An International Journal

Nature and Science

ISSN 1545-0740

Volume 5 - Number 4 (Cumulated No. 17), December 1, 2007

Marsland Press, Michigan, The United States

Nature and Science

ISSN: 1545-0740

Volume 5 - Number 4 (Cumulated No. 17), December 1, 2007

Contents

Marsland Press, P.O. Box 21126, Lansing, Michigan 48909, The United States
Edmondson Intercultural Enterprises, P.O. Box 1324, East Lansing, Michigan 48826, USA
(347) 789-4323

Disinfection effect of chlorine dioxide on air quality control in Armed Forces General Hospital of Taiwan

Kuen Song Lin[1], Ming June Hsieh[1], Ming Jer Liou[2], Sheau Long Lee[2], Cheng-Kuo Lai[3]

[1]Department of Chemical Engineering & Materials Science/Fuel Cell Center, Yuan Ze University, Chungli City, Taoyuan, Taiwan 320, R.O.C.
[2]Department of Chemistry, ROC Military Academy, Fengshan, Taiwan 830, R.O.C.
[3]Army NBC Protection Research Center, Taoyuan, Taiwan 320, R.O.C.
leesheaulong@gmail.com

Abstract: Under the increasing threat of various global infectious diseases, the importance of epidemic prevention and air quality control in hospital is accented. Four disinfectants were prepared and tested to verify the disinfection effect of air environment in Taoyuan Armed Forces General Hospital (TAFGH). STB bleach powder (1417 ppm), Type 82 disinfectant (4877 ppm), NaOCl bleacher (1386 ppm) and chlorine dioxide disinfectant (193 ppm) were all capable to sterilize medical disposal of 3.2×10^5 CFU/mL with disinfection efficiency higher than 99.9% were observed from the environmental specimen and disinfection tests in the physician out-patient department. Before sterilization, the average residual colony was 180 per handset, which were higher than the value of 15 on door knob. After spraying 1 mL of 200 ppm chloride dioxide solution twice onto the surfaces of different objects using the hand-held sprayer, the comparison for average disinfection efficiencies of the samples was door knob (100%) = handset of telephone (100%) > chair cushion (90.3%) > floor (20.5%) in series. In addition, the background data of biological aerosols also revealed that the comparison of average space colony numbers was semi-closed out-patient area in the physician department (318 CFU/m^3) > semi-closed out-patient area in the surgical department (183 CFU/m^3) > open-space emergency ward (58 CFU/m^3) in series. After using ultrasonic aerosol and handheld sprayer ways to sprinkle the chlorine dioxide solution into hospital spaces for 30 minutes, the average colony number in the physician out-patient area decreased from 421 to 21 CFU/m^3, approaching to a disinfection efficiency of 95.0 %. The disinfection efficiency of chlorine dioxide in gas or solution phase is notably affirmative and available for the infection control of hospital. [Nature and Science. 2007;5(4):94-99].

Keywords: disinfectant; chlorine dioxide; air quality control; hospital; biological aerosol

1. Introduction

Chlorine dioxide is a disinfectant recommended by the World Health Organization (WHO) of the United Nations (UN) and the Food and Agriculture organization (FAO). It is also a disinfectant listed on the Guideline for Laboratory Biosafety of the WHO[1]. The disinfection efficacy of chlorine dioxide is not affected by the pH and its advantage of not generating carcinogen (THMs)[2], make Chlorine dioxide a green disinfectant highly praised by the european countries and the United States. As early as in 1946, Ridenour et al. reported that Chlorine dioxide is plausible to deactivate and even kill the virus that cause poliomyelituss[3]. And in 1973, Smith et al. found that Chlorine dioxide has a higher disinfection rate than Chloride against various viruses, like Echo viruses Type 7, Coxsackie virus B3 and Sendai virus [4]. In 1980, Roberts et al. reported thst after exposure of chlorine and chlorine dioxide for 2 minutes, the survival rates of Polioviruses type 1 are 63.1 % and 6.3 %, respectively[5].

