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Disinfection Characteristics of Waterborne Pathogenic Protozoa *Giardia lamblia*

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Abstract: *Giardia lamblia* is a parasitic protozoa which is transmitted in the form of a cyst through untreated water and also treated drinking water. Since its presence in water has led to frequent outbreaks of giardiasis and death in many countries, the removal and disinfection of this protozoan cyst from the water supply are of great concern for public health. This study examined the disinfection characteristics of *G. lamblia* cysts isolated from a Korean patient with giardiasis. When using sodium hypochlorite including 5 or 10 ppm chlorine, the killing rate was initially rapid, however, the disinfection slowed down and a 3log reduction could not be achieved even after 2 h. The disinfection effectiveness was also reduced at a lower temperature, thereby implying that the risk of a giardiasis outbreak will be higher in the winter season. A CT (concentration · time) curve was constructed based on the results with sodium hypochlorite for use in designing and predicting disinfection performance. The organic chlorination disinfectant SDIC (sodium dichloroisocyanurate) produced a lower pH and a much higher residual effect than sodium hypochlorite. The disinfection of cysts by SDIC continued steadily throughout 2 h of contact, although the initial killing rate was lower than that with sodium hypochlorite.

Keywords: Giardia lamblia, disinfection, sodium hypochlorite, SDIC, protozoa

INTRODUCTION

Giardia lamblia is a worldwide parasitic protozoa which is transmitted to humans and animals in the form of a cyst, a dormant form, through untreated water and also treated drinking water [1]. The ingestion of *G. lamblia* cysts is followed by the excystation of the cysts into trophozoites and subsequent reproduction and colonization in the upper small intestine, where they cause diarrheal diseases and gastroeneteritis, known as giardiasis [2]. *G. lamblia* has been a contributing cause of death for some immuno-compromised people. Before being released into the environment from the host, trophozoites are transformed in the distal ileum or the large intestine into cysts (encystation), a form that is resistant against harsh environmental conditions [3]. These cysts are 6-8 μm ovals and covered with a well-defined filamentous wall which makes them impervious to inactivation by drinking water disinfectants [1,4].

Since their presence in water has led to frequent outbreaks of giardiasis in many countries, methodologies for monitoring and removing this protozoan cyst from the water supply are of great concern for public health. In the majority of water treatment facilities, *G. lamblia* cysts are mostly removed from the water through chemical coagulation/precipitation or porous-media filtration, and it is generally believed that the final safety level can be achieved by disinfection. However, many countries, including developed countries, have reported frequent outbreaks of giardiasis over the last decade even with treated water supplies, plus researchers have found out that certain viruses and the major protozoan species, such as *Giardia* and *Cryptosporidium*, are resistant to common disinfectants like chlorine [4,5].

Accordingly, this study examined and compared the survival characteristics of *G. lamblia* cysts, isolated from a Korean patient with giardiasis, based on chlorination with sodium hypochlorite and the organic disinfectant SDIC (sodium dichloroisocyanurate). The effects of the chlorine dosage, contact time, and temperature on the disinfection effectiveness was investigated and a CT curve was constructed to correlate the disinfection effectiveness relative to the chlorine concentration and contact time.

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MATERIALS AND METHODS

Cultivation of *G. lamblia* Trophozoites

The trophozoites and cysts of *G. lamblia* isolated from the feces of a Korean patient with chronic symptomatic giardiasis were obtained from EDiT, Inc., Korea. The trophozoite cells were routinely cultivated in a filter-sterilized Diamond TYI-S-33 medium [6] supplemented with 10% adult bovine serum and 0.5 mg/mL

of bovine bile at pH 7.1. When the attached cells formed a confluent monolayer, subcultures were made in 15 x 125 mm (working volume 14 mL) screw-capped borosilicate glass tubes at 37°C. These subcultures were then chilled in an ice bath to make the cells dislodge and 2% of the inoculum was transferred into a tube with fresh medium. A lag phase existed for the initial 50 h, followed by an exponential growth phase up to 90 h during which a cell density of over 10^7 cells/ mL was obtained [7]. The number of trophozoite cells was counted using a hemocytometer under a phase contrast microscope after iodine staining.

