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AKS Accredited testing laboratory
Registration number: xxx
National Accreditation Centre Hannover

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ZLG Central locations of the countries for health protection with regards to medication and medicinal products
ZLG-P-470.06.02

Expert report on efficacy of Trustwater Ecaflo Systems for a short-period sanitization of biofilm-contaminated water- bearing systems

Client: Trustwater Group, Ireland
Written order from 25.07.2007
Examination duration: 02.08.2007 – 22.11.2007
DMT – Number: DMT-2007-246

Aim of the examination

To determine the efficacy of a biocidic active ingredient produced by a Trustwater-Ecaflo™ generator, based on active chlorine with the trade name Ecasol to be documented over a short-term treatment of biofilm in drinking water pipes (according to EG regulation 2032/2003, 1451/2007 EG and according to paragraph 11 drinking water regulation).

1. Introduction

1.1 Biofilms

Biofilms are extremely successful biotic communities that enable microbial life embedded in a matrix made out of extra cellular polymer substances (EPS). Nearly all microorganisms live in such synergic communities. The synergic way of life and the different habitats in a biofilm enable the co-habitation of mixed populations of differing species (Flemming and Wingender 2001, Flemming and Wingender 2002). When the biofilm has reached a certain size it switches to a balanced state of new growth and ablation of biofilm particles. Individual microorganisms and whole biofilm fragments can be discharged by local hydrolysis of the EPS and physical forces (e.g. currents), respectively, which can result in the release of microorganisms into the flowing water (Costerton 1995, Costerton *et al.* 1999).

The possible contamination by facultative pathogenic microorganisms, especially by *Pseudomonas aeruginosa* (Reuter *et al.* 2002, Anaissie *et al.* 2002), *Legionella pneumophila*, Acinetobacter, a typical mycobacteria or *Serratia ssp.* presents a risk of infection for water supply systems (Rahal and Urban 2000, Trautmann *et al.* 2001, Langsrud *et al.* 2003, Hall-Stoodley and Stoodley 2005, Exner *et al.* 2005).

The difficulty of biofilms in drinking water systems and connected systems is gaining increased importance (Exner *et al.* 2005, Donlan and Costerton 2002, Reuter *et al.*2002). They can lead to the contamination of drinking water in the distribution network even when the drinking water has left the water suppliers in immaculate quality. Entry routes for biofilm organisms present themselves during the extraction and processing of drinking water, after the processing through pipe leaks in the pipe network, during repairs, maintenance and cleaning processes but also through retrograde contamination of drinking water extraction points and not least through employed personnel (Nagy and Olsen 1985, Le Chavallier *et al.* 1987, Block 1992).

Particularly installation in buildings of medical institutions may be a source of nosocomial infections; the underestimated importance of this became apparent in a recent publication by Exner *et al.* 2007. Some critical areas of the installation in buildings are, for example, long supply pipes, areas of stagnation, accumulations of sediment, dead-end pipes as well as warm-water containers. Since the new legislation on water quality for human consumption (drinking water regulation 2001) came into force on 1 January 2003, hospitals – as operators of installations in buildings – have, for example, been assigned independent responsibility for the water quality within their building's installation system (Exner and Kistemann 2004). The same is true for all other operators of buildings' installations, for example in the public domain for swimming pools, or for surgical day clinics and dental surgeries as well.

1.2 The Trustwater-Ecaflo™ Procedure

An activated aqueous disinfection solution with oxidative properties is produced via electrochemical route by the Trustwater – Ecaflo™ procedure which, according to the company, is patented worldwide. The liquid offered under the brand name Ecasol™ is a disinfection means which is produced directly onsite. With a pH of 7 the product lays in the pH-neutral range and it is virtually odourless. The produced solutions can show substance concentrations of ≥ 1000 mg free chlorine at a pH of 7. According to the company the neutral pH value allows safe user handling. Additionally the danger of corrosion of surfaces like, for example, fluid-bearing metal pipe lines, and material compatibility of PVC and PU hoses in dental units at a neutral pH range is minimised. According to the company the usage of Ecasol™ is unobjectionable to the law in line with the EG regulations 98/8/EG and 2032/2003 and 1451/2007 EG as well as paragraph 11 of the German drinking water regulation and its efficacy has been documented extensively in international reports. The company GESUNDLEBEN GmbH & Co. KG has already successfully used the Trustwater-Ecaflo™ procedure in the Krankenhaus (Hospital) Bad Doberan in Germany (www.hygiene.ag).

2. Materials and Methods

2.1 Quantitative suspension trial

The quantitative suspension trial is used to ascertain bactericidal and fungicide efficacy of a disinfection means and was performed in a modified manner according to standard methods of the DGHM (Deutsche Gesellschaft fuer Hygiene and Mikrobiologie – German Association for Hygiene and microbiology) for the testing of chemical disinfection methods, 2001. *P. aeruginosa* with a germ count of 10^6 per ml was used as test organism. Neutralisation of the disinfection means was performed (according to pre-established regulation) with 0.3% sodium thiosulphate and 0.1% catalase. The tests were performed for different reaction times and at different concentrations.

All tests were performed without and with defined organic loads (0.03% bovine serum albumin BSA), respectively.

2.2 Silicone Hose Model

The company Deutsch & Neumann provided silicone hoses for the tests in which biofilms had been generated through continuous drinking water flow (according to Otte, 2006). At the beginning of the examinations the silicone hose was covered in a three-year-old biofilm with up to 8.4×10^7 colony-building units (KBE) per cm^2. The used silicone hose with biofilm measures 25 metres in length, has an inner diameter of 0.4 cm and a thickness of 1 mm.

2.2.1 Circuit system in the silicone hose model for stimulation of immediate measures

The efficacy of Ecasol™ was examined in a closed circuit system. A pre-defined concentration of the biocide (100 and 200 mg/l (ppm)) was pumped into 2 litres of fresh water through a biofilm-contaminated silicone hose over a defined time period (180 and 360 minutes). After the treatment the biomass was scraped out of the silicone hose, homogenised and incubated in R2A Agar at 20℃ for seven days. Germ count was ascertained by heterotrophic plate count (HPC). Afterwards the bacterial load was converted into KBE per cm^2. The circulation of the fluid occurred continuously over the entire test period. The flow rate was 400ml per minute during all trials. At the beginning and end of each test the potency, pH value and redox potential were recorded.

The circuit trials were performed at differing temperatures (22℃ and 37℃).

3. Results

3.1 Quantitative suspension trial without and with organic loads

In order to document the general efficacy of Ecasol suspension trials were initially performed without and with organic loads (0.03% BSA) at room temperature. The *P. aeruginosa* concentration measure 10^6 KBE/ml.

The results without organic loads show that an Ecasol concentration of 22 ppm is sufficient to inactivate the bacteria beyond the detