

SYSTEM AND METHOD OF CLEANING AN ENVIRONMENT

FIELD OF THE INVENTION

5 The present invention relates generally to a system and method of cleaning an environment, including but not limited to cleaning an environment of microorganisms, particularly viruses.

BACKGROUND TO THE INVENTION

10 The following discussion of the background to the invention is intended to facilitate an understanding of the present invention. However, it should be appreciated that the discussion is not an acknowledgment or admission that any of the material referred to was published, known or part of the common general knowledge in any
15 jurisdiction as at the priority date of the application.

 It is known to use chemical disinfectants to clean an environment of microorganisms which include but are not limited to fungi, bacteria and viruses. Oxidising disinfectants such as hydrogen peroxide target cellular material and attenuate and/or
20 stop the functions of microorganisms. However, such disinfectants may have damaging effects to a surrounding and/or users because of their strong oxidising characteristics, thereby reducing their disinfectant efficacy. Non-oxidising disinfectants such as alcohol (e.g. ethyl alcohol and isopropyl alcohol), which may be less damaging to the surroundings and users, typically have different modes of action depending on the
25 microorganism. The effectiveness in inactivating and/or attenuating microorganisms' activities depends on the chemical disinfectant used and different chemical disinfectants can have different ranges of effect on microorganisms. Therefore, it is known to combine different chemical disinfectants to improve the effectiveness of a disinfecting cleaner. However, this can involve substantial preparation time, infrastructure and costs, to
30 produce suitable mixtures of chemical disinfectants for cleaning an environment.

 Viruses are known to be difficult to inactivate and eradicate. It is also difficult to prevent or reduce their spread in an environment. There has been a recent increase in viral diseases, which include severe acute respiratory syndrome, ebola virus disease,
35 and hand foot and mouth disease (HFMD). Viruses are easily transmitted and can stay active *ex vivo* for a considerable amount of time. This increases the need to properly clean an environment, especially when the environment or premise is frequently used by individuals who are susceptible to viral infection, which include children and the elderly.

40 Methods of cleaning an environment typically involve routine cleaning of the environment using cleaning fluids which can contain chemical disinfectants. Such methods can involve cleaning at different time intervals and/or frequency. The frequency of cleaning can affect the actual cleanliness of an environment and the amount of microorganisms in the environment, e.g. infrequent cleaning may result in fouled
45 premises, while cleaning too frequently can increase the work load of cleaning personnel and increase costs due to high use and consumption of cleaning fluids. Many methods do not involve a particular routine and may be a result of reacting to the state of the environment being cleaned. Accordingly, there is little quality control in the cleanliness of the environment, which can be particularly important if the environment is utilized by
50 individuals who are susceptible to viral infection, which include children and the elderly.

 Chlorine dioxide has shown to be effective against various bacteria and viruses due to its strong oxidising ability. However chlorine dioxide is unstable, highly reactive

and explosive. It is therefore known in the art that chlorine dioxide should be produced and/or generated in a well-ventilated area. The Occupational Safety and Health Administration (OSHA), an agency of the United States Department of Labor, has set an 8-hour permissible exposure limit of 0.1 ppm in air (0.3 mg/m³) for people working with chlorine dioxide. Further, aqueous solutions of chlorine dioxide, especially activated chlorine dioxide solutions, should also always be stored and used in a well-ventilated area. As a result, chlorine dioxide solutions cannot be safely stored and used in enclosed, indoor environments, especially those frequently used by children and the elderly. Moreover, chlorine dioxide has a similar smell to chlorine which may cause discomfort to individuals in an enclosed environment.

JP 2003-266079 A discloses a system for injecting chlorine dioxide into water to be treated, where the gaseous chlorine dioxide is dissolved in water in a second storage means for purposes of retaining as much chlorine dioxide in the water to be treated as possible. Any excess chlorine dioxide that cannot be dissolved in water is released via a vent. This system however cannot be used indoors, especially in enclosed environments because water is not an efficient neutralizer of chlorine dioxide – the water simply acts as a solvent for chlorine dioxide in this apparatus. Furthermore, excess gaseous chlorine dioxide is vented out from the apparatus which can pose a danger to the individuals of the environment in which the apparatus of JP 2003-266079 A is installed. Therefore the apparatus of JP 2003-266079 A has to be installed in a well-ventilated area.

Therefore there is a need to alleviate problems in the prior art, which include but are not limited to improving the cleanliness of an environment, particularly from microorganisms, and improving the storage and use of chlorine dioxide in an enclosed environment.

SUMMARY OF THE INVENTION

The above mentioned need is met at least in part and an improvement in the art is made by a system and method in accordance with this invention.

According to an aspect of the present invention, there is a dosing system comprising a housing enclosing: a first closed container adapted to contain a solution of a first oxychlorine compound, the first closed container including a first conduit and a second conduit; and a second closed container adapted to contain a neutralizer, the second closed container in fluid communication with the first closed container via the second conduit, wherein the first conduit is adapted to convey the solution of the first oxychlorine compound or part thereof to mix with a fluid to obtain and dispense a cleaning fluid, and wherein the neutralizer is adapted to neutralize emissions of the solution of the first oxychlorine compound.

The fact that the first and second containers are closed containers, and the neutralizer is adapted to neutralize emissions of the solution of the first oxychlorine compound, allows the dosing system to be used in an indoor, optionally enclosed, environment.

Preferably, the first oxychlorine compound is chlorine dioxide and the emissions are gaseous chlorine dioxide.

Preferably, the solution of the first oxychlorine compound is a stabilized chlorine dioxide solution or an activated chlorine dioxide solution.

Preferably, the chlorine dioxide in the solution of the oxychlorine compound is in a concentration range of about 20,000 ppm to about 60,000 ppm. More preferably, the concentration of the chlorine dioxide in the solution of the oxychlorine compound is 50,000 ppm.

Preferably, the neutralizer comprises sodium thiosulfate solution.

Preferably, one end of the second conduit is arranged to be in contact with the sodium thiosulfate solution and near a surface level of the sodium thiosulfate solution.

Preferably, the volume of the second closed container is one-tenth the volume of the first closed container.

Preferably, the first closed container further includes a third conduit comprising a one-way valve, the third conduit adapted to only permit fluid flow into the first closed container.

Preferably, the second closed container further includes a fourth conduit comprising a one-way valve, the fourth conduit adapted to only permit fluid flow out of the second closed container.

According to another aspect of the present invention, there is a method of cleaning an environment, the method comprising: a) providing a dosing system comprising a housing enclosing: a first closed container containing a solution of a first oxychlorine compound, the first closed container including a first conduit and a second conduit; and a second closed container containing a neutralizer, the second closed container in fluid communication with the first closed container via the second conduit, wherein the neutralizer neutralizes emissions of the solution of the first oxychlorine compound; b) conveying the solution comprising the first oxychlorine compound via the first conduit and dosing a fluid to obtain a first cleaning fluid; c) applying on and cleaning at least one surface in the environment at least once a day with the first cleaning fluid; d) generating and releasing a gaseous second oxychlorine compound via a release system into the environment; and e) fogging the environment with a fog comprising a third oxychlorine compound.

Preferably, the method further comprises the step of f) spraying a second cleaning fluid comprising a fourth oxychlorine compound via at least one hand-held spray means on at least one surface in the environment.

Preferably, the fourth oxychlorine compound is chlorine dioxide, and wherein the second cleaning fluid comprising a fourth oxychlorine compound is a stabilized chlorine dioxide solution.

Preferably, the chlorine dioxide in the second cleaning fluid is in a concentration range of about 500 ppm to about 900 ppm.

Preferably, the first oxychlorine compound is chlorine dioxide, and wherein the solution comprising the first oxychlorine compound is a stabilized chlorine dioxide solution.

Preferably, the method further comprises the step of activating the stabilized chlorine dioxide solution prior to step b).

Preferably, the chlorine dioxide in the solution comprising the first oxychlorine compound, is in a concentration range of about 20,000 ppm to about 60,000 ppm. More preferably, the concentration of the chlorine dioxide in the solution of the oxychlorine compound is 50,000 ppm.

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Preferably, the chlorine dioxide in the first cleaning fluid is in a concentration range of about 50 ppm to about 300 ppm.

10 Preferably, the release system comprises a precursor of the gaseous second oxychlorine compound and a dry hygroscopic material, wherein the hygroscopic material is adapted to generate and release the gaseous second oxychlorine compound in the presence of water, and wherein the gaseous second oxychlorine compound is gaseous chlorine dioxide.

15 Preferably, the hygroscopic material further comprising an activator adapted to react with the precursor in the presence of water to generate and release the gaseous second oxychlorine compound, wherein the precursor is a metal chlorite and the activator is an acid.

20 Preferably, the method comprises generating and releasing the gaseous chlorine dioxide over 24 to 48 hours. More preferably, the method comprises mounting the release system onto a surface in the environment, near to a vent.

25 Preferably, the third oxychlorine compound is chlorine dioxide and wherein the chlorine dioxide in the fog comprises a third oxychlorine compound is in a concentration range of about 500 ppm to about 900 ppm.

Preferably, step d) comprises fogging the environment once a month.

30 Preferably, the method further comprising fumigating the environment with gaseous chlorine dioxide in a concentration range of about 1 ppmv to 300 ppmv.

35 Preferably, the method is for cleaning an environment of one or more viruses selected from the group comprising Coxsackievirus A16, Coxsackievirus B2 and Enterovirus 71.

Preferably, the environment is an indoor environment. More preferably, the environment is a childcare centre.

40 According to another aspect of the present invention, there is a method of cleaning an environment, the method comprising: a) providing a dosing system comprising a housing enclosing a first closed container containing a stabilized solution of a first oxychlorine compound, the first closed container including a first conduit; b) conveying the stabilized solution comprising the first oxychlorine compound via the first conduit and dosing a fluid to obtain a first cleaning fluid; c) applying on and cleaning at
45 at least one surface in the environment at least once a day with the first cleaning fluid; d) generating and releasing a gaseous second oxychlorine compound via a release system into the environment; and e) fogging the environment with a fog comprising a third oxychlorine compound.