In vitro Encystation

After the trophozoites were grown in the confluent monolayer for 80 to 90 h in a normal TYI-S-33 medium containing 0.5 mg/mL of bovine bile at pH 7.1, they were transferred to an encystation medium containing 10 mg/mL of bovine bile with pH 7.8. This high bile condition for the induction of encystation was to mimic the physiological condition of the distal ileum and large intestine of a human host where the natural differentiation into cysts is initiated [8]. After being incubated in the high-bile encystation medium for 48 h at 37°C [7], the cells were then further incubated back in the normal TYI-S-33 medium for another 24 h. The cells were dislodged by chilling at 4°C, the medium removed, and then the undifferentiated trophozoites extracted by hypotonic lysis in distilled water. The remaining cysts were enumerated using a microscope after a vital staining with fluorescein diacetate and propidium iodide [9].

Disinfection

All disinfectant solutions and required glassware were prepared with chlorine-demand-free water to quantify the exact concentration of chlorine used or remaining. The chlorine-demand-free water was made by boiling distilled deionized water for 30 min, then irradiating with UV light for 24 h. The chlorine disinfectant solutions were prepared by diluting the stock solution of sodium hypochlorite (NaOCl) with chlorine-demand-free water of pH 6.5. The chlorine concentration was determined using the iodometric method for the % range and by the DPD (*N,N*-diethyl-*p*-phenylenediamine) colorimetric method for the ppm range [10].

The organic disinfectant SDIC (sodium dichlroisocyanurate) was the powdered product from Shikoku Chemical Co., Japan. When it was dissolved in water, the solution pH was within a range of 6.5 to 7. The effective chlorine content of SDIC was 62-65% (w/w).

The disinfection was carried out in 45-mL centrifuge tubes with 20 mL chlorine solution of a known concentration. The *G. lamblia* cysts in 1 mL were transferred and contacted for a given time period. The disinfection was terminated by adding 2.4 mL of 0.01 N sodium thiosulfate, which exhausted all the residual chlorine. The disinfection tube was then centrifuged at 2,000 rpm and 4°C to concentrate into a final volume of 1 mL. The surviving *G. lamblia* cysts were enumerated using a phase contrast microscope and hemacytometer after vital staining. The experiments were triplicated and the results averaged.

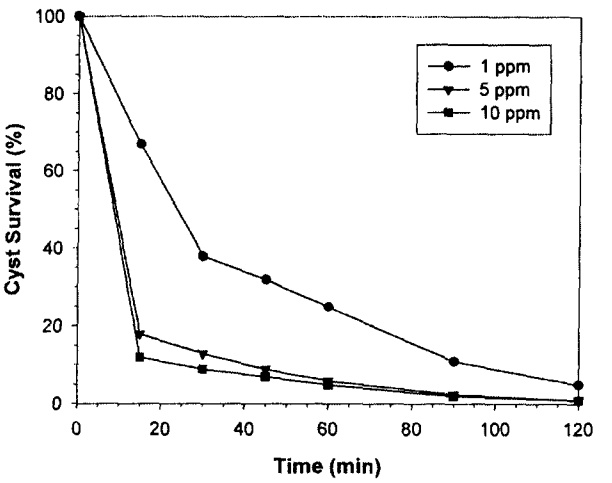

Fig. 1. Disinfection of *G. lamblia* cysts using sodium hypochlorite at 25°C. Sodium hypochlorite solution of 20 mL was spiked with 10^4 cysts.

RESULTS AND DISCUSSION

Effects of Chlorine Dosage and Temperature

For the disinfection of *G. lamblia* cysts with chlorine, 10^4 cysts were spiked into 20 mL of a sodium hypochlorite solution in a centrifuge tube. The chlorine contents of the sodium hypochlorite solutions were 1, 5, and 10 ppm as free chlorine. The spiked solution was mixed in a rotational mixing device. After a specific contact time, the disinfection was terminated by adding sodium thiosulfate and the mixture was centrifuged to concentrate the remaining cysts into 1 mL. The number of surviving *G. lamblia* cysts was enumerated using a phase contrast microscope and hemacytometer after vital staining.

Fig. 1 shows the result of disinfection performed at 25°C. A rapid killing was observed during the initial 15 to 30 min, then the killing rates slowed down. The dosage of 1 ppm exhibited a poor killing rate, as about 25% of the cysts survived even after 1 h of contact. Disinfection with 1 ppm chlorine was carried out as a reference. This dosage is not practical in reality because the typical chlorine dosage and residual chlorine levels employed in water treatment facilities are within a range of 2 to 10 ppm [11]. Disinfection with dosages of 5 and 10 ppm chlorine killed more than 80% of the cysts within 15 min, however, thereafter the number of remaining cysts only decreased very slightly. As such, cysts of a $\sim 10^0$ range still survived with 5 ppm and 10 ppm chlorine even after 2 h of contact, which means

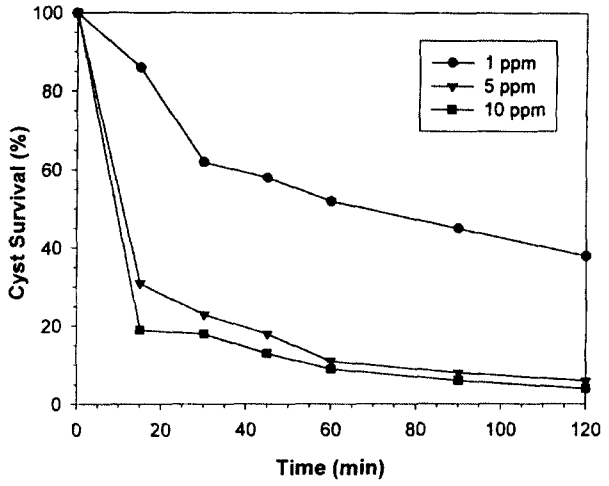

Fig. 2. Disinfection of *G. lamblia* cysts using sodium hypochlorite at 4°C. Sodium hypochlorite solution of 20 mL was spiked with 10^4 cysts.

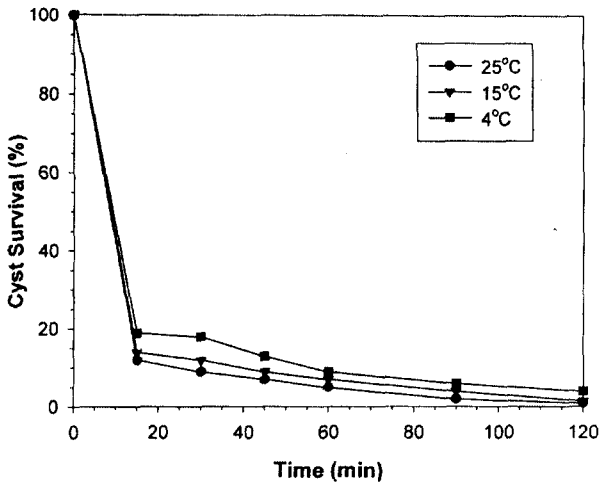

Fig. 3. Disinfection of *G. lamblia* cysts using 10 ppm sodium hypochlorite. Sodium hypochlorite solution of 20 mL was spiked with 10^4 cysts.

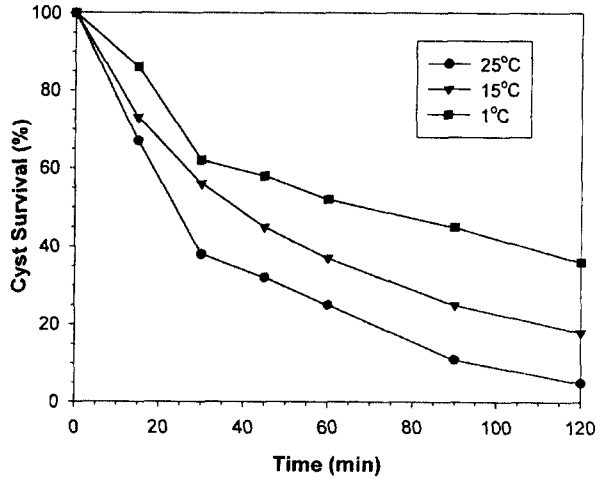

Fig. 4. Disinfection of *G. lamblia* cysts using 1 ppm sodium hypochlorite. Sodium hypochlorite solution of 20 mL was spiked with 10^4 cysts.

only a 2log reduction was achieved. The reason for such low disinfection effectiveness was maybe because the concentration of cysts employed in the disinfection tests (10^4 cysts in 20 mL) was much higher than that found in real water treatment systems. More than 3log killing is typically obtained within 20-30 min for bacteria when disinfection is carried out with 5 ppm of chlorine [11].

Fig. 2 shows the disinfection results performed at 4°C. The disinfection effectiveness was greatly reduced at a lower temperature when comparing Figs. 1 and 2. At 4°C, only 90-95% of the cysts were killed after 2 h with 10 ppm chlorine and only 60% with 1 ppm. The effect of temperature can be further visualized in Figs. 3 and 4. Fig. 3 shows the result of disinfection with 10 ppm chlorine carried out at three different temperatures, and Fig. 4 shows the result with 1 ppm. As reported previously [11], the disinfection effectiveness in killing *G. lamblia* cysts with chlorine decreased as the water temperature was lowered, and such a tendency was exacerbated with a low chlorine dosage. This observation implies that the risk of outbreaks of water-transmitted giardiasis will be higher in the winter than in other seasons, thus more careful attention should be paid to the disinfection process in water treatment facilities at this time of year.

CT Curves

A CT curve is a plot of the product of the residual disinfectant concentration (C) and contact time (T) versus a particular operating condition of disinfection, such as temperature, pH, etc. This type of plot is useful in designing the disinfection conditions to obtain a specific level of killing effectiveness [11,12]. The disinfection tests were performed with different chlorine dosages and temperatures to construct CT curves for the *G. lamblia* cysts used. Twenty mL water samples were spiked with 10^3 cysts in these experiments to obtain a higher log reduction. Fig. 5 shows the various CT curves for the chlorine disinfection of the *G. lamblia* cysts so as to envision the effect of temperature and the relationship between the disinfectant concentration and the contact time for the purpose of obtaining a specific killing effectiveness. The interpretation of this plot requires an assumption that a similar extent of killing effectiveness is obtained if the values of the CT product obtained from different disinfection tests are similar. This assumption may not true for the disinfection of microorganisms other than *G. lamblia*, plus a low C and high T are generally more effective than the reverse, for a given CT value [11].

According to Fig. 5, the required CT value for a spe-

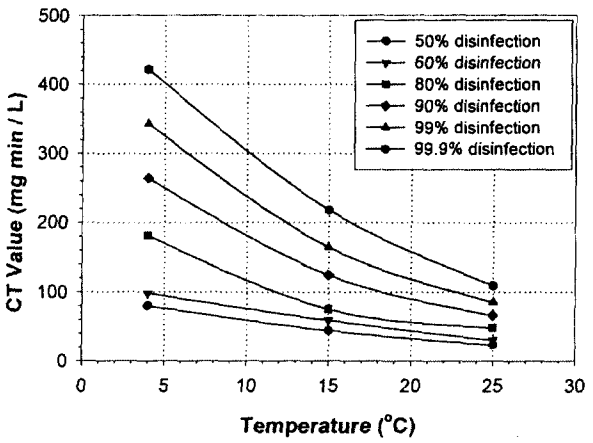

Fig. 5. CT curve for dininfection of *G. lamblia* cysts using sodium hypochlorite. Sodium hypochlorite solution of 20 mL was spiked with 10^3 cysts.

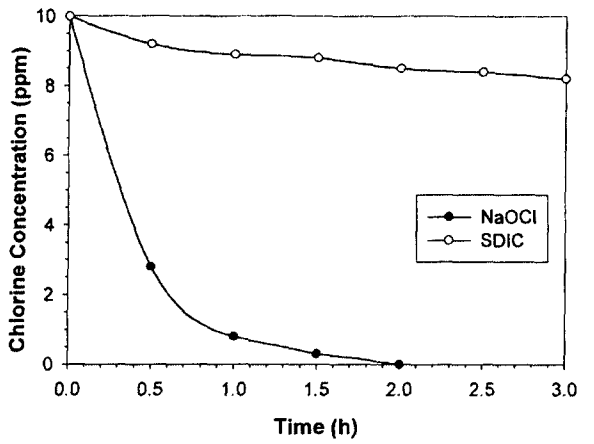

Fig. 6. Difference in residual effects of sodium hypochlorite and SDIC with sunlight and mild stirring.

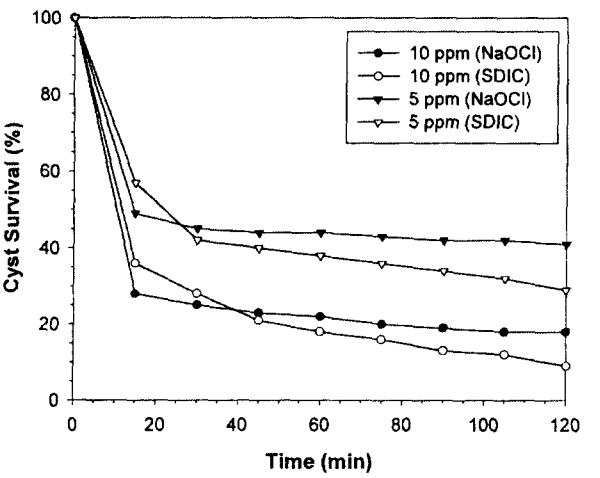

Fig. 7. Comparison of disinfection of *G. lamblia* cysts when using sodium hypochlorite and SDIC at 15°C. Sodium hypochlorite solution of 20 mL was spiked with 10^5 cysts.

cific killing effectiveness increased as the temperature was lowered. The CT values were approximately doubled when the temperature was lowered by 10°C. As an example, the CT values to obtain a 99% disinfection (2log reduction) were 80 at 25°C, 165 at 15°C, and 345 at 4°C. A 99.9% disinfection (3log reduction) required CT values of 110 at 25°C, 225 at 15°C, and 425 at 4°C. The CT value of 110 at 25°C represents that, to obtain a 99.9% killing, 22 min of contact time is required with 5 ppm chlorine or 11 min with 10 ppm of chlorine. Meanwhile at 4°C, 86 min is required with 5 ppm or 43 min with 10 ppm of chlorine to obtain a 99.9% killing.

Use of Organic Disinfectant SDIC

Although inorganic chlorination is the most popular chlorination practice currently used in water treatment due to its low cost and established track record, one of the major drawbacks of inorganic chlorine disinfectants is that their residual effect is not so high. Inorganic chlorine disinfectants, like chlorine gas, sodium hypochlorite, and calcium hypochlorite, all generate hypochlorous acid (HOCl) in water. Hypochlorous acid is the species that performs the actual oxidative disinfection. The disinfection strength is weakened at an alkaline pH because hypochlorous acid is a weak acid and can dissociate into hypochlorous ions and hydrogen ions in the following equilibrium relationship [12,13]:

$$HOCl \rightarrow H^+ + OCl^- \; ; \; pK_a=7.6 \text{ at room temperature.}$$

In addition, the disinfection activity in water is easily lost without any oxidative consumption because it is easily stripped out or evaporated by agitation, high temperature, or sunlight.

The organic disinfectant SDIC (sodium dichlroisocyanurate) has been recently considered as an alternative chlorination disinfectant to enhance disinfection effectiveness in water. SDIC has been used in the sterilization of food, feeding bottles, and some swimming pools [14,15]. When SDIC is dissolved in water, the solution pH is within a range of 6.5 to 7. This pH is relatively acidic compared to the pH of a sodium hypochlorite solution, thereby implying that the disinfection strength of SDIC is higher than that of sodium hypochlorite. Furthermore, SDIC exhibits a much higher residual effect than sodium hypochlorite.

The difference in the residual effects of sodium hypochlorite and SDIC was compared in Fig. 6. Each disinfectant solution of 100 mL with 10 ppm as free chlorine was exposed to sunlight and slow magnetic stirring in a 500-mL beaker. The chlorine content of the sodium hypochlorite solution decreased rapidly and no residual chlorine remained after 2 h. Meanwhile, the SDIC solution showed a slow decrease in the chlorine content and a residual effect of 70-80% remained even after 3 h. Fig. 7 shows a comparison of sodium hypochlorite and SDIC in the disinfection of *G. lamblia* cysts. Twenty

mL of water samples were spiked with 10^5 cysts and the disinfection was carried out at 15°C. The initial killing rate with sodium hypochlorite was higher than that with SDIC. However, the killing rate with sodium hypochlorite was decreased quickly after 15 min, and any further cyst removal was negligible. This was possibly because the residual effect of sodium hypochlorite was poor, as mentioned above, plus an increased number of cysts was spiked in the solution for the current tests. In contrast, with SDIC, the cyst survival was steadily decreased and the killing conti-nued even after 2 h of disin-fection due to the stable residual effect and lower pH.

