/5	2/5	3.5
16	20.8	0.29	139.0	39.9	0.9	9	90	0/5	0/5	3/4	3.3
17	0.31	0.33	15.0	5.0	1	10	100	1/5	1/5	1/5	2.8
18	5.0	0.32	16.0	5.1	1	10	100	0/5	0/5	2/5	3.8
19	0.34	0.32	123.0	39.9	1	10	100	0/5	0/5	0/5	>4.0
20	4.8	0.31	131.0	40.1	1	10	100	0/5	0/5	2/5	3.8
21	9.9	0.30	17.0	5.1	1	10	100	0/5	1/4	5/5	3.0
22	19.9	0.28	18.0	5.0	1	10	100	0/5	1/5	5/5	3.1
23	9.9	0.29	137.0	39.9	0.5	5	50	0/5	0/5	1/5	3.8
24	19.8	0.27	146.0	39.9	0.7	0.7	0.7	0/5	0/5	4/5	3.6

Notes: oocyst concentration in all suspensions was 3×10^4 mL^{-1}.

experiments done with batch 2 cysts was not substantially different than in the experiments done with batch 1 and 2 cysts despite the fact that inactivation in the batch 2 controls ranged from 1.1 to 1.4 $\log_{10}$ (Figure 5). The batch 2 results, therefore, were included in further analysis. Complete elimination of infectivity (i.e. inactivation >4 $\log_{10}$) was observed in only three out of twenty-four exposure trials with *C. parvum* (Table 2).

The inactivation results suggest that, although UV exposure resulted in high levels (>3 $\log_{10}$) of inactivation for both parasites, inactivation was not complete. Low levels of infection were observed in the mice at all exposure conditions tested, suggesting that a small fraction (approx. $<0.1\%$) of the parasite population either escaped or survived UV exposure. This finding is consistent with the tailing effect in the UV fluence–inactivation curves of *G. muris* and *C. parvum* reported previously by the authors (Craik *et al.* 2000, 2001). Other research teams that have used collimated beam protocols to study UV inactivation of protozoan parasites and animal infectivity assays have reported more complete inactivation of *Giardia* spp. cysts (Linden *et al.* 2002; Hayes *et al.* 2003) or *C. parvum* oocysts (Clancy *et al.* 2000). Evidence of tailing, however, has been observed. Infections were reported in Mongolian gerbils inoculated with human-derived *G. lamblia* cysts exposed to a UV fluence of 40 mJ/cm² (Campbell & Wallis 2002). Another study reported possible evidence of tailing in the UV inactivation curves of *Giardia* spp. cysts at lower UV fluences (3.1 and 6.0 mJ/cm²). However, the authors noted that the tailing may have been due to the variability inherent in the animal infectivity assay used (Mofidi *et al.* 2002). Infections were also reported in neonatal CD-1 mice infected with *C. parvum* oocysts suspended in deionized water and exposed to UV fluence up to 33 mJ/cm² (Clancy *et al.* 2000). Explanations for tailing in UV inactivation curves include shielding of oocysts due to clumping and adherence to particles, insufficient mixing in the UV-exposed suspensions and inherent biological variation or the presence of a resistant sub-population (Campbell & Wallis 2002). Differences in the extent of tailing observed in the various studies may be due to differences in experimental procedures. These include, but are not necessarily limited to, procedures used to prepare parasite suspensions, the type of water matrixes used, the UV exposure protocols

and mixing conditions and the methods used to detect infection in the animals.

Interestingly, the average level of *G. muris* inactivation measured at 40 mJ/cm² in this study (4.2 $\log_{10}$, Table 1) was more than 1 $\log_{10}$ greater than the inactivation previously reported for similarly exposed *G. muris* cysts (Craik *et al.* 2000). In the earlier study, the cyst preparations were purified by sucrose flotation only and contained considerably more residual fecal matter than the cyst preparations used in the present study, which were additionally purified by Percoll-sucrose flotation. This finding suggests that UV inactivation of parasites may be influenced partly by the method of parasite purification and sheds some light on the tailing phenomenon. It also raises questions regarding the UV resistance of naturally occurring parasites as compared to the purified parasite suspensions that were used in this and other collimated beam UV exposure studies.