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According to another aspect of the present invention, there is a system of cleaning an environment, the system comprising: a) a dosing system comprising a housing enclosing: a first closed container adapted to contain a solution of a first

oxychlorine compound, the first closed container including a first conduit and a second conduit; and a second closed container adapted to contain a neutralizer, the second closed container in fluid communication with the first closed container via the second conduit, wherein the first conduit is adapted to convey the solution of the first oxychlorine compound or part thereof to mix with a fluid to obtain and dispense a first cleaning fluid for cleaning at least one surface in the environment, and wherein the neutralizer is adapted to neutralize emissions of the solution of the first oxychlorine compound; b) a release system adapted to generate and release a gaseous second oxychlorine compound into the environment; c) a fog generator adapted to fog the environment with a fog comprising a third oxychlorine compound; and d) at least one hand-held spray means adapted to spray a second cleaning fluid comprising a fourth oxychlorine compound on at least one surface in the environment.

Preferably, the first, second, third and fourth oxychlorine compounds comprise chlorine dioxide.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 illustrates an embodiment of a dosing system of the present invention.

Figure 2a and 2b illustrate a cross-sectional and top view of a portion of an embodiment of a dosing system of the present invention.

Figure 3 illustrates an embodiment of a dosing system of the present invention.

Figure 4 is a photograph of a dosing system of Figure 3.

Figure 5 shows a flow chart of an embodiment of the present invention.

Figure 6 shows a graph of weekly incidence of Air/Droplet-Bourne Diseases, 2015-2016, obtained from the Ministry of Health, Singapore.

Figure 7 illustrates occurrences of Hand Foot and Mouth disease (HFMD) cases in a childcare centre before and after application of the method of the present invention.

Figure 8 provides a graph showing the efficacy of a 50 ppm chlorine dioxide solution on enterovirus (Enterovirus 71) causing Hand, Foot and Mouth Disease (HFMD). The graph is based on TCID₅₀ (TCID₅₀/mL) against Time (min). Time 0 represents the TCID₅₀ for the positive control.

Figure 9 provides a graph showing the efficacy of a 100 ppm chlorine dioxide solution on enterovirus (Coxsackie B2) causing Hand, Foot and Mouth Disease (HFMD). The graph is based on TCID₅₀ (TCID₅₀/mL) against Time (min). Time 0 represents the TCID₅₀ for the positive control.

Other arrangements of the invention are possible and, consequently, the accompanying drawings are not to be understood as superseding the generality of the preceding description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Particular embodiments of the present invention will now be described with reference to the accompanying drawings. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention. Other definitions for selected terms used herein may be found within the detailed description of the invention and applied throughout. Additionally, unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Where possible, the same reference numerals are used throughout the figures for clarity and consistency.

Definitions

Throughout the specification, unless the context requires otherwise, the word “comprise” or variations such as “comprises” or “comprising”, are to be construed as inclusive and not exhaustive.

Furthermore, throughout the specification, unless the context requires otherwise, the word “include” or variations such as “includes” or “including”, are to be construed as inclusive and not exhaustive.

As used herein, the term “about” typically means $\pm 5\%$ of the stated value, more typically $\pm 4\%$ of the stated value, more typically $\pm 3\%$ of the stated value, more typically $\pm 2\%$ of the stated value, even more typically $\pm 1\%$ of the stated value, and even more typically $\pm 0.5\%$ of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as a limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. Ranges are not limited to integers, and can include decimal measurements where applicable. This applies regardless of the breadth of the range.

As used throughout the specification, the term “environment” refers to the physical surroundings that a particular activity or operation is carried out. An “environment” includes but is not limited to a premise, space, building, room or part thereof. An “environment” may be an indoor or outdoor environment. An indoor environment refers to an environment which is situated, conducted, or used within a building or under cover, and it may be open (which includes but is not limited to substantially open) or enclosed (which includes but is not limited to substantially enclosed). An example of an enclosed indoor environment is a room located in a building accessible via for example a door. In a preferred embodiment, an environment includes but is not limited to one that is frequented by individuals who are susceptible to viral infection, such as children and the elderly. Such an environment may be a childcare centre (includes but is not limited to kindergarten and nurseries), school, elderly home, or playground.

The term "fluid" used throughout the specification has ordinary meaning in the art and includes but is not limited to liquids and gases. The term "water" used throughout the specification refers to water in any one of its different states, which includes but is not limited to liquid water and gaseous water vapour.

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Oxychlorine compounds include but are not limited to dichlorine monoxide (Cl_2O), chlorine oxide (ClO), dichlorine trioxide (Cl_2O_3), chlorine dioxide (ClO_2), dichlorine pentoxide (Cl_2O_5), chlorine trioxide (ClO_3), dichlorine heptoxide (Cl_2O_7), chlorite (ClO_2^-) and chlorate (ClO_3^-). The inventors of the present invention have found that an environment, especially an indoor environment frequented by individuals who are susceptible to viral infection, can be effectively cleaned by using chlorine dioxide. Advantageously, chlorine dioxide does not react with organic materials to form chlorinated hydrocarbons, which are increasingly avoided because of health concerns. Chlorine dioxide is a neutral compound of chlorine in the +4 oxidation state. It disinfects by oxidation and can function as a strong, but highly selective oxidant due to its unique, one-electron transfer mechanism in which it is reduced to chlorite. Due to its strong oxidising ability, chlorine dioxide may be used in pulp bleaching and as a disinfectant, mainly in water treatment.

For the purposes of the present invention, chlorine dioxide may be used in the form of a gas. At room temperature and pressure, chlorine dioxide exists as a greenish yellow to orange gas with a characteristic pungent chlorine-like odour, and may be unstable, highly reactive and explosive. In addition, gaseous chlorine dioxide may be detonated by sunlight or heat. Therefore chlorine dioxide is typically stored and used in a well-ventilated area, and not in indoor environments, especially not in indoor enclosed environments.

More preferably, aqueous solutions of chlorine dioxide (or aqueous chlorine dioxide) may be used in the present invention. Chlorine dioxide is water soluble and as such, can be kept as aqueous solutions. Preferably, such solutions are kept cool, in the dark and in a closed vessel. As aqueous solutions of chlorine dioxide may be unstable, they are preferably produced at or near the point of use. Such aqueous solutions of chlorine dioxide may deteriorate by chemical degradation into chlorine, oxygen, chlorite, chlorate, or other decomposition products. Consequently, the storage times may be limited.

Aqueous solutions of chlorine dioxide used in the present invention may contain an additive, such as a stabilizer known to those skilled in the art. The stabilizer may stabilize the aqueous solution of chlorine dioxide to form a stabilized chlorine dioxide solution. Such solutions may be more storage-stable, and thus more suitable for transportation. Advantageously, deterioration of the levels of chlorine dioxide in such solutions either do not occur or occur at very low rates. Such solutions may be diluted by water and/or activated by lowering of the pH of the solution, for example by the addition of an acid, such as citric acid.

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As used herein, the term "activated aqueous chlorine dioxide" refers to an aqueous solution of chlorine dioxide that is activated by the lowering of the pH of the solution, for example, by the addition of an acid. It will be appreciated that the strength of the acid can affect the rate of generation of chlorine dioxide via activation, e.g. a strong acid will generate chlorine dioxide faster than a weak acid.

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Alternatively, a chlorine dioxide-generating species such as a metal chlorite may be dissolved in water to form an aqueous solution, and upon contact with an activator,

form chlorine dioxide. An activator of a chlorine dioxide-generating species includes but is not limited to acids. For instance, the chlorine dioxide-generating species may be sodium chlorite, which can be dissolved in water to form aqueous sodium chlorite, which is stable and suitable for transportation. Chlorine dioxide can be generated from the aqueous sodium chlorite solution via an acid displacement reaction.

Therefore, as used throughout the specification and unless specifically stated, the term "chlorine dioxide" will refer to its gaseous form and/or its aqueous form. In addition, the term "chlorine dioxide solution" includes aqueous solutions of chlorine dioxide (or aqueous chlorine dioxide) that may optionally contain a stabilizer, and aqueous solutions of a chlorine dioxide-generating species. In a preferred embodiment, the chlorine dioxide solution is a stabilized chlorine dioxide solution.

As used throughout the specification, the term "metal chlorite" includes but is not limited to an alkali metal chlorite salt, such as a chlorous acid salt of lithium, sodium, potassium, rubidium or caesium, or an alkaline earth metal chlorite salt, such as a chlorous acid salt of magnesium, calcium, strontium or barium.

As used herein, the unit "ppm" refers to parts per million by weight and the unit "ppmv" refers to parts per million by volume. It would be appreciated by a person skilled in the art that the concentration of the oxychlorine compound, particularly chlorine dioxide, will depend on factors such as, the temperature of the solution and humidity.

In an embodiment of the present invention, the concentration of chlorine dioxide in a chlorine dioxide solution may be about 100 ppm to about 60,000 ppm, about 100 ppm to about 50,000 ppm, about 1,000 ppm to about 40,000 ppm, about 1,000 ppm to about 3000 ppm, about 200 ppm to about 50,000 ppm, about 400 ppm to about 50,000 ppm, about 5000 ppm to about 40,000 ppm, about 5000 ppm to about 30,000 ppm, and about 20,000 ppm to about 60,000 ppm. Preferably, the concentration of chlorine dioxide in a chlorine dioxide solution is about 50,000 ppm.

The pH of the chlorine dioxide solution may be acidic or alkaline. In a preferred embodiment, the pH of the stabilized chlorine dioxide solution used in the present invention is about 9.2 to about 9.8, while the pH of the activated chlorine dioxide solution used in the present invention is about 4.0 to about 5.0. In addition, the chlorine dioxide solution used in the present invention can effectively clean an environment over a wide range of pH and temperatures. Advantageously, the range of pH values that the chlorine dioxide solution can effectively clean an environment is from 2.0 to 12.0, 2.0 to 10.0, 2.0 to 8.0, 4.0 to 12.0, 4.0 to 10.0 and 2.5 to 11.5.

In an embodiment, the range of temperatures that the chlorine dioxide solution can effectively clean an environment is about -5°C to about 80°C, -5°C to about 70°C, about 0°C to about 80°C, about 0°C to about 70°C, about 10°C to about 80°C, about 10°C to about 70°C, about 20°C to about 70°C, about 30°C to about 70°C, about 30°C to about 60°C, about 30°C to about 50°C, about 30°C to about 40°C and about 25°C to about 35°C. In a preferred embodiment, the chlorine dioxide solution can effectively clean an environment at a temperature range of about 25°C to about 35°C.

As used throughout the specification, the term "bacteriostat" refers to a substance that stops bacteria from reproducing, while not necessarily killing them. For example, a stabilized chlorine dioxide solution as described herein can function as a bacteriostat.

As used throughout the specification, the term “bactericide” refers to a substance that kills bacteria. For example, an activated chlorine dioxide solution as described herein can function as a bactericide.

5 DOSING SYSTEM

Figure 1 provides an embodiment of a dosing system 100 of the present invention. Figures 2a and 2b provide a cross-sectional and top view of a portion of the dosing system 100. The dosing system 100 comprises a housing 180, a first closed container 110, second closed container 120 and conduits 111, 112, 130, 121. The dosing system 100 also includes a mixing chamber 160 that is connected to the first container 110 via conduit 112 (first conduit). The mixing chamber 160 further includes an inlet conduit 161 and outlet conduit 162. The conduits 111, 112, 130, 121, 161, 162 are preferably pipes having a circular cross-section, but it will be appreciated that the cross-sectional shape of these conduits may differ depending on the application and requirements. The dosing system 100 may further comprise components such as piping, bolts and screws that would be readily appreciated by a person skilled in the art and are contemplated as being within the scope of the present invention and can be readily used in alternate embodiments without undue experimentation.

It will be understood that the first container 110 and second container 120 are considered closed containers because in operation, these containers 110, 120 are sealed via covers 114, 124, and the contents of these containers 110, 120 are only accessible via conduits 111, 112, 130, 121. The first closed container 110 is adapted to store an oxychlorine compound, preferably aqueous chlorine dioxide 140, and more preferably activated aqueous chlorine dioxide 140, in a first chamber 113, while the second closed container 120 is adapted to store a neutralizer 150. The neutralizer 150 is adapted to neutralize the emissions of the oxychlorine compound that enters the second chamber 123. In a preferred embodiment, the neutralizer 150 is adapted to neutralize gaseous chlorine dioxide that enters the second chamber 123. The neutralizer 150 includes but is not limited to activated carbon and sodium thiosulfate. The neutralizer 150 is preferably sodium thiosulfate solution. The first closed container 110 has a first conduit 112 that extends substantially into the aqueous chlorine dioxide 140 and said conduit 112 is adapted to convey in direction B, the aqueous chlorine dioxide 140 to the mixing chamber 160 where the aqueous chlorine dioxide 140 is dosed and mixed with a fluid that enters the mixing chamber 160 from inlet conduit 161, to obtain and dispense a cleaning fluid (first cleaning fluid) via outlet conduit 162. The fluid that enters the mixing chamber 160 from inlet conduit 161 can comprise water or water mixed with detergent. The drawing in of the fluid via inlet conduit 161 and the aqueous chlorine dioxide 140 via conduit 112, and the dispensing of the mixed first cleaning fluid via outlet conduit 162 may be automated by suitable motors and pumps (not shown). The mixing chamber 160 may be configured to mix the aqueous chlorine dioxide and the fluid entering inlet conduit 162 in a pre-determined ratio. Alternatively, the mixing chamber 160 may comprise suitable controls which allow a user to decide on the amount and concentration of chlorine dioxide in the first cleaning fluid that is dispensed from outlet conduit 162. Preferably, the first cleaning fluid dispensed via conduit 162 comprises chlorine dioxide in a concentration range of about 50 ppm to about 900 ppm, preferably about 50 ppm to about 300 ppm, more preferably about 50 ppm to about 150 ppm and even more preferably about 100 ppm.

The aqueous chlorine dioxide 140 may be a stabilized chlorine dioxide solution or an activated chlorine dioxide solution. A stabilized chlorine dioxide solution can function as a bacteriostat by removing the biofilm in water logged areas where

microorganisms such as bacteria and viruses are typically found. The removal of the biofilm reduces and prevents the spread of microorganism because without the biofilm, such microorganisms cannot grow and survive. Accordingly, a stabilized chlorine dioxide solution would be suitable to maintain the cleanliness of an environment from microorganisms. An activated chlorine dioxide solution has strong oxidising properties and can function as a bactericide which kills bacteria. An activated chlorine dioxide solution is also capable of attenuating viruses. Accordingly, an activated chlorine dioxide solution would be suitable to clean an environment to a desired state suitable for use by individuals, such as children and the elderly. The concentration of chlorine dioxide in the aqueous chlorine dioxide 140 is about 20,000 ppm to about 60,000 ppm, preferably about 30,000 ppm to about 60,000 ppm, and more preferably about 50,000 ppm.

The first closed container 110 and the second closed container 120 are in fluid communication via second conduit 130. Chlorine dioxide is unstable, highly reactive and explosive. It is known in the art that aqueous solutions of chlorine dioxide should always be used in a well-ventilated area, especially activated chlorine dioxide solutions. In the present invention, chlorine dioxide solutions may be used in an enclosed environment with little ventilation because the first closed container 110 is in fluid communication with the second closed container 120 via second conduit 130. Any chlorine dioxide gas and/or emissions from the activated aqueous chlorine dioxide 140 can travel through the second conduit 130 to the second closed container 120 in the direction C and be neutralized by the sodium thiosulfate 150 when in operation. Therefore in operation, the first container 110, second container 120 and second conduit 130 forms a substantially closed system, which prevents chlorine dioxide emissions from leaking into the environment where the dosing system 100 is used. Chlorine dioxide emissions can pose a danger to individuals in the environment.

The first closed container 110 further includes a third conduit 111 having a one-way valve, where the third conduit 111 is adapted to convey atmospheric air in the direction A into the first closed container 110. The second closed container 120 includes a fourth conduit 121 having a one-way valve, where the fourth conduit is adapted to convey air and any gases or emissions in the chamber 123 out, in direction A', into the atmosphere. The third and fourth conduits 111, 121 equalize the pressure in the first and second closed containers 110, 120 and the second conduit 130, so as to ensure that gaseous chlorine dioxide from the first closed container 110 will travel in direction C via second conduit 130 to second container 120. It will however be appreciated that conduits 111, 121 are optional components. Therefore in various embodiments, the dosing system 100 does not comprise conduits 111, 121, and the first closed container 110, the second closed container 120 and the second conduit 130 form a closed system, where in operation, the contents of the system 100 can only be accessed via mixing chamber 160 and first conduit 112. This improves the protection against leakage of chlorine dioxide emissions into an environment where the dosing system 100 is used.

Neutralizer 150 is a sodium thiosulfate solution 150. Second conduit 130 has an outlet 130' that is in contact with the sodium thiosulfate solution 150 and which is near the fluid surface level of said solution 150. Preferably, the outlet 130' is submerged in the sodium thiosulfate solution 150. As significant pressure resulting from the chlorine dioxide gas emitted from the aqueous chlorine dioxide may not build up in the first closed container 110 and second conduit 130, the outlet 130' being located near and preferably below the fluid surface level of the sodium thiosulfate solution will prevent exerting a significant counter pressure that prevents chlorine dioxide gas from travelling in direction C. As the outlet 130' is in contact with the sodium thiosulfate solution 150, the solution 150 will be sufficient to neutralize, preferably completely, any chlorine dioxide gas that

enters the second closed container 120. The volume of the second chamber 123 is preferably one-tenth of the volume of the first chamber 113, due to low pressure of the chlorine dioxide gas in the chamber 113. The volume of the chamber 123 being one-tenth the volume of the chamber 113 is sufficient to neutralize the chlorine dioxide gas emitted in the first closed container 110.

The first and second closed containers 110, 120, mixing chamber 160 and conduits 111, 112, 130, 121, 161, 162 are preferably made from material resistant to being oxidised by chlorine dioxide. Preferably, such material comprises polyethylene terephthalate (PTE) and/or polytetrafluoroethylene (PTFE).

Suitable attachments may be provided to inter-connect the first container 110, second container 120, mixing chamber 160 and conduits 111, 112, 130, 121, 161, 162 such that the first container 110 and second container 120 are removable from the dosing system 100 for replacement and/or replenishment, without removing the dosing system 100 itself.

Figures 3 and 4 provide another embodiment of a dosing system 200 of the present invention. The dosing system 200 comprises a housing 280, a first container 210 and a mixing chamber 260. The mixing chamber 260 is in fluid communication with the chamber 213 of the first container 210 via conduit 212. The mixing chamber further includes inlet conduit 261 and outlet conduit 262. The chamber 213 of the first container 210 contains stabilized chlorine dioxide solution 240. The dosing system 260 is not suitable for use with activated chlorine dioxide solution because any chlorine dioxide emissions from the solution 140 can be unstable, highly reactive and explosive, thereby posing a danger to users of the environment where the dosing system 260 is used. Stabilized chlorine dioxide solution minimally emits gaseous chlorine dioxide, therefore the dosing system 260 is suitable for stabilized chlorine dioxide solutions.

When in operation, the first conduit 212 which extends substantially into the stabilized chlorine dioxide solution 240, conveys the stabilized chlorine dioxide solution 140 to the mixing chamber 260 where the chlorine dioxide solution 240 is dosed and mixed with a fluid that enters the mixing chamber 260 from inlet conduit 261, to obtain and dispense a cleaning fluid via outlet conduit 262. The fluid that enters the mixing chamber 260 from inlet conduit 261 can comprise water or water mixed with detergent. The drawing in of the fluid via inlet conduit 261 and the stabilized chlorine dioxide solution 240 via conduit 212, and the dispensing of the mixed first cleaning fluid via outlet conduit 262 may be automated by suitable motors and pumps (not shown). The mixing chamber 260 may be configured to mix the stabilized chlorine dioxide solution and the fluid entering inlet conduit 262 in a pre-determined ratio. Alternatively, the mixing chamber 260 may comprise suitable controls which allow a user to decide on the amount and concentration of chlorine dioxide in the first cleaning fluid that is dispensed from outlet conduit 262.