As early as in 1967, the EPA of the US has cataloged the solution of Chlorine dioxide as a disinfectant or sanitizer. In 1988, the US EPA has further classified the Chlorine dioxide gas as a class 3 sterilant[6]. Besides, according to Hoehn's report, there are more than 700 waterworks worldwide that use Chlorine dioxide to replace Chloride in drinking water disinfection[7]. The number of waterworks that use Chlorine dioxide has grown to over 2000 up to date because of the advantage that Chlorine dioxide does not induce the generation of THMs. According to the reports concerning the disinfection efficency of Chlorine dioxide published by Huang et al. in 1997 and the US EPA at 1999, it has been shown that Chlorine dioxide has a higher disinfection rate than ozone and Chloride. Chlorine dioxide has a very prominent disinfection effect against Coxsackievirus, Echo viruses, Polioviruses, Herpes simplex virus (HSV), Hepatitis B virus, Newcastle disease virus, Bacteriophage, Vaccinia virus, Poliomyelitis virus and Sendai virus et al. in water[8,9].

In a series of anthrax attacks following the 911 terrorism attack, Chlorine dioxide was chosen for

disinfection because of its excellent disinfection efficiency. The US EPA, for the first time, successfully used Chlorine dioxide gas of 500ppm to disinfect Bacillus anthrax in the building where Senator Hart's office located in the Capitol Hill in the US. In the following year, the US EPA used Chlorine dioxide steam again to successfully disinfect a locker in the Brentwood mail process and delivery center at Washington DC[6]. During the SARS outbreak, the national troops of ROC also used Chlorine dioxide solution, with concentration ranging from 500 ppm to 1000 ppm fro the surveillance of the contamination control on the passage between the disinfection area and the clear area in Taipei Veterans General Hospital and Taipei City Hospital He-Ping and Yang-Ming branche. 5ppm of chlorine dioxide was also added to the water system of the personnel decontamination station.

The present report was based on the environment of Taoyuan Armed Forces General Hospital. We report this disinfection efficiency of Chlorine dioxide under different environments. The results can be of great value for reference use in hospitals.

2. Material and Methods

Instruments and chemicals in using were listed as follows:

Microbe incubation (autoclave, laminar flow, temperature-control incubator, colony counter).

Iodine titration.

Record the values from Chlorine dioxide spectrometer (ODYSSEY DR/2500) and analyze by the built-in CRP and DPD methods.

Handheld sprayer (1 liter)

EP606 ultrasonic aerosol (with frequency of 17000 times per second)

XMX/2AL Aerosol Concentrator (Dycor, Canada)

EP606 Two part system chlorine dioxide (Gosh corporation, ROC Taiwan)

2.1 Experiment procedure

1. This study was focused on the sampling and disinfection of the wastewater, out-patient area and the waiting area in Taoyuan Armed Forces General Hospital. The main disinfectant used was the EP606 two part system chlorine dioxide purchased from Gosh (ROC Taiwan). For the quality analysis of effective chlorine and Chlorine dioxide, we referred to the iodometric titration analysis and the spectrophotometry in the "Standard methods for the examination of water and wastewater" published by the American Public Health Association (APHA)[10].

ClO_2 content=(V-V0)xCx0.01349xD/(W)x100%

where V and V0 are the volume of sodium thiosulfate used to titrate the sample solution and the control solution, respectively. And W is the amount of sample in gram, while C is the equivalent concentration of sodium thiosulfate and D is the dilution fold.

2. Medical wastewater treatment: We first collect the wastewater and then used four biochemical disinfectants, namely super tropic bleaching powder (STB ; calcium hypochlorite), ROC Army disinfectant Type 82 (sodium dichloroisocyanurate), home-use bleacher (sodium hypochlorite) and EP606 disinfectant (Chlorine dioxide) to test the disinfection efficiency, followed by using mixing dilution method (NIEA E204.51).

3. The door knob, handset of telephone, chair cushion and the floor: soak sterilized cotton with dilution solution or distilled water to rub the surface of the door knob, handset of telephone, chair cushion and the floor, respectively to collect the background value of the bacteria in the environment. The samples were labeled sequentially. The same areas were disinfected by 200 ppm Chlorine dioxide and the concentration of bacteria were examined again by the same procedures describe above.

4. The space of the out-patient area: By using Aerosol concentrator, the bacteria concentration in the air were collected before and after the disinfection by using EP 606 containing 200 ppm of Chlorine dioxide.

3. Results and Discussion

3.1 Disinfection of wastewater

A series concentration of chlorine dioxide solution and NaOCl solution were added to the wastewater of hospital with a 9:1 ratio, 1 mL of samples were then incubated after a period of 10 minutes incubation time. The result was shown in Table 1. The total colony of the control was $3.2x10^5$ CFU/mL while the E. coli colony was $2.0x10^4$ CFU/mL. The disinfection rate of 10 ppm Chlorine dioxide and 30 ppm NaOCl solution were as good as 99.81 % and 99.93 %, respectively. It is similar to the results published by Huang *et al* at 1997, which demonstrate that 0.6 ppm Chlorine dioxide solution and 1.2 ppm NaOCl solution can disinfect 90 % of *Bacillus subtilis*.

The disinfection rate of Chlorine dioxide solution and NaOCl solution were still 63.41 % and 86.06 % if the concentration decreased to as low as 0.07 ppm and 0.173 ppm, respectively. According to the current medical wastewater disposal standard (coliform conc. < $2x10^5$ CFU/100 mL), the discharged wastewater can conform to the standard if even only treated by the lowest concentration of Chlorine dioxide and NaOCl in Table 1.

The disinfection ability of Chlorine dioxide to bacteria were proposed to initiated through changing the permeability of the bacteria membrane. Chlorine dioxide can then penetrate the membrane and oxidize the –SH group of glucose oxidase to an –S-S- group. Because the oxidizability of Chlorine dioxide is about 2.5 times high as that of liquid chlorine, Chlorine dioxide can efficiently inactivate the enzyme activity and cause the bacterium death. In the case of conventional treatment by liquid chlorine, the effect component of which is hypochlorate (HOCl). However, HOCl and OCl- coexist in water and the bacteriacidal efficiency of OCl- is only 1/80 of HOCl[11]. When the higher the pH is, the higher the ration of OCl- is and the weaker the disinfectability of liquid chlorine is. Hence, the disinfection efficiency of Chlorine dioxide under neutral condition is at least two to three times higher than sodium hypochlorate.

To compare the disinfection ability of various disinfectants, the disinfectant solutions were added to the un-pretreated wastewater, followed by incubation times of 2, 5 and 10 minutes. The results are shown in Table 2. It showes that various biochemical disinfectants can achieve disinfection rates of higher than 99.9 % when incubated for 10 minutes. And the efficiency of Chlorine dioxide is the best among these four disinfectants, which can achieve equivalent disinfection rate when the concentration is only 1/7 to 1/25 of other disinfectants.

3.2 Environmental disinfection test in physician out-patient area

Table 3 showes that the average bacteria number on each handset of telephone is 180, which is higher than the average bacteria number of 15 CFU/m^3 on the door knob. This is caused by the fact that telephones were placed at positions where doctors, nurses and patients can access. And further, various bacteria colonies can accumulate if the one who use telephone has bacteria on his/her hands or they didn't use a mask when use a telephone. In table 3, we also observed that the disinfection rate in order is door knob (100 %) = handset of telephone (100 %) > chair cushion (90.3 %) > floor (20.5 %). The disinfection rate is better when the surface of the subject is smooth and is not absorbent. It is not very suitable to use spray to disinfect floor which is very dirty, full of dust and very absorbent. The disinfection rate will increase if the floor was disinfected by wiping with a mop or sprinkling.

After spraying 1 mL of 200 ppm Chlorine dioxide solution twice on the surface of door knob, handset of telephone and chair cushion, the disinfection rates reaches 90 %. The point is that the surface of these objects is smooth and not absorbent plastic surface, which makes the Chlorine dioxide solution can soak the bacteria efficiently. The disinfection rate on the fllor is lower, which is only 20.5 %, might cause by the fact that surface of the floor is abrasive and absorbent. In 1967 Bernade[12] reported that main mechanism of microorganism inactivation is protein break down. And in 1986 Aieta and Berg[13] suggested the imbalance of osmosis can distroy the outer membrane of a cell, which causes final degradation of pathogens. In 2006 Ison *et al*. summarized the possible mechanisms of the disinfection effects of chlorine dioxide[14]. The bases mentioned above are only possible when an efficient contact exist. Therefore, when using chlorine dioxide spray to disinfect an object, the smoothness, toughness and absorbance are important determinant factors.

3.3 Disinfection of aerosols in the physician out-patient area

Aerosols is an important transfer media of bacteria and viruses in hospital. It is difficult to sample representative aerosols in a hospital because of the sampling process may disturbed by factors like time, space, temperature, humidity and circulation. We use random sampling first bu operating the XMX/2AL

aerosols collecting machine (1000 L/min) to gather background value to assess the bacteria content of different areas. The bacteria in the open-space emergency department is 58 CFU/m^3, 183 CFU/m^3 in the semi-closed out-patient area in the surgical department and 318 in the out-patient area in the physician department. We chose the out-patient area of the physician department as the target for Chlorine dioxide disinfection.