Inactivation of *G. muris* and *C. parvum* is plotted as a function of suspension turbidity for turbidity up to 20 NTU in Figures 6 and 7, respectively. UV treatment of an unfiltered drinking water supply would not normally be considered for a water supply with a turbidity as high as 20 NTU. An upper turbidity target of 20 NTU was used in this study as a conservative value and also to increase the probability of detecting a measurable turbidity effect. In addition, low turbidity surface water supplies, and groundwater supplies under the direct influence of surface water, may be vulnerable
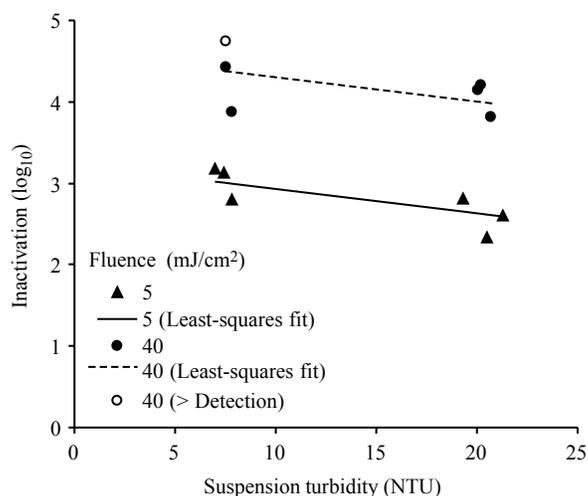

Figure 6 | Inactivation ($-\log_{10}d/d_0$) of *Giardia muris* ocysts in UV exposure experiments as a function of turbidity.
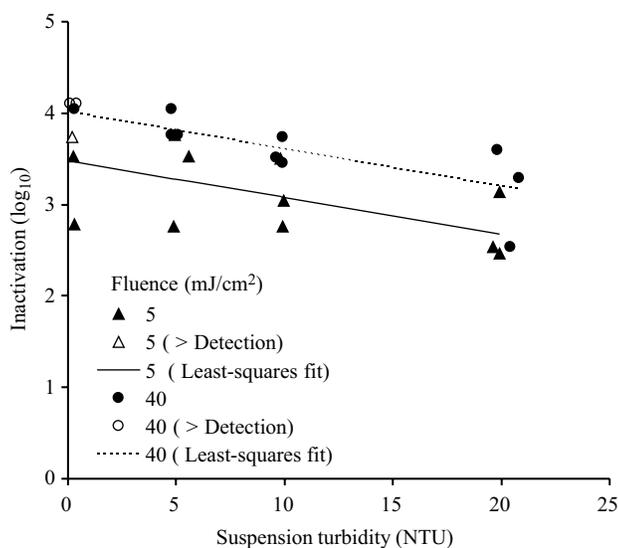
Figure 7 | Inactivation ($-\log_{10} d/d_0$) of *Cryptosporidium parvum* oocysts in UV exposure experiments as a function of suspension turbidity.

to occasional short-term turbidity spikes of several NTU. For these sources, it would be of interest to understand the capabilities of the UV system for parasite inactivation during these events. Both sets of plots suggest an inverse relationship between inactivation and particle concentration (measured as turbidity) at both experimental UV fluence levels. Interpretation of protozoan inactivation results, however, is often limited by the variation inherent in the infectivity assays. To address the experimental variability, UV exposure trials at different turbidities and UV doses were carried out in triplicate and in random order for both parasites (see Tables 1 and 2). The results of all UV exposure trials with each parasite were combined and the combined dataset was analyzed using multiple linear regression with least-squares criteria. By combining the results, the statistical sample size ($n = 24$ and $n = 12$ for *C. parvum* and *G. muris*, respectively) was increased and the standard error and statistical uncertainty were reduced. For the purpose of fitting Equation (3) to the combined data, the outcomes of those trials for which the infectivity result was beyond detection ($>$) were set at the detection limit values.

The calculated regression model coefficients and associated statistics are provided in Table 3 and the model fits are shown in Figures 6 and 7. The computed p values for the regression model (Equation (3)) coefficient in Table 3

indicate that UV fluence and turbidity were statistically significant factors at a 5% significance level when the entire data set was considered (i.e. up to 20 NTU). It was concluded that the linear relationships between turbidity and inactivation observed in Figures 6 and 7 were statistically significant. An increase in particle concentration that resulted in an increase in turbidity from 0.25 to 20 NTU was associated with a 0.8 $\log_{10}$ decrease in *C. parvum* inactivation. An increase in particle concentration that resulted in an increase in turbidity from 7.5 to 20 NTU was associated with a 0.4 $\log_{10}$ decrease in *G. muris* inactivation. For *C. parvum*, the model-predicted mean decreases in inactivation at 10 NTU and 5 NTU were 0.4 log and 0.2 log, respectively. When the trial results at 10 NTU or less were considered separately, the turbidity effect was marginally insignificant (p value = 0.06). When only those trials at 5 NTU or less were considered, the turbidity effect was clearly insignificant (p value = 0.6). This suggests that the experimental determined turbidity effect was detectable only if the entire data set (up to 20 NTU) was considered. This does not necessarily mean that there was no effect of turbidity on inactivation at turbidities less than 10 NTU, but rather that the magnitude of the effects was small compared to the variability in the data.

The relationship between UV fluence and inactivation was also statistically significant. An increase in the UV fluence from 5 to 40 mJ/cm^2 was associated with 0.5 and 1.4 $\log_{10}$ increases in inactivation of *G. muris* and *C. parvum*, respectively. This limited increase in inactivation, despite an eight-fold increase in average UV fluence, is consistent with the previously discussed tailing effect. The fluence–turbidity interaction term was not statistically significant, indicating that the particle concentration effect was the same at either fluence.

Interpretation of the particle effect

An increase in particle concentration resulted in a statistically significant decrease in inactivation for both parasites even though the fluence was maintained constant. One explanation for this finding is that the true average UV fluence rate in the suspensions was overestimated at the higher turbidity levels due to scattering of UV radiation by particles in the suspension. The use of conventional

Table 3 │ Results of multiple linear regression modeling

	Cryptosporidium parvum			*Giardia muris*
	0.1–5 NTU	0.1–10 NTU	0.1–20 NTU	7–20 NTU
Fluence rate coefficient, a_1	0.017 (0.02)	0.016 (0.002)	0.015 (0.0003)	0.04 (0.00001)
Turbidity coefficient, a_2	−0.02 (0.62)	−0.04 (0.06)	−0.04 (0.0009)	−0.03 (0.03)

Note: values in parentheses are *p* values for the test

transmission spectroscopy on unfiltered samples to estimate fluence rate using the Beer–Lambert law, however, tends to underestimate rather than overestimate the true average fluence rate in a particulate suspension (Linden & Darby 1998). This explanation, therefore, is unsatisfactory. An alternative hypothesis is that some fraction of the cysts and oocysts added to the particle suspensions became attached to particles of sufficient size and surface irregularity such that the attached parasites were effectively shielded from UV exposure. Little has been reported in the literature about the tendency of cysts or oocysts to attach to particulate matter present in surface water: however, *C. parvum* oocysts and *G. lamblia* cysts added to wastewater have been observed to attach physically to biological flocs (Medema *et al.* 1998). A scenario in which most (about 98–99.9%) of cysts and oocysts were freely suspended and fully exposed to UV, and as few as 0.1% were shielded from UV through particle attachment, is consistent with the trends observed in Figures 6 and 7. Since the added parasite concentrations in these experiments were 2×10^3 to 4×10^3 cysts/mL and 3×10^4 oocysts/mL for *G. muris* and *C. parvum*, respectively, the concentration of associated particle and shielded parasites would need to have been of the order of 2–4 cysts/mL and 30 oocysts/mL to explain the experimental observations. To provide effective shielding, a particle would probably need to be considerably larger than the attached parasite. Emerick *et al.* (2000) found that a minimum particle size of approximately 10 μm determined the ability of wastewater particles to shield coliform bacteria from UV exposure. Considering that *C. parvum* oocysts are about 3–5 μm in size and *G. muris* cysts are about 8–12 μm, particles would likely need to be greater than 25 μm to provide shielding.

The information provided in Figure 1 was used to estimate the concentration of particles in this size range present in the exposed suspensions. Figure 1 is the particle size distribution of the particulate material concentrated from the lake and not of the suspensions themselves. The concentration procedure tended to select for larger particles that were more readily captured and eluted from the 1 μm filter than were smaller particles. These experiments, therefore, were primarily examining the effect of the addition of larger (5–25 μm) particles on parasite inactivation. Smaller particles and colloids will reduce the efficiency of the UV inactivation process primarily by absorbing UV radiation and are not likely to provide effective shielding of the parasites through a particle attachment mechanism. Considering the 0.3% dilution factor of the suspended particulate concentrate, the concentration of particles in the size range >25 μm in the 20 NTU suspensions was approximately 130 mL^{-1} (excluding added cysts or oocysts). This is greater than the required number of shielded cysts and oocysts determined above. The postulation of shielding by attachment to particles is, therefore, plausible. The preceding analysis assumes one parasite attached per particle and does not consider the potential for the formation of multiple particle–oocyst aggregates. Evidence of parasite–particle attachment or aggregation was not observed directly in this study. During microscopic examination of the centrifuged suspensions, the enumerated parasites appeared freely dispersed, much like those depicted in Figure 3. Given the concentration of larger (>25 μm) particles in the suspension, and with a level of parasite shielding of as low as 0.1%, the phenomenon would likely have escaped detection by conventional microscopic examination. If low level parasite attachment

and shielding (i.e. 0.1%) did occur in the higher turbidity samples it is unlikely that this would have been detected in the control trials (Figure 5). The response in the non-UV-exposed controls would be dictated mainly by the high concentration of live mono-dispersed parasites present in the suspension. Following UV exposure, most of the mono-dispersed parasites would be inactivated and the response in the mice would then be dominated by parasites that were shielded from UV exposure.

One of the limitations of collimated beam UV exposure studies is that large numbers of prepared parasites must be added to relatively small volumes of suspension in order to measure high levels (>4 $\log_{10}$) of inactivation. Despite modifications to the UV exposure methods, the concentrations of parasites in the experimental suspensions were several orders of magnitude greater than what would be expected in even highly contaminated untreated surface water. As a result, only a small fraction ($<0.1\%$) of the particles would have had the opportunity to attach to particles of sufficient size (e.g. >25 μm) and to be shielded effectively from the incident UV radiation. In addition, the experimental oocysts were not actively embedded in the particles. Rather, they were contacted with the experimental water matrix with gentle stirring for one hour prior to UV exposure. Oocysts and cysts naturally present in surface water may have greater opportunity to interact with and to become embedded in particulate matter than artificially added parasites. Particulate matter, therefore, might have an even greater effect on UV inactivation of naturally occurring parasites in contaminated surface water. Further research is required to confirm this.