SYSTEM AND METHOD OF CLEANING AN ENVIRONMENT

Figure 5 shows a method 1 of the present invention for cleaning an environment. At step 2, a cleaning personnel doses a fluid via a dosing system, preferably the dosing system 100 or dosing system 200 of the present invention, with a first oxychlorine compound to obtain a first cleaning fluid. The first cleaning fluid will also include water and can include detergents and/or other additives to make it suitable as a cleaning fluid. The cleaning personnel at step 3, then uses the first cleaning fluid to apply on (or disperse) and clean at least one surface in the environment at least once a day. At step

4, a release system releases a second oxychlorine compound in gaseous form into the environment, and at step 5, the environment is fogged with a fog comprising a third oxychlorine compound, preferably on a periodic basis, and more preferably once a month. Step 6 involves spraying a second cleaning fluid comprising a fourth oxychlorine compound via at least one hand-held spray means (preferably a bottle) on at least one surface in the environment. This step 6 may be done concurrently with any one of step 2 to 5 or 7, or in between steps 2 to 5 and 7. When oxychlorine compounds are applied to an environment in accordance with method 1, the environment can be adequately cleaned, decontaminated and be rid of microorganisms, particularly bacteria and viruses, without the overuse of oxychlorine compounds. Overuse of oxychlorine compounds may be dangerous due to the explosive nature of oxychlorine compounds, thereby causing danger to individuals that frequent the environment. Furthermore, inhalation of high levels of oxychlorine compounds can be unpleasant and overbearing for the users because oxychlorine compounds can have a pungent odour. Method 1 provides for a substantially high level of cleanliness in the environment, which reduces the amount and spread of microorganisms that cause diseases in individuals who are more susceptible to such diseases, such as children and the elderly, in that environment. This method 1 can therefore be used for safely cleaning areas or spaces where children and/or elderly frequent, thereby substantially reducing the occurrence of disease, particularly those resulting from microorganism infections. When there is an outbreak of a disease in an environment, method 1 will continue to step 7 which involves fumigating the environment with concentrated gaseous chlorine dioxide. The fumes in step 7 preferably comprise chlorine dioxide in the concentration range of about 1ppmv to about 300 ppmv.

While method 1 shows steps 2 to 5 (and to 7 in the event of an outbreak) in a sequential manner, it will be appreciated that certain steps may be done concurrently with another. For example, steps 2, 3 or 5 may be done concurrently with step 4 since step 4 involves a release system to generate and release chlorine dioxide, and does not involve manual labour unless when materials within the release system have to be replenished. Preferably, materials within the release system are replenished once a month.

Preferably, equipment in method 1 that will be in contact with chlorine dioxide during operation are made from material resistant to oxidation by chlorine dioxide. Preferably, such material comprises polyethylene terephthalate (PTE) and/or polytetrafluoroethylene (PTFE).

Method 1 is useful and effective in cleaning an environment having at least one virus from the genus *Enterovirus*, which is responsible for HFMD in children. In particular, method 1 is effective in cleaning an environment against Coxsackievirus A16, Coxsackievirus B2 and/or Enterovirus 71. Figure 6 shows that there was a significant increase of 33% in the total number of HFMD cases in week 13 of year 2016 compared to week 13 of year 2015. Therefore, there is a need to curb the rise of viral disease occurrences, especially those involving children and the elderly.

Figure 7 shows that the occurrences of HFMD cases in a childcare centre were significantly reduced after the application of method 1. Further, any occurrences of HFMD after the application of method 1 were due to a new strain of HFMD virus that had infected one of the users of the childcare centre where method 1 was applied.

Steps 2 and 3 - Dosing

Preferably, the first oxychlorine compound in the dosing system of step 2 comprises chlorine dioxide solution. More preferably, the chlorine dioxide in the solution comprising the first oxychlorine compound is in a concentration range of about 20,000 ppm to about 60,000 ppm, preferably about 30,000 ppm to about 60,000 ppm, and more preferably about 50,000 ppm.

The aqueous chlorine dioxide in the dosing system of step 2 preferably comprises stabilized chlorine dioxide solution because stabilized chlorine dioxide solution is safe for transportation. The stabilized chlorine dioxide solution may be activated prior to dosing a fluid to obtain the first cleaning fluid. It will be understood that activation involves lowering of the pH of the stabilized chlorine dioxide solution, which includes but is not limited to the addition of an acid to the stabilized chlorine dioxide solution. Activation of the stabilized chlorine dioxide solution may be done on-site at the intended environment, or off-site before transportation to the intended environment. It will be appreciated that if it is the latter arrangement, the distance between the site of activation and the intended environment should preferably not be a substantial distance since activated aqueous chlorine dioxide can generate and emit chlorine dioxide gas that is unstable, reactive and explosive.

Depending on the application and requirements, the chlorine dioxide solution of step 2 may be used as a stabilized chlorine dioxide solution or an activated chlorine dioxide solution. A stabilized chlorine dioxide solution can function as a bacteriostat by removing the biofilm in water logged areas where microorganisms such as bacteria and viruses reside. The removal of the biofilm reduces and prevents the spread of microorganisms because without the biofilm, such microorganisms cannot grow and survive. Accordingly, a stabilized chlorine dioxide solution would be suitable to maintain the cleanliness of an environment from microorganisms. An activated chlorine dioxide solution has strong oxidising properties and can function as a bactericide which kills bacteria. An activated chlorine dioxide solution is also capable of attenuating viruses. Accordingly, an activated chlorine dioxide solution would be suitable to clean an environment to a desired state suitable for use by individuals, such as children and the elderly.

The oxychlorine compound is diluted in step 2. Therefore, after dosing the fluid with the first oxychlorine compound, the first cleaning fluid in step 3 preferably comprises chlorine dioxide in a concentration range of about 50 ppm to about 900 ppm, preferably about 50 ppm to about 300 ppm, more preferably about 50 ppm to about 150 ppm and even more preferably about 100 ppm. This concentration range of chlorine dioxide is safe for users in the environment and effective against microorganisms.

The cleaning personnel at step 3, then uses the first cleaning fluid to apply on (or disperse) and clean at least one surface in the environment at least once a day.

Step 4 – Release System

In step 4, the release system generates and releases gaseous oxychlorine compound, preferably gaseous chlorine dioxide into the environment. The release system preferably comprises a precursor of the oxychlorine compound and a dry hygroscopic material adapted to generate and release the oxychlorine compound in the presence of water, wherein the oxychlorine compound is chlorine dioxide. Preferably, the hygroscopic material comprises an activator adapted to react with the precursor in the

presence of water to generate and release the second oxychlorine compound, wherein the precursor is a metal chlorite and the activator is an acid. The use of a hygroscopic material comprising an acid allows for the absorption of water, in the form of water vapour, which provides a medium for the acid to react with the metal chlorite to generate chlorine dioxide, particularly in its gaseous form. Preferably, the release system is adapted to generate and release chlorine dioxide gas when the relative humidity in the environment is above 70%. Therefore, the type of hygroscopic material (i.e. having different measures of hygroscopicity) used and the strength of the acid can affect the period of release of the chlorine dioxide from the release system, for example, a release system with a hygroscopic material having a weak activator (e.g. weak acid) will generate less chlorine dioxide compared to a release system with a hygroscopic material having a strong activator (e.g. strong acid). However, a release system with a hygroscopic material having a weak activator (e.g. weak acid) will generate chlorine dioxide over a longer period of time compared to a release system with a hygroscopic material having a strong activator (e.g. strong acid). Accordingly, the duration and amount of chlorine dioxide gas released can be controlled by manipulating the materials (e.g. the hygroscopic material, precursor and activator) in the release system according to the given relative humidity percentage of the environment. Depending on the application and requirements, the precursor of the oxychlorine compound and the hygroscopic material (which can include an acid) can be provided in the form of a cartridge which allows the release system to be replenished on a periodic basis. For example, a cartridge which no longer generates and releases a desired amount of gaseous chlorine dioxide can be replaced by a new and fresh cartridge.

The release system can be a slow control sustainable release system which is configured to slowly release the gaseous chlorine dioxide into the environment. Preferably, the concentration of the gaseous chlorine dioxide released into the environment by the slow control sustainable release system is less than 0.1 ppmv.

The release system can also be a fast control sustainable release system which is configured to generate and release the gaseous chlorine dioxide over 24 to 48 hours. Such fast control sustainable release systems can be used over a weekend where the environment is unused by individuals.

The release system can be mounted at various sites in the environment depending on the application of the present invention. A slow control sustainable release system is preferably mountable onto a surface in the environment, near to a vent (i.e. air vent). It will be understood that "near" would mean a distance that allows for the generated chlorine dioxide gas to be vented and circulated in the environment. It will be appreciated that this distance will depend on the rate of generation of chlorine dioxide, whereby if chlorine dioxide gas is generated quickly and in substantially large volumes, the release system may be located further away from the vent compared to a release system that generates chlorine dioxide gas slowly and in small amounts. Preferably, the release system is mounted on the vent. The gaseous chlorine dioxide from release system is useful in the prevention of mould and mildew which typically accumulate around air vents. A fast control sustainable release system can be mounted in confined areas at specific sites in the environment to effectively decontaminate such sites, for example, in cupboards and in boxes to destroy mould and microorganisms.

Step 5 - Fogging

Step 5 involves fogging the environment via a fog machine or fog generator, with a third oxychlorine compound where the third oxychlorine compound comprises chlorine

dioxide. Preferably, the chlorine dioxide in the fog is in a concentration range of about 500 ppm to about 1,000 ppm, preferably about 500 ppm to about 900 ppm, and more preferably about 800 ppm to about 900 ppm. Fogging of the environment according to step 5 preferably occurs once a month and is done by trained fogging personnel.

The fog according to step 5 is generated from a chlorine dioxide solution, where the fog machine or generator aerosolizes the chlorine dioxide solution into a fine spray or mist of micro-droplets for fogging the environment. Depending on the application and requirements, the chlorine dioxide solution may be a stabilized chlorine dioxide solution or an activated chlorine dioxide solution. After fogging, any fog residual on the surfaces of the environment, may be allowed to dry up, i.e. the water in the chlorine dioxide solution evaporates. This provides a protective coating of chlorine dioxide on the surfaces of the environment which prevents and/or minimizes the growth and spread of microorganisms in the environment.

Step 6 - Spray

The method 1 also includes a further step 6 of spraying a second cleaning fluid comprising a fourth oxychlorine compound via at least one hand-held spray means (preferably a bottle) on at least one surface in the environment. This step 6 may be done concurrently with any one of step 2 to 5 or 7, or in between steps 2 to 5 and 7. This step 6 allows for a user of the environment to efficiently and effectively address any soiling, fouling, dirtying, etc. of a surface of the environment, to maintain a clean condition of the environment. Preferably, the fourth oxychlorine compound in step 6 comprises stabilized chlorine dioxide solution, and more preferably, the chlorine dioxide in the second cleaning fluid is in a concentration range of about 500 ppm to about 1,000 ppm, preferably about 500 ppm to about 900 ppm, and more preferably about 800 ppm. This concentration range of chlorine dioxide is safe for users in the environment and effective against microorganisms.

Step 7 – Fumigation

When there is an outbreak of a disease in an environment, method 1 will continue to step 7 which involves fumigating the environment with concentrated gaseous chlorine dioxide. The fumes in step 7 preferably comprise chlorine dioxide in the concentration range of 1ppmv to 300 ppmv. During fumigation, the environment is sealed off from users, and gaseous chlorine dioxide is introduced into the sealed environment. The gaseous chlorine dioxide is strongly oxidising and will effectively kill and attenuate microorganisms in the environment. Consequently, step 7 decontaminates the environment.

EXAMPLES

1. Dermal Irritation Test

A safety test was conducted to assess and evaluate the potential of a stabilized chlorine dioxide solution at the concentration of 50,000 ppm ("Test Sample 01") in inducing irritation or corrosion to the skin after intimate contact with the stabilized chlorine dioxide solution. Information derived from such test indicates the existence of possible health hazards associated with contact exposure of skin to the stabilized chlorine dioxide solution. The safety test was conducted in accordance with the procedures as outlined in Hygienic Standard for Cosmetics (2002) issued by Ministry of Health, P.R. China, which is incorporated by reference herein, and was conducted on albino New Zealand

rabbits. The rabbits were observed for at least 3 days for signs of illness or disease prior to initiating the tests.

Materials and Methods

5

Animal and Husbandry

10 The albino New Zealand rabbit (License No. CXK (JING) 2002-0005) supplied by Beijing Keyu Animal Breeding Centre is the preferred species for these tests and the New Zealand strain rabbit is the most often used. Animals were observed for at least 3 days for signs of illness or disease prior to initiating tests. Animals were cared for in the following standard practices.

15 Upon delivery, each animal (1.8 – 2.0 kg) is kept in a separated case with raised flooring suspended over drip pans lined with absorbent paper bedding. Food and water are available *ad libitum*. Food rations are dispensed daily provided by Chinese Academy of Medical Sciences, Experimental Animal Research Institute Affiliated Fodder Company. Water supplied was tap water. Animals were housed in a conditioned room kept at 23°C±2°C, relative humidity 55% ± 5%.

20

Procedure

Preparation of Test Animals

25 Four albino rabbits (2 females, 2 males) were selected for the test procedure. Before 24 hours of sample administration, the fur of the skin at both sides of the backbone with area of 6 cm² (3 x 2 cm) was shaved. Care was taken to avoid abrading the skin when shaving. Fur removal cream was applied to ensure all fur was removed. The test sites were examined for any wound. Each of the four animals was treated with
30 Test Sample 01 on one side of the shaved skin, opposite site was treated with sterilized distilled water as control.

Sample Administration

35 An aliquot of 0.5 g of Test Sample 01 was evenly spread on a 2.5 x 2.5 cm² test skin site (left). Same amount of sterilized distilled water were applied to the control site (right). The patches were contained under an adhesive dressing to hold the Test Sample 01 in place. The trunks of the animals were then wrapped with an elastic bandage and tape to prevent the animals from removing or ingesting the Test Sample 01. Animals
40 were not restrained during or after the 2 hours exposure period.

Clinical Observations and Scaling

45 At the end of the exposure period of 2 hours, the adhesive patches were removed and the skin was cleaned of the Test Sample 01 using warm water. The test skin sites were evaluated at 1, 24, 48 and 72 hours post exposure observation times as part of the basis for the following weighed rating scale (Table 1a):

50

Table 1a: Weighed rating scale for Table 1.

Erythema and Eschar Formation	Weighed Value
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema to slight eschar formation	4
Edema Formation	Weighed Value
No edema	0
Very slight edema	1
Slight edema	2
Moderate edema (area raised approximately 1mm)	3
Severe edema (area raised more than 1mm and extending beyond area of exposure)	4
Total maximum score for primary irritation	8

Results

5

According to Table 1, the stabilized chlorine dioxide solution at the concentration of 50,000 ppm is considered to be a non-skin irritant (see Appendix 1). Consequently, an environment can be cleaned of microorganisms, particularly bacteria and viruses, without causing skin irritation to individuals who may be exposed to the stabilized chlorine dioxide solution.

10

Table 1: Dermal Irritation test for stabilized chlorine dioxide solution at 50,000 ppm, conducted on albino New Zealand rabbits.

Irritation response	Observation Period (hrs)	Groups	Individual Animals Test Values				Average
			1	2	3	4	
Erythema-Eschar	1	Test	0	0	0	0	0
		Control	0	0	0	0	0
Edema	1	Test	0	0	0	0	0
		Control	0	0	0	0	0
Erythema-Eschar	24	Test	0	0	0	0	0
		Control	0	0	0	0	0
Edema	24	Test	0	0	0	0	0
		Control	0	0	0	0	0
Erythema-Eschar	48	Test	0	0	0	0	0
		Control	0	0	0	0	0
Edema	48	Test	0	0	0	0	0
		Control	0	0	0	0	0

15

Appendix 1: Classification of Skin Irritant According to Irritation Test Value for Table 1

Category	Average Test Value
No skin irritant	0 – <0.5
Slight skin irritant	0.5 – <2.0
Moderate skin irritant	2.0 – <6.0
Severe skin irritant	6.0 – 8.0

2. Acute Oral Toxicity Study in KM Mice

A safety test was conducted to determine if acute health hazards are associated with ingestion of a stabilized chlorine dioxide solution having a concentration of 50,000 ppm ("Test Sample 02"). The measure of acute toxicity can be expressed as the median lethal dose (LD50), a statistically derived value that estimates the dose that would theoretically kill 50% of the test animal group. The safety test was conducted in accordance with the procedures as outlined in Guidelines of New Traditional Chinese Medicine study, Toxicology Study Section (1994) issued by Ministry of Health, P.R. China, which is incorporated by reference herein.

Materials and Methods

Test Animals

Species: Mice
 Strain: KM mice clean animal
 Source: Institute of Experimental Animals, Chinese Academy of Medical Sciences
 Diet: Institute of Experimental Animals, Chinese Academy of Medical Sciences
 Quality Certificate No.: SCXK-11-00-0006
 Date Received: 23/04/2004

Upon arrival, an equal number of male and female were randomly assigned to a control group or treatment group. Animals were housed by sex in the observation battery rack and stained the hair on different part of the body for animal identification. Animals were observed for at least 2 days for signs of illness or disease prior to initiating tests.

Procedure

White KM mice (male and female, Quality Certificate No.: SCK-11-00-0006), each weighing between 18 and 20 grams were selected for each dosage. The animals were housed in plastic cages with stainless steel wire mesh caps, the floors of the cages were put with softwood sawdust. The temperatures of animal room was 23±2°C, humidity 50±10%. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness. Animals were maintained on a commercial mouse food diet and water was available *ad libitum*. Twelve hours prior to dosing, all food was removed to fast the animals before initiating the test. On the day of the test, animals were identified and body weights recorded. The dosage to be administered was calculated based on the animal's body weight. The maximum volume is 0.4ml/10g body weight. The control group was treated with the same amount of distilled water.

For test articles that are liquids or could be administered as solutions, suspensions or extracts, appropriate doses were administered to animals using a feeding

needle and syringe. For certain solid-form test articles, doses were administered by incorporating the material into a feed mix that was fed to laboratory animals over 24 hours period. The method of sample administration used for the Test Sample 02 is outlined in the Sample Preparation section herein.

In Sighting Study, 5 female and 5 male mice per group were treated with the Test Sample 02 diluted to different dosage concentration in distilled water. The mice were observed for mortality and clinical signs after dosing. The dosage for 0% and 100% mortality were estimated.

Based on the Sighting Study, the Main Study was carried out with 5 groups, each group consisting of 5 female and 5 male mice treated with single dose from 1148.18 mg/kg to 1750 mg/kg body weight for test animals. The control group was treated with the same amount of distilled water.

Animals were closely observed for mortality and gross toxicological effects immediately after a single dose administration of the Test Sample 02 and then daily for a 7-day observation period. Necropsies of dead, moribund or surviving animals were performed if indicated during the progression of the study.

Sample Preparation

A test solution was prepared by dissolving the Test Sample 02 with distilled water to a concentration of 0.25 mg/ml. A single dose administration of 0.4 ml/10g body weight using needle and syringe was given. Each animal was fasted 12 hours prior to selection and test initiation and then continuously for 3 hours after treatment. The control group was treated with the same amount of distilled water.

Clinical Signs

Each mouse was observed for mortality and clinical signs at 1, 3 and 6 hours after administration during Day 1 and thereafter daily for a period of 7 consecutive days. Any mental status, mortality, food consumption, activity and gross anatomy were recorded.

Statistical Analysis

LD50 was determined using SPSS 10.0 or Windows.

Results

In Sighting Study, a bluish discoloration of the skin (cyanosis) in experimental animals were observed after the Test Sample 02 was given orally. Animal death happened within 10 minutes to 24 hours after treatment at the dosage of 1547.96 mg/kgbw. The colour of liver and blood turned to purple were observed under gross anatomy examination. There were no noticeable changes in other organs. The dosage of 100% mortality was 1547.96 mg/kgbw and the maximum tolerated dose was 1148.18 mg/kgbw. No sexual differences in toxicity were observed. So in Main Study, each group consisted of 5 female and 5 male mice.

In Main Study, the observations were the same as that in the Sighting Study. The number of dead animals in each dosage are shown in Table 2 below.

Table 2: Result of acute toxicity for aqueous chlorine dioxide in 50,000 ppm, conducted in KM mice.

Dose (mg/kgbw)	No. of test animal	No. of animal died	LD50 and its 95% confidence interval
1750.00	10	8	1535.93 mg/kgbw, (1433.91 mg/kg – 1682.98 mg/kg)
1575.00	10	5	
1417.50	10	4	
1275.75	10	2	
1148.18	10	0	

5 Appendix 2: Acute Toxicity (LD50) Classification (GB 15193.3-94, Appendix D) for Table 2.

Category	LD50 (mg/kg)
Extremely toxic	1.0 or less
Highly toxic	1.0 – 50.0
Moderately toxic	51.0 – 500.0
Slightly toxic	501.0 – 5000.0
Practically non-toxic	5001.0 – 15,000.0
Non-toxic	>15,000

10 When tested as specified, the LD50- of the submitted Test Sample 02 was found to be 1535.93 mg/kgbw with 95% confidence limits of 1433.91 mg/kg (lower) and 1682.98 mg/kg (upper). According to Table 2, the stabilized chlorine dioxide solution at the concentration of 50,000 ppm is considered to be slightly toxic (see Appendix 2).

3. Acute Oral Toxicity Study in Rats

15 An acute oral toxicity study of a 900 ppm ("Test Sample 03") stabilized chlorine dioxide solution was conducted on rats.

Materials and Methods

20 Preparation of Test Substance

Test Sample 03 was used as test substance and used for dosing directly without any pre-treatment.

25 A single dose was administered orally to each animal based on the body weight using gavage feeding needle.

Test Animals

Species:	Rats
Strain:	SD
Microbiological status:	Murine Pathogen Free (MPF)
Age:	8-12 weeks old
Sex:	Female
Number:	6
Source:	InVivos Pte Ltd 9 Perahu Road, Lim Chu Kang,

Animal Holding Facility: Singapore 718793
 Animal Holding Unit
 TÜV SÜD PSB Pte Ltd
 No 1 Science Park Drive
 Singapore 118221
 Housing Condition: OptiMICE Caging System
 Temperature: 19 - 25°C
 Humidity: 30 – 70%
 Food: Altromin Maintenance Diet #1324
 Water: Tap water
 Animal ID: 7191136416-01-G1-1~3
 7191136416-01-G2-1~3

Test Conditions

Preparation of test animals

5

The test animals were acclimatised for at least 5 days before the test was conducted.

10

The test animal were fasted overnight before dosing. Feed, but not water was withheld. The animals were weighted prior to dosing. Test Sample 03 was then administered by gavage feeding needle at a starting dose level of 2000 mg/kg body weight based on the body eight of each test animal.

15

Rationale of selection of stating dose

According to OECD Guideline for testing of Chemicals 423, the limit dose of 2000 mg/kg is used for the test item which the toxicity or mortality is not expected. Thus, starting dose of 2000 mg/kg was selected according to the requirement of sponsor.

20

Administration level at 2000 mg/kg body weight

Administration route: Oral route by gavage feeding needle
 Dose level: 2000 mg/kg body weight
 Dose interval: Single dose

25

The details for each animal are as follows:

Table 3A: Dosage of Group 1

Group	Animal ID	Dosing Date	Body weight (g)	Amount of test substance used during dosing (mg)
Group 1	7191136416-01-G1-1	04 May 2016	180	360
	7191136416-01-G1-2		176	352
	7191136416-01-G1-3		190	380

Table 3B: Dosage of Group 2

Group	Animal ID	Dosing Date	Body weight (g)	Amount of test substance used during dosing (mg)
Group 2	7191136416-01-G2-1	05 May 2016	190	380
	7191136416-01-G2-2		178	356
	7191136416-01-G2-3		192	384

Feed and water frequency

Animals were fasted overnight before dosing. Feed, but not water was withheld.

Feed was given about 3 hours after dosing and throughout the observation period. Feed was given in the chamber in the cage.

Water was given ad libitum during dosing and observation period. Water was given through plastic bottle.

Observation, body weight measurement and necropsy

The observation was conducted on each animal during the first 30 minutes, 1, 2 and 4 hours, and daily thereafter to 14 days.

The body weight of each animal was measured once a week.

On the termination day, all the test animals were euthanized by CO₂ inhalation. Gross necropsy was conducted on each test animal.

*Test Results*Observation of each Test Animal

Table 3C: Observation of Group 1

Group	Animal ID	Observation during 14-day period
Group 1	7191136416-01-G1-1	No adverse effects observed
	7191136416-01-G1-2	No adverse effects observed
	7191136416-01-G1-3	No adverse effects observed

Table 3D: Observation of Group 2

Group	Animal ID	Observation during 14-day period
Group 2	7191136416-01-G2-1	No adverse effects observed
	7191136416-01-G2-2	No adverse effects observed
	7191136416-01-G2-3	No adverse effects observed

Body weight (BW, in gram) and Body weight changes (CH, in gram) of each animal

Table 3E: Body weight and body weight change of Group 1

Group	Animal ID	Day 0 (before dosing) (04 May 2016)	Day 7 (11 May 2016)		Day 14 (Termination Day) (18 May 2016)	
		BW	BW	CH	BW	CH
Group 1	7191136416-01-G1-1	180	214	+34	218	+4
	7191136416-01-G1-2	176	204	+28	216	+12
	7191136416-01-G1-3	190	220	+30	220	±0

5

Table 3F: Body weight and body weight change of Group 2

Group	Animal ID	Day 0 (before dosing) (04 May 2016)	Day 7 (11 May 2016)		Day 14 (Termination Day) (18 May 2016)	
		BW	BW	CH	BW	CH
Group 2	7191136416-01-G2-1	190	210	+20	220	+10
	7191136416-01-G2-2	178	208	+30	218	+10
	7191136416-01-G2-3	192	234	+42	228	-6

No obvious body weight loss (>10%) was found in all animals.

10

Death Prior to Endpoint

No animals died before the endpoint, i.e. 14 days after dosing.

15

Onset of Toxicity and Reversal

No toxicity effect was observed on all the test animals during dosing and observation period.

20

Necropsy Findings

No abnormality was observed on all the test animals.

Discussion

25

Based on the above study,

- No animal died during the study.
- No adverse effect was observed on all the test animals during the study.
- No obvious body weight loss (>10%) was observed during the study.
- No abnormality was observed on all animals during necropsy.

Conclusion

Under the condition of this study and based on Global Harmonised Classification System (GHS) for acute toxicity hazard categories, the acute oral toxicity of the Test Sample 03 is considered as Category 5 or unclassified; the LD₅₀ cut-off value of the Test Sample 03 is 5000 mg/kg body weight or unclassified.

4. Antimicrobial Activity Evaluation – 50 ppm of Chlorine Dioxide Solution

Materials and Methods

Test Sample

50 ppm of stabilized chlorine dioxide solution ("Test Sample 04").

Method

BS EN 1040 : 2005

"Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics – Test method and requirements (phase 1).

The test microorganisms used were:

- *Staphylococcus aureus* (ATCC 6538)
- *Pseudomonas aeruginosa* (ATCC 15442)
- *Escherichia coli* (ATCC 8739)
- Methicillin Resistant *Staphylococcus aureus* (NCTC 12493)

Dilution tested: Neat

Contact Time: 5 minutes and 10 minutes

Neutralization broth: D/E Neutralization broth

Results

With reference to Table 4, the chlorine dioxide solution shall be deemed to have passed the test if it demonstrates a 5 Log reduction or more (at least >99.999% kill) in viability within 5 minutes or less under the conditions defined by this test when the test organisms are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Table 4: Antimicrobial activity evaluation of a 50 ppm chlorine dioxide solution

Organism	Concentration / Contact Time	Initial Count of Test Microorganism per ml of Test Mixture		Count of Surviving Test Microorganism per ml		Log Reduction	Percentage Kill of Test Microorganism
		CFU per ml	Log ₁₀	CFU per ml	Log ₁₀		
<i>Staphylococcus aureus</i> (ATCC 6538)	Neat 5 minutes	81 000 000	7.91	Less than 10	Less than 1.00	More than 6.91	More than 99.999988
	10 minutes	81 000 000	7.91	Less than 10	Less than 1.00	More than 6.91	More than 99.999988
<i>Pseudomonas aeruginosa</i> (ATCC 15442)	Neat 5 minutes	110 000 000	8.04	Less than 10	Less than 1.00	More than 7.04	More than 99.99991
	10 minutes	110 000 000	8.04	Less than 10	Less than 1.00	More than 7.04	More than 99.99991
<i>Escherichia coli</i> (ATCC 8739)	Neat 5 minutes	150 000 000	8.18	Less than 10	Less than 1.00	More than 7.18	More than 99.999993
	10 minutes	150 000 000	8.18	Less than 10	Less than 1.00	More than 7.18	More than 99.999993
Methicillin Resistant <i>Staphylococcus aureus</i> (NCTC 12493)	Neat 5 minutes	130 000 000	8.11	Less than 10	Less than 1.00	More than 7.11	More than 99.999992
	10 minutes	130 000 000	8.11	Less than 10	Less than 1.00	More than 7.11	More than 99.999992

5. Antimicrobial Activity Evaluation – 100 ppm of Chlorine Dioxide Solution

Materials and Methods

5 Test Sample

100 ppm of stabilized chlorine dioxide solution ("Test Sample 05").

10 Method

BS EN 1040 : 2005

15 "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics – Test method and requirements (phase 1).

The test microorganisms used were:

- *Staphylococcus aureus* (ATCC 6538)
- *Pseudomonas aeruginosa* (ATCC 15442)
- 20 - *Escherichia coli* (ATCC 8739)
- Methicillin Resistant *Staphylococcus aureus* (NCTC 12493)

Dilution tested: Neat

Contact Time: 5 minutes and 10 minutes

25 Neutralization broth: D/E Neutralization broth

Results

30 With reference to Table 5, the chlorine dioxide solution (Test Sample 05) shall be deemed to have passed the test if it demonstrates a 5 Log reduction or more (at least >99.999% kill) in viability within 5 minutes or less under the conditions defined by this test when the test organisms are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Table 5: Antimicrobial activity evaluation of a 100 ppm chlorine dioxide solution

Organism	Concentration / Contact Time	Initial Count of Test Microorganism per ml of Test Mixture		Count of Surviving Test Microorganism per ml		Log Reduction	Percentage Kill of Test Microorganism
		CFU per ml	Log ₁₀	CFU per ml	Log ₁₀		
<i>Staphylococcus aureus</i> (ATCC 6538)	Neat 5 minutes	81 000 000	7.91	Less than 10	Less than 1.00	More than 6.91	More than 99.999988
	10 minutes	81 000 000	7.91	Less than 10	Less than 1.00	More than 6.91	More than 99.999988
<i>Pseudomonas aeruginosa</i> (ATCC 15442)	Neat 5 minutes	110 000 000	8.04	Less than 10	Less than 1.00	More than 7.04	More than 99.99991
	10 minutes	110 000 000	8.04	Less than 10	Less than 1.00	More than 7.04	More than 99.99991
<i>Escherichia coli</i> (ATCC 8739)	Neat 5 minutes	150 000 000	8.18	Less than 10	Less than 1.00	More than 7.18	More than 99.999993
	10 minutes	150 000 000	8.18	Less than 10	Less than 1.00	More than 7.18	More than 99.999993
Methicillin Resistant <i>Staphylococcus aureus</i> (NCTC 12493)	Neat 5 minutes	130 000 000	8.11	Less than 10	Less than 1.00	More than 7.11	More than 99.999992
	10 minutes	130 000 000	8.11	Less than 10	Less than 1.00	More than 7.11	More than 99.999992

6. Antimicrobial Preservatives – Effectiveness of a 100 ppm of Chlorine Dioxide Solution (“Test Sample 06”)

Materials and Methods

Antimicrobial Preservatives – Effectiveness was conducted with reference to the United States Pharmacopeia, The National Formulary, USP 26, 2003, General Chapter 51. Duration of time was modified to 60 seconds.

Tested Organisms:

- *Candida albicans* (ATCC No. 10231)
- *Escherichia coli* (ATCC No. 8739)
- *Pseudomonas aeruginosa* (ATCC No. 9027)
- *Staphylococcus aureus* (ATCC No. 6538)
- *Salmonella* (ATCC 13076)
- *Legionella pneumoniae* (ATCC 33152)
- *Klebsiella pneumoniae* (ATCC 4352)

Results

Table 6: Antimicrobial effectiveness of a 100 ppm chlorine dioxide solution (Test Sample 06).

Organisms	Inoculum used (cfu/ml)	Inoculum recovered after 60 secs* (cfu/ml)	% reduction
<i>Candida albicans</i> (ATCC No. 10231)	2.1 x 100000	<1	>99.9
<i>Escherichia coli</i> (ATCC No. 8739)	5.9 x 1000000	<1	>99.9
<i>Pseudomonas aeruginosa</i> (ATCC No. 9027)	8.9 x 100000	<1	>99.9
<i>Staphylococcus aureus</i> (ATCC No. 6538)	1.0 x 1000000	<1	>99.9
<i>Salmonella</i> (ATCC No. 13076)	8.0 x 100000	2.3 x 100	>99.9
<i>Legionella pneumoniae</i> (ATCC No. 33152)	7.3 x 1000000	<1	>99.9
<i>Klebsiella pneumoniae</i> (ATCC No. 4352)	9.6 x 100000	<1	>99.9

Note:

< = Less than

> = More than

cfu = colony forming unit

* The testing was made on the diluted sample (100 ppm) prepared from diluting 50,000 ppm of chlorine dioxide solution.

7. Efficacy study of a 50 ppm of chlorine dioxide solution on enterovirus (Enterovirus 71) causing Hand, Foot and Mouth Disease (HFMD)

Materials and Methods

Test Product

50 ppm of chlorine dioxide solution ("Test Sample 07")

5 Test Viruses and Host Cells

Virus: Enterovirus 71 (EV71)

Cell line used: Rhabdomyosarcoma (RD) cells

10 Test Conditions

Host RD cells were left to grow in 96-well plates to reach 100% confluency for the test. Reagents were prepared – 5 ml pen-strep was added into a 500 ml MEM, which was then filtered through 0.2 µm filter. This MEM was then added with heat-inactivated fetal bovine serum to concentration of 2%. This 2% MEM is the media used for maintenance of culture, as well as a diluent. Neutraliser prepared by dissolving 0.06g of lab-grade sodium thiosulfate in 2% MEM.

20 Test Methods

Test Methods were as follows:

A) The following procedures were done in 15 ml falcon tubes.

- 25 - Test:
1. Equal parts of test virus (1 ml) and Test Sample 07 (1 ml) were mixed and left incubated for 5 time points: 15 sec, 1 min, 2 mins, 5 mins, and 30 mins.
 2. At each time point, 0.2 ml of the virus-product solution was collected into 0.1 ml of neutraliser.
 3. The final solution was serially diluted; 1 part solution in 9 parts diluent.
- 30 - Product Control:
1. 0.1 ml of Test Sample 07 was mixed with 0.2 ml diluent.
 2. The solution was serially diluted; 1 part solution in 9 parts diluent.
- 35 - Neutraliser Control:
1. 0.1 ml of neutraliser was added with 1.9 ml 2% MEM.
 2. The solution was serially diluted; 1 part solution in 9 parts diluent.
- 40 - Virus (positive) Control:
1. 0.1 ml virus was added with 1.9 ml 2% MEM.
 2. The solution was serially diluted; 1 part solution in 9 parts diluent.
- 45 - Each final solution (test, product control, neutraliser control and virus control) was serially diluted 8 times; 1 part solution in 9 parts diluent.
- 50 B) Media from the cell culture was removed and washed 1X with phosphate buffered saline (PBS). PBS was then removed.
- C) 0.2 ml from each dilution tube was inoculated into each well, accordingly. Controls were added into the wells without replicates. Test solutions were

added in 8 replicates. For negative control, 2% MEM was added in to the wells.

Results

The plates were checked after an overnight incubation, and results were tabulated with regards to the degree of infection per well. All the wells were observed for cytopathic effects (CPE; cell death) under the light microscope. Each well is graded with level of CPE (%).

Table 7A – Time Point: 15 seconds

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	80	70	50	0	0	0	0	0
	100	70	50	10	0	0	0	0
	100	80	50	5	0	0	0	0
	100	70	50	5	0	0	0	0
	90	80	50	10	0	0	0	0
	90	70	60	10	0	0	0	0
	90	80	60	10	5	0	0	0
	90	80	60	5	0	0	0	0

Table 7B – Time point: 1 minute

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	80	50	20	0	0	0	0	0
	80	50	20	0	0	0	0	0
	80	65	30	5	0	0	0	0
	80	60	10	0	0	0	0	0
	70	65	10	0	0	0	0	0
	80	65	10	0	0	0	0	0
	80	70	10	0	0	0	0	0
	80	70	5	0	0	0	0	0

Table 7C – Time point: 2 minutes

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	70	40	5	0	0	0	0	0
	70	40	0	0	0	0	0	0
	70	45	0	0	0	0	0	0
	70	40	0	0	0	0	0	0
	70	50	0	0	0	0	0	0
	70	40	0	0	0	0	0	0
	70	45	0	0	0	0	0	0
	70	50	5	0	0	0	0	0

Table 7D – Time point: 5 minutes

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	70	45	10	0	0	0	0	0
	65	45	5	0	0	0	0	0
	70	40	0	0	0	0	0	0
	70	50	0	0	0	0	0	0
	65	45	0	0	0	0	0	0
	70	50	0	0	0	0	0	0
	70	50	0	0	0	0	0	0
	70	40	0	0	0	0	0	0

Table 7E – Time point: 30 minutes

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

5 Results for positive and negative wells

Table 8A – Positive control

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	100	90	70	30	0	0	0	0

Table 8B – Negative control

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	0	0	0	0	0	0	0	0

The degree of CPE of the cells was recorded to determine the TCID₅₀ which means median tissue culture infectious dose. TCID₅₀ provides us with the viral titre (amount of virus). To put it simply, the lower the TCID₅₀, the lesser virus present, which also means the more effective the test solution is – vice versa. Results of the test were compared against the positive control.

Based on the results tabulated above, TCID₅₀ was then determined by following a set of calculations, the final calculated results are charted in Figure 8. Time 0 represents the TCID₅₀ for the positive control. After exposure of virus to the test solution, a decrease in TCID₅₀ can be seen until it reaches 0 at 30 minutes.

In Figure 8, time 0 represents the viral titre before the exposure to the Test Sample 07. After 15 seconds exposure, the viral titre decreases from 3.5 TCID₅₀/mL to 3.07 TCID₅₀/mL, and generally continues until 30 minutes where viral titre is 0. The slight increase at the 5 minutes time point. Based on the tabulated CPE levels, the values between 2 minutes and 5 minutes are similar. Therefore, this increase is not significant.

The test solution is therefore proven to be effective in virucidal activity.

8. Efficacy study of a 100 ppm of chlorine dioxide solution on enterovirus (Coxsackie B2) causing Hand, Foot and Mouth Disease (HFMD)

Materials and Methods

5

Test Product

100 ppm of chlorine dioxide solution ("Test Sample 08")

10 Test Viruses and Host Cells

Virus: Coxsackie B2 (CB2)

Cell line used: Rhabdomyosarcoma (RD) cells

15 Test Conditions

Host RD cells were left to grow in 96-well plates to reach 100% confluency for the test. Reagents were prepared – 5 ml pen-strep was added into a 500 ml MEM, which was then filtered through 0.2 µm filter. This MEM was then added with heat-inactivated fetal bovine serum to concentration of 2%. This 2% MEM is the media used for maintenance of culture, as well as a diluent. Neutraliser prepared by dissolving 0.24g of lab-grade sodium thiosulfate in 40 mL 2% MEM.

25 Test Methods

Test Methods were as follows:

A) The following procedures were done in 15 ml falcon tubes.

- 30 - Test:
1. Equal parts of test virus (1 ml) and Test Sample 08 (1 ml) were mixed and left incubated for 5 time points: 15 sec, 1 min, 2 mins, 5 mins, and 30 mins.
 - 35 2. At each time point, 0.2 ml of the virus-product solution was collected into 0.1 ml of neutraliser.
 3. The final solution was serially diluted; 1 part solution in 9 parts diluent.
- Product Control:
- 40 1. 0.1 ml of Test Sample 07 was mixed with 0.2 ml diluent.
 2. The solution was serially diluted; 1 part solution in 9 parts diluent.
- Neutraliser Control:
- 45 1. 0.1 ml of neutraliser was added with 0.2 ml 2% MEM.
 2. The solution was serially diluted; 1 part solution in 9 parts diluent.
- Virus (positive) Control:
- 50 1. 0.1 ml virus was added with 0.2 ml 2% MEM.
 2. The solution was serially diluted; 1 part solution in 9 parts diluent.
- Each final solution (test, product control, neutraliser control and virus control) was serially diluted 8 times; 1 part solution in 9 parts diluent.

B) Media from the cell culture was removed and washed 1X with phosphate buffered saline (PBS). PBS was then removed.

5 C) 0.1 ml from each dilution tube was inoculated into each well, accordingly. Controls were added into the wells without replicates. Test solutions were added in 4 replicates. For negative control, 2% MEM was added in to the wells.

Results

10 The plates were checked after an overnight incubation, and results were tabulated with regards to the degree of infection per well. All the wells were observed for cytopathic effects (CPE; cell death) under the light microscope. Each well is graded with level of CPE (%).

15 Results for positive wells

Table 8A – Positive control

Dilution	-1	-2	-3	-4	-5	-6	-7	-8
CPE (%)	100	100	95	90	80	10	0	0
	100	100	100	90	85	10	0	0
	100	100	100	90	85	10	0	0
	100	100	100	90	85	5	0	0

20 Results for test plates

Table 8B – Time point: 15 seconds

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	100	100	95	90	70	5	0	0
	100	100	100	95	70	10	0	0
	100	100	100	95	85	5	0	0
	100	100	100	95	85	5	0	0

Table 8C – Time point: 1 minute

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	100	100	95	95	50	5	0	0
	100	100	100	100	70	5	0	0
	100	100	100	95	80	10	5	0
	100	100	100	95	20	10	0	0

25 Table 8D – Time point: 2 minutes

Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
CPE (%)	100	100	95	90	55	10	0	0
	100	100	100	90	60	10	10	0
	100	100	95	95	80	5	0	0
	100	100	100	90	30	5	0	0

Table 8E – Time point: 5 minutes

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
CPE (%)	100	100	100	90	10	5	0	0
	100	100	95	95	30	10	0	0
	100	100	95	95	70	10	0	0
	100	100	100	95	75	10	0	0

Table 8F – Time point: 30 minutes

Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
CPE (%)	100	100	95	90	60	5	0	0
	100	100	95	90	35	5	0	0
	100	100	95	90	30	15	0	0
	100	100	95	90	70	10	0	0

5 The degree of CPE of the cells was recorded to determine the TCID₅₀ which means median tissue culture infectious dose. TCID₅₀ provides us with the viral titre (amount of virus). To put it simply, the lower the TCID₅₀, the lesser virus present, which also means the more effective the test solution is – vice versa. Results of the test were compared against the positive control.

10 Based on the results tabulated above, TCID₅₀ was then determined by following a set of calculations, the final calculated results are charted in Figure 9. Time 0 represents the TCID₅₀ for the positive control. After exposure of virus to the test solution, a decrease in TCID₅₀ can be seen until 30 minutes time point.

15 In Figure 9, time 0 represents the viral titre before the exposure to the Test Sample 08. After 15 seconds exposure, the viral titre decreases from 2.82×10^5 to 2.39×10^5 TCID₅₀/mL, and generally continues until 30 minutes where viral titre is 9.49×10^4 TCID₅₀/mL. The slight increase at the 2 minutes time point. Based on the tabulated CPE levels, the values between 1 minute and 2 minutes are very close. Therefore, this increase is not significant.

The test solution is therefore proven to be effective in virucidal activity.

25 It is to be understood that the above embodiments have been provided only by way of exemplification of this invention, such as those detailed below, and that further modifications and improvements thereto, as would be apparent to persons skilled in the relevant art, are deemed to fall within the broad scope and ambit of the present invention described. In particular, the following additions and/or modifications can be made without departing from the scope of the invention:

- 35 • Step 2 of the method 1 should be followed by step 3, while steps 3, 4 and 5 may take place independent of one another, for example, the release system of step 4 may be introduced into an environment first before a dosing system in step 2 and 3 are provided into that same environment.
- The first and second containers may have different shapes and sizes, but it is preferable that the volume of the second container is one-tenth the volume of the first container.
- 40 • A system comprising the steps of the method 1 may be provided to an environment for cleaning that environment.

- The dosing system can be configured to be a manual or automatic dosing system.
- The openings of the inlet conduits 161, 261 and the outlet conduits 162, 262 may be located on any exterior surface of the housing 180, 280.

Furthermore, although individual embodiments have been discussed it is to be understood that the invention covers combinations of the embodiments that have been discussed as well.

Claims

1. A dosing system comprising a housing enclosing:
5 a first closed container adapted to contain a solution of a first oxychlorine compound, the first closed container including a first conduit and a second conduit; and
a second closed container adapted to contain a neutralizer, the second closed container in fluid communication with the first closed container via the second conduit,
10 wherein the first conduit is adapted to convey the solution of the first oxychlorine compound or part thereof to mix with a fluid to obtain and dispense a cleaning fluid, and wherein the neutralizer is adapted to neutralize emissions of the solution of the first oxychlorine compound.
- 15 2. The dosing system of claim 1, wherein the first oxychlorine compound is chlorine dioxide and the emissions are gaseous chlorine dioxide.
- 20 3. The dosing system of claim 2, wherein the solution of the first oxychlorine compound is a stabilized chlorine dioxide solution or an activated chlorine dioxide solution.
- 25 4. The dosing system of claim 2 or 3, wherein the chlorine dioxide in the solution of the oxychlorine compound is in a concentration range of about 20,000 ppm to about 60,000 ppm.
5. The dosing system of claim 4, wherein the concentration of the chlorine dioxide in the solution of the oxychlorine compound is 50,000 ppm.
- 30 6. The dosing system of any one of the preceding claims, wherein the neutralizer comprises sodium thiosulfate solution.
- 35 7. The dosing system of claim 6, wherein one end of the second conduit is arranged to be in contact with the sodium thiosulfate solution and near a surface level of the sodium thiosulfate solution.
- 40 8. The dosing system of any one of the preceding claims, wherein the volume of the second closed container is one-tenth the volume of the first closed container.
- 45 9. The dosing system of any one of the preceding claims, wherein the first closed container further includes a third conduit comprising a one-way valve, the third conduit adapted to only permit fluid flow into the first closed container.
10. The dosing system of any one of the preceding claims, wherein the second closed container further includes a fourth conduit comprising a one-way valve, the fourth conduit adapted to only permit fluid flow out of the second closed container.

11. A method of cleaning an environment, the method comprising:
a) providing a dosing system comprising a housing enclosing:
5 a first closed container containing a solution of a first oxychlorine compound, the first closed container including a first conduit and a second conduit; and
a second closed container containing a neutralizer, the second closed container in fluid communication with the first closed container via the second conduit,
10 wherein the neutralizer neutralizes emissions of the solution of the first oxychlorine compound;
b) conveying the solution comprising the first oxychlorine compound via the first conduit and dosing a fluid to obtain a first cleaning fluid;
c) applying on and cleaning at least one surface in the environment at
15 least once a day with the first cleaning fluid;
d) generating and releasing a gaseous second oxychlorine compound via a release system into the environment; and
e) fogging the environment with a fog comprising a third oxychlorine compound.
- 20 12. The method of claim 11 further comprising the step of f) spraying a second cleaning fluid comprising a fourth oxychlorine compound via at least one hand-held spray means on at least one surface in the environment.
- 25 13. The method of claim 12, wherein the fourth oxychlorine compound is chlorine dioxide, and wherein the second cleaning fluid comprising a fourth oxychlorine compound is a stabilized chlorine dioxide solution.
- 30 14. The method of claim 13, wherein the chlorine dioxide in the second cleaning fluid is in a concentration range of about 500 ppm to about 900 ppm.
15. The method according to any one of claims 11 to 14, wherein the first oxychlorine compound is chlorine dioxide, and wherein the solution comprising the first oxychlorine compound is a stabilized chlorine dioxide solution.
- 35 16. The method of claim 15, wherein the method further comprises the step of activating the stabilized chlorine dioxide solution prior to step b).
- 40 17. The method of claim 15 or 16, wherein the chlorine dioxide in the solution comprising the first oxychlorine compound, is in a concentration range of about 20,000 ppm to about 60,000 ppm.
18. The method of claim 17, wherein the concentration of the chlorine dioxide in the solution of the oxychlorine compound is 50,000 ppm.
- 45 19. The method of any one of claims 11 to 18, wherein the chlorine dioxide in the first cleaning fluid is in a concentration range of about 50 ppm to about 300 ppm.
- 50 20. The method according to any one of claims 11 to 19, wherein the release system comprises a precursor of the gaseous second oxychlorine compound and a dry hygroscopic material, wherein the hygroscopic material is adapted to generate and release the gaseous second oxychlorine compound in the presence of water, and wherein the gaseous second oxychlorine compound is gaseous chlorine dioxide.

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21. The method of claim 20, the hygroscopic material further comprising an activator adapted to react with the precursor in the presence of water to generate and release the gaseous second oxychlorine compound, wherein the precursor is a metal chlorite and the activator is an acid.

5

22. The method of claim 20 or 21, the method comprising generating and releasing the gaseous chlorine dioxide over 24 to 48 hours.

23. The method of any one of claims 11 to 22, the method comprising mounting the release system onto a surface in the environment, near to a vent.

10

24. The method according to any one of claims 11 to 23, wherein the third oxychlorine compound is chlorine dioxide and wherein the chlorine dioxide in the fog comprises a third oxychlorine compound is in a concentration range of about 500 ppm to about 900 ppm.

15

25. The method according to any one of claims 11 to 24, wherein step d) comprises fogging the environment once a month.

20

26. The method according to any one of claims 11 to 25, the method further comprising fumigating the environment with gaseous chlorine dioxide in a concentration range of about 1 ppmv to 300 ppmv.

27. The method according to any one of claims 11 to 26, the method for cleaning an environment of one or more viruses selected from the group comprising Coxsackievirus A16, Coxsackievirus B2 and Enterovirus 71.

25

28. The method according to any one of claims 11 to 27, wherein the environment is an indoor environment.

30

29. The method according to claim 28, wherein the environment is a childcare centre.

30. A system of cleaning an environment, the system comprising:

35

a) a dosing system comprising a housing enclosing:

a first closed container adapted to contain a solution of a first oxychlorine compound, the first closed container including a first conduit and a second conduit; and

a second closed container adapted to contain a neutralizer, the second closed container in fluid communication with the first closed container via the second conduit,

40

wherein the first conduit is adapted to convey the solution of the first oxychlorine compound or part thereof to mix with a fluid to obtain and dispense a first cleaning fluid for cleaning at least one surface in the environment, and wherein the neutralizer is adapted to neutralize emissions of the solution of the first oxychlorine compound;

45

b) a release system adapted to generate and release a gaseous second oxychlorine compound into the environment;

c) a fog generator adapted to fog the environment with a fog comprising a third oxychlorine compound; and

50

d) at least one hand-held spray means adapted to spray a second cleaning fluid comprising a fourth oxychlorine compound on at least one surface in the environment.

31. The system of claim 30, wherein the first, second, third and fourth oxychlorine compounds comprise chlorine dioxide.