CONCLUSION

The disinfection characteristics of pathogenic protozoa *Giardia lamblia* cysts isolated from a Korean patient with giardiasis were studied. When using sodium hypochlorite including 5 or 10 ppm chlorine, rapid disinfection was observed during the initial 15 min of contact, thereafter the disinfection rate slowed down. A 3log reduction could not be achieved even after 2 h of contact. The disinfection effectiveness was also reduced at a lower temperature, thereby implying that the risk of a giardiasis outbreak will be higher in the winter season, therefore, more careful attention should be paid to water treatment practices at this time of year. A CT curve was plotted based on the results of the disinfection tests with sodium hypochlorite for utilization in designing and predicting disinfection performance for the purpose of obtaining a specific level of disinfection effectiveness.

To improve the disinfectant effectiveness and stable residual effect, the organic chlorination disinfectant SDIC (sodium dichlorosiocyanurate) was compared to sodium hypochlorite. SDIC exhibited a lower pH when dissolved in water and a much higher residual effect. The killing of cysts by SDIC continued steadily throughout 2 h of contact, although the initial killing rate was lower than that with sodium hypochlorite.

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REFERENCES

[1] Despommier, D. D., R. W. Gwadz, and P. J. Hotez (1995) *Parasitic Diseases.* 3rd ed., pp. 144-150. Springer-Verlag, NY, USA.

[2] Kulda, J. and E. Nohynkova (1995) *Giardia* in humans and animals. In: J. P. Kreier (ed.). *Parasitic Protozoa.* Vol. 10, pp. 258-324. Academic Press, San Diego, USA.

[3] Lujan, H. D., M. R. Mowatt, and T. E. Nash (1997) Mechanisms of *Giardia lamblia* differentiation into cysts. *Microbiol. Mol. Biol. Rev.* 61: 294-304.

[4] Galal-Gorchev, H. (1996) Chlorine in water disinfection. *Pure Appl. Chem.* 68: 1731-1735.

[5] LeChevallier, M. W., W. D. Norton, and R. G. Lee (1991) *Giardia* and *Cryptosposidium* spp. in filtered drinking water supplies. *Appl. Environ. Microbiol.* 57: 2617-2621.

[6] Keister, D. B. (1983) Axenic cultivation of *Giardia lamblia* in TYI-S-33 medium supplemented with bile. *Trans. Royal. Soc. Trop. Med. Hyg.* 77: 487-488.

[7] Hong, W. S., K. J. Kim, and K. Lee (2000) Optimized conditions for *in vitro* high density encystation of *Giardia lamblia*. *J. Microbiol. Biotechnol.* 10: 529-531.

[8] Gillin, F. D., S. E. Boucher, S. S. Rossi, and D. S. Reiner (1989) *Giardia lamblia*: The roles of bile, lactic acid, and pH in the composition of the life cycle *in vitro*. *Exp. Parasitol.* 69: 164-174.

[9] Schupp, D. G. and S. L. Erlandsen (1987) A new method to determine *Giardia* cyst viability: Correlation of fluorescein diacetate and propidium iodide staining with animal infectivity. *Appl. Environ. Microbiol.* 53: 704-707.

[10] APHA-AWWA-WPCF (1995) *Standard Methods for the Examination of Water and Wastewater.* 19th ed., Section 4500.

[11] Office of Water (1999) *Alternative Disinfectants and Oxidants Guideline Manual.* Chapter 2. US EPA 815-R-99-014.

[12] AWWA (1999) *Water Quality and Treatment.* 5th ed., Chapter 14. McGraw-Hill, NY, USA.

[13] Stumm, W. and J. J. Morgan (1981) *Aquatic Chemistry.* 2nd ed., Chapter 7. John Wiley & Sons, NY, USA.

[14] Oie, S. and A. Kamiya (1995) Disinfection of feeding bottles by sodium hypochlorite or sodium dichloroisocyanurate. *Biomed. Lett.* 51: 57-62.

[15] Nicholl, P. and M. Prendergast (1998) Disinfection of shredded salad ingredients with sodium dichloroisocyanurate. *J. Food. Process. Preserv.* 22: 67-68.

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