SUMMARY AND CONCLUSIONS

UV inactivation of *C. parvum* oocysts and *G. muris* cysts in the presence of suspended particulate matter concentrated from a lake was examined at average UV fluence levels of 5 and 40 mJ/cm^2 using collimated-beam exposure protocols. The addition of particulate matter was correlated with statistically significant reductions in *C. parvum* and *G. muris* inactivation of 0.8 $\log_{10}$ and 0.4 $\log_{10}$, even after the fluence was adjusted for increased absorbance due to the presence of the particles. The corresponding increases in turbidity

were 0.3–20 NTU for *C. parvum* and 7.5–20 NTU for *G. muris*. The magnitude of the effect was a function of particle concentration, measured as suspension turbidity, but was independent of fluence. In the suspensions studied in this research, the effect of turbidity on inactivation was small and difficult to measure when the turbidity was less than 10 NTU. This suggests that, at these turbidity levels, the effect of turbidity on inactivation would likely be unimportant in water treatment practice. The results of this study also suggested that the limited *G. muris* reported in an earlier study (Craik *et al.* 2000) might have been due in part to the purity of the parasite preparations.

A mechanism to explain the observed effect of particles on UV inactivation of these parasites was not determined. However, particle size and concentration measurements were consistent with a hypothesis based on the shielding of particle-attached parasites from UV exposure. The results of this study suggest that the presence of particulate matter in natural surface waters will have a negative impact on inactivation of oocysts and cysts in UV disinfection reactors even after absorbance has been accounted for in the determination of the average fluence rate. The potential effect of particulate matter should, therefore, be considered carefully if UV is to be used as the primary disinfection barrier against *C. parvum* and *G. lamblia* contamination in unfiltered surface water supplies. It should be cautioned that the results of this study should not be used to make general conclusions regarding the effectiveness of UV for inactivating waterborne parasites at specific water turbidity levels. The observed turbidity effect may not necessarily apply to all waters since the characteristics of the particles that compose the turbidity, and, therefore, how the particles interact with parasites, may be vary considerably between water sources.

ACKNOWLEDGEMENTS

Marcia Browne at the City of Kelowna Waterworks Department for helping to coordinate the experiments and for arranging shipment of samples, and Cezary Kucharski for his invaluable technical assistance in the parasitology aspects of the work.

NOMENCLATURE

a_1, a_2, a_{12}	least-squares regression model parameters
ANOVA	Analysis of Variance
α_0, α_1	parameters of the *Giardia muris* mouse dose–response
β_0, β_1	parameters for the *Cryptposporidium parvum* mouse dose–response
d	number of infectious parasites administered to each mouse
d_0	total number of parasites administered to each mouse
E_g	average germicidal UV fluence rate (mW/cm^2)
H_g	average germicidal UV fluence (mJ/cm^2)
ID_{50}	dose at which 50% of mice were positive for infection
LP	latent period prior to onset of *Giardia muris* infection in mice (d)
n	statistical sample size
NTU	nephelometric turbidity unit
p	probability of making a type I error in a statistical hypothesis test
P	proportion of mice scoring positive for infection in a cohort
t	UV exposure time (s)
logit	$\ln[P/(1-P)]$

REFERENCES

Batch, L. F., Schulz, C. R. & Linden, K. G. 2004 Evaluating water quality effects on UV disinfection of MS2 coliphage. *J. AWWA* **97**, 75–87.

Bukhari, Z., Hargy, T. M., Bolton, J. R., Dussert, B. & Clancy, J. L. 1999 Medium-pressure UV for oocyst inactivation. *J. AWWA* **91**, 86–94.

Campbell, A. T. & Wallis, P. 2002 The effect of UV irradiation on human-derived *Giardia lamblia* cysts. *Wat. Res.* **36**, 963–969.

Clancy, J. L., Bukhari, Z., Hargy, T. M., Bolton, J. R., Dussert, B. W. & Marshall, M. M. 2000 Using UV to inactivate *Cryptosporidium*. *J. AWWA* **92**, 97–104.

Craik, S. A., Finch, G. R., Bolton, J. R. & Belosevic, M. 2000 Inactivation of *Giardia muris* cysts using medium-pressure ultraviolet radiation in filtered drinking water. *Wat. Res.* **34**, 4325–4332.