The spatial volume of the physician out-patient area is about 445.5 cubic meter. We released 1090 mL of Chlorine dioxide solution into the space, which corresponded to the efficient concentration of 200 ppm, to test the disinfection rate. EP 606 ultrasonic aerosol machine was used, together with handheld sprayer to spray on the walls and the air. Because the boiling point of Chlorine dioxide is 11^{o}C, which is lower than the room temperature of 24^{o}C, the Chlorine dioxide solution was nebulized immediately after which the Chlorine dioxide molecules were evaporized and were capable to destroy bacteria in the space. The maximum concentration of chlorine dioxide in the space after 30 minutes is 0.8 ppm which is lower than 0.10 ppm after 8 hours in the US OSHA bulletin. The results are summarized in Table 4.

The collection volume of the aerosols collecting machine is 15000 liters, which equals to 15 cubic meter. Therefore the background value of the physician out-patient area was 421 CFU/m^3, which was in accord with the normal distribution of the hospital aerosol which ranges between 370 CFU/m^3 and 740 CFU/m^3. The variation of the bacteria at a same smpling spot is correlated to the number of waiting patients in the out-patient area. When sampling for te first time, there were 20 patients in the waiting area, which resulted in 83 CFU/m^3. While sampling for the second time, there were around 70 patients in the waiting area, which resulted in 758 CFU/m^3. We therefore speculate that more patients in the waiting area will result in more bacteria in the aerosol and higher transmission rate. The background value drop from 421 CFU/m^3 to 21 CFU/m^3, reached a high disinfection rate of 94.9%, after treated by EP 606 Chlorine dioxide gas. The value is far lower than standard of high quality air in Singapore (500 CFU/m^3) or in Japan (300 CFU/m^3).

According to the reports published by Guo et al.[15], the possible pathogens in aerosol in hospital include Acinetobacter spp., Burkholderia, E. coli, Enterococcus, Klebsiella spp., Ps. Aeruginosa, S. aureus and Sta. epidermidis. Infection through aerosol can be very remarkable. Other potential pathogens in the aerosol can cause an even larger threat to medical staff and patients. Influenza virus, Enteroviruses and SARS virus can also be transmitted through aerosol. Thus, monitoring and disinfection of aerosol cannot be overlooked in infection control.

Table 1. Disinfection rate of chloride dioxide and bleacher solution

EP 606 Chlorine dioxide solution				NaOCl bleacher solution			
Conc. (ppm)	Total colony (CFU/mL)	Coliform (CFU/100mL)	Disinfect. rate (%)	Concentration (ppm)	Total colony (CFU/mL)	Coliform (CFU/100mL)	Disinfect. rate (%)
Control	3.2 x 10^5	2.0 x 10^4	-	Control	3.2 x 10^5	2.0 x 10^4	-
100	0	0	100	200	0	0	100
51	150	0	99.95	139	0	0	100
10	600	80	99.81	30	216	20	99.93

Table 2. Time effect on the disinfection rate of four disinfectants

Disinfectant	STB		Type 82		NaOCl bleacher		EP 606 ClO_2 disinfectant	
Conc.	1417 ppm		4877 ppm		1386 ppm		193 ppm	
microbe	Total※	Coliform※	Total	Coliform	Total	Coliform	Total	Coliform
Control	3.2 x 10^5	2.0 x 10^4	3.2 x 10^5	2.0 x 10^4	3.2 x 10^5	2.0 x 10^4	3.2 x 10^5	2.0 x 10^4
2 min.	500	0	250	0	150	0	500	0
5 min.	300	0	250	0	0	0	400	0
10 min	250	0	200	0	0	0	300	0

Disinfection rate (10 min.)	99.92 %	100 %	99.94 %	100 %	100 %	100 %	99.91 %	100 %

※ Unit for total colony is CFU/mL; Unit for coliform is CFU/100mL

Table 3. Disinfection rate of chlorine dioxide (200ppm) in the physician department

Room no. Test against		7	8	9	10	11	12	13	Average	Disinfection rate (%)
Knob (m^2)	Background (CFU)	2	50	4	50	0	2	0	15	100
	Disinfected (CFU)	0	0	0	0	0	0	0	0	
Handset (m^2)	Background (CFU)	360	264	38	300	98	150	50	180	100
	Disinfected (CFU)	0	0	0	0	0	0	0	0	

Out-patient area		Sampling spot A	Sampling spot B	Average	Disinfection rate
Cushion (m^2)	Background (CFU)	40	82	62	90.3
	Disinfected (CFU)	2	10	6	
Floor (m^2)	Background (CFU)	306	16	161	20.5
	Disinfected (CFU)	245	11	128	

Table 4. Disinfection rate of chlorine dioxide (200ppm) of the aerosol in physician department

Physician out-patient	Sampling spot A		Sampling spot B		Average	Averaged disinfection rate
Background (CFU/m^3)	50	581	117	936	421	94.9 %
Disinfected (CFU/m^3)	23	6	50	5	21	

4. Conclusion

The chlorous disinfectants investigated in this study revealed disinfection rates higher than 99.9 % for highly contaminated medical wastewater. To reach the same disinfection rate, the concentration of Chlorine dioxide is only 1/7 to 1/25 of other chlorous disinfectants. In different surfaces in the physicina department, we observed the disinfection rate of door knob of 100 %. At handset of telephone was also 100 % in comparison to chair cushion of 90.3 % and floor of 20.5 %. Disinfection by spraying has better effect on smooth and non-absorbant surface. Meanwhile, sprinkling or wiping with mop provides a better effect on surfaces which is dirty, full of dust and absorbant.

The background values of bacteria in aerosol are semi-closed out-patient area in the physician department (318 CFU/m^3) > semi-closed out-patient area in the surgical department (183 CFU/m^3) > open-space emergency department (58 CFU/m^3). The air quality of above areas all conformed to the standard bacteria number in the aerosol. After distribution of 1090 mL of 200 ppm Chlorine dioxide solution into the air by using ultrasonic aerosol and handheld sprayer for thirty minutes, the average number of bacteria drop from 421 CFU/m^3 to 21 CFU/m^3. Chlorine dioxide is very efficient to disinfect bacteria in aerosol and can be used as a refernce.

Correspondence to:
Sheau Long Lee, Ph.D.
Associate Professor, ROC Military Academy
Fengshan, 830 Taiwan, R.O.C.
886-7742-9442
leesheaulong@gmail.com

6. References

1. World Health Organization: Laboratory Biosafety Manual: Disinfection and sterilization, 2nd ed. Geneva: WHO. 2003; Chapter 14:59-66.
2. Li, J.W., Yu, Z., Cai, X., et al, "Trihalomethanes Formation In Water Treated With Chlorine dioxide." Wat. Res. 30(10), pp. 2371-2376 (1996)
3. Ridenour, G.M., Ingols, R., "Inactivation of Poliomyelitis Virus by Free Chlorine." Amer Public Health. 36:639 (1946)
4. Smith, J.E., McVey, J.L., "Virus Inactivation by Chlorine dioxide and Its Application to Storm Water Overflow." Proceeding, ACS Annual Meeting. 13(2), pp. 177 (1973)
5. Roberts, P.V., Aieta, E.M., Berg, J.D., et al, "Chlorine dioxide for Wastewater Disinfection: A Feasibility Evaluation. Stanford University Technical Report." 1980, October: 251.
6. USEPA, "Guidance Manual Alternative Disinfectants and Oxidants: 4. Chlorine dioxide.", US EPA 815-R-99-014, (April 1999).
7. Hoehn, R.C., Rosenblatt, A.A., Gates, D.J., "Considerations for Chlorine dioxide Treatment of Drinking Water." Conference proceedings, AWWA Water Quality Technology Conference, 1996,Boston, MA.US
8. Huang, J., Wang, L., Ren, N., Ma, F., Juli., "Disinfection effect of chlorine dioxide on Viruses, Algae and Animal Plankton in Water." Wat. Res., 31(3), pp. 455-460 (1997)
9. US EPA (1999, April). Guidance Manual Alteration Disinfectants and Oxidants: 4. Chlorine dioxide. US EPA(815-R-99-014) http://www.epa.gov/safewater/mdbp/mdbptg.html
10. APHA, AWWA, WEA: Standard Methods for the Examination of Water and Wastewater. 1998; Chapter 4: 73-8.
11. Huang, J., Wang, L., Ren, N., Ma, F., Juli., "Disinfection effect of chlorine dioxide bacteria in water." Wat. Res., 33(3), pp. 607-613 (1997)
12. Bernade, M.A., Snow, W.B., Olivieri, V.P., "Chlorine Dioxide Disinfection Temperature Effects" Jour. Appl. Bacteriol., 30, pp. 159-165 (1967)
13. Aieta, E.M., Berg, J.D., "Review of Chlorine Dioxide in Drinking Water Treatment" Jour. Amer. Wat. Works. Assoc. (AWWA), 78(6), pp. 62-72 (1986)
14. Ison, A., Odeh, I.N., Margerum, D.W., "Kinetics and Mechanisms of Chloride Dioxide and Chlorite Oxidations of Cysteine and Glutathione." Inorg. Chem., 45(21), pp. 8768-8775 (2006
15. Guo, C.I., Liang, C.S., Chen, Y.J., "Research of Biological Aerosol in Hospitals" Conference proceedings, US Safety and Health Conference, 2003,Taipei, Taiwan ROC

Nature and Science

ISSN 1545-0740

The ***Nature and Science*** is an international journal with a purpose to enhance our natural and scientific knowledge dissemination in the world under the free publication principle. Any valuable papers that describe natural phenomena and existence or any reports that convey scientific research and pursuit are welcome, including both natural and social sciences. Papers submitted could be reviews, objective descriptions, research reports, opinions/debates, news, letters, and other types of writings that are nature and science related.

1. General Information

(1) Goals: As an international journal published both in print and on internet, *Nature and Science* is dedicated to the dissemination of fundamental knowledge in all areas of nature and science. The main purpose of *Nature and Science* is to enhance our knowledge spreading in the world under the free publication principle. It publishes full-length papers (original contributions), reviews, rapid communications, and any debates and opinions in all the fields of nature and science.
(2) What to Do: *Nature and Science* provides a place for discussion of scientific news, research, theory, philosophy, profession and technology - that will drive scientific progress. Research reports and regular manuscripts that contain new and significant information of general interest are welcome.
(3) Who: All people are welcome to submit manuscripts in any fields of nature and science.
(4) Distributions: Web version of the journal is freely opened to the world, without any payment or registration. The journal will be distributed to the selected libraries and institutions for free. For the subscription of other readers please contact with: editor@americanscience.org or americansciencej@gmail.com or editor@sciencepub.net.
(5) Advertisements: The price will be calculated as US$400/page, i.e. US$200/a half page, US$100/a quarter page, etc. Any size of the advertisement is welcome.

2. Manuscripts Submission

(1) Submission Methods: Electronic submission through email is encouraged and hard copies plus an IBM formatted computer diskette would also be accepted.
(2) Software: The Microsoft Word file will be preferred.
(3) Font: Normal, Times New Roman, 10 pt, single space.
(5) Manuscript: Don't use "Footnote" or "Header and Footer".
(6) Cover Page: Put detail information of authors and a short title in the cover page.
(7) Title: Use Title Case in the title and subtitles, e.g. "**Debt and Agency Costs".**
(8) Figures and Tables: Use full word of figure and table, e.g. **"Figure 1.** Annul Income of Different Groups**", Table 1. Annual Increase of Investment".**
(9) References: Cite references by "last name, year", e.g. "(Smith, 2003)". References should include all the authors' last names and initials, title, journal, year, volume, issue, and pages etc.

Reference Examples:

Journal Article: Hacker J, Hentschel U, Dobrindt U. Prokaryotic chromosomes and disease. Science 2003;301(34):790-3.
Book: Berkowitz BA, Katzung BG. Basic and clinical evaluation of new drugs. In: Katzung BG, ed. Basic and clinical pharmacology. Appleton & Lance Publisher. Norwalk, Connecticut, USA. 1995:60-9.

(10) Submission Address: editor@sciencepub.net, Marsland Company, P.O. Box 21126, Lansing, Michigan 48909, The United States.
(11) Reviewers: Authors are encouraged to suggest 2-8 competent reviewers with their name and email.

2. Manuscript Preparation

Each manuscript is suggested to include the following components but authors can do their own ways:
(1) Title page: including the complete article title; each author's full name; institution(s) with which each author is affiliated, with city, state/province, zip code, and country; and the name, complete mailing address, telephone number, facsimile number (if available), and e-mail address for all correspondence.
(2) Abstract: including Background, Materials and Methods, Results, and Discussions.
(3) Keywords.
(4) Introduction.
(**5) Materials and Methods**.
(**6) Results**.
(**7) Discussions**.
(8) Acknowledgments.
(9) References.

Journal Address:

Marsland Company
P.O. Box 21126
Lansing, Michigan 48909
The United States
Telephone:(517) 349-2362
E-mail: editor@sciencepub.net;
naturesciencej@gmail.com
Websites: http://www.sciencepub.org

ISSN 1545-0740