Craik, S. A., Finch, G. R., Bolton, J. R. & Belosevic, M. 2001 Inactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. *Wat. Res.* **35**, 1387–1398.

Eaton, A. D., Clesceri, L. S. & Greenberg, A. E. (eds) 1995 *Standard Methods for the Examination of Water and Wastewater*, 19th edn. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC.

Emerick, R. W., Loge, F. J., Ginn, T. & Darby, J. L. 2000 Modeling the inactivation of particle-associated coliform bacteria. *Wat. Environ. Res.* **72**, 432–438.

Gyürék, L. L., Li, H., Belosevic, M. & Finch, G. R. 1999 Ozone inactivation kinetics of *Cryptosporidium parvum* in phosphate buffer. *J. Environ. Engng.* **125**, 913–924.

Hayes, S. L., Rice, E. W., Ware, M. W. & Schaefer, F. W., III 2003 Low pressure ultraviolet studies for inactivation of *Giardia muris* cysts. *J. Appl. Microbiol.* **94**, 54–59.

Linden, K. G. & Darby, J. L. 1998 Ultraviolet disinfection of marginal effluents: determining ultraviolet absorbance and subsequent estimation of ultraviolet intensity. *Wat. Environ. Res.* **70**, 214–223.

Linden, K. G., Shin, G. A., Faubert, G. M., Cairns, W. L. & Sobsey, M. D. 2002 UV disinfection of *Giardia lamblia* cysts in water. *Environ. Sci. Technol.* **36**, 2519–2522.

Loge, F. J., Emerick, R. W., Thompson, D. E., Nelson, D. C. & Darby, J. L. 1999 Factors influencing ultraviolet disinfection performance. Part I: Light penetration to wastewater particles. *Wat. Environ. Res.* **71**, 377–381.

Malley, J. P. J., Shaw, J. P. & Ropp, J. R. 1995 *Evaluation of By-Products Produced by Treatment of Groundwaters With Ultraviolet Radiation*. American Water Works Association Research Foundation, Denver, CO.

Medema, G. J., Schets, F. M., Teunis, P. F. M. & Havelaar, A. H. 1998 Sedimentation of free and attached *Cryptosporidium* oocysts and *Giardia* cysts in water. *Appl. Environ. Microbiol.* **64**, 4460–4466.

Mofidi, A. A., Meyer, E. A., Wallis, P. M., Chou, C. I., Meyer, B. P., Ramalingam, S. & Coffey, B. M. 2002 The effect of UV light on the inactivation of *Giardia lamblia* and *Giardia muris* cysts as determined by animal infectivity assay. *Wat. Res.* **36**, 2098–2108.

Neumann, N. F., Gyürék, L. L., Gammie, L., Finch, G. R. & Belosevic, M. 2000 Comparison of animal infectivity and nucleic acid staining for assessment of *Cryptosporidium parvum* viability in water. *Appl. Environ. Microbiol.* **66**, 406–412.

Nieminski, E. C. & Ongerth, J. E. 1995 Removing *Giardia* and *Cryptosporidium* by conventional treatment and direct filtration. *J. AWWA* **87**, 96–106.

Passantino, L., Malley, J., Knudson, M., Ward, R. & Kim, J. 2004 Effect of low turbidity and algae on UV disinfection performance. *J. AWWA* **96**, 128–137.

Qualls, R. G., Flynn, M. P. & Johnson, J. D. 1983 The role of suspended particles in ultraviolet disinfection. *J. Wat. Pollut. Control Feder.* **55**, 1280–1285.

Roberts-Thomson, I. C., Stevens, D. P., Mahmoud, A. A. F. &

Warren, K. S. 1976 Giardiasis in the mouse: an animal model. *Gastroenterology* **71**, 57–61.

Shin, G. A., Linden, K. G., Arrowood, M. J. & Sobsey, M. D. 2001 Low-pressure UV inactivation and DNA repair potential of *Cryptosporidium parvum* oocysts. *Appl. Environ. Microbiol.* **67**, 3029–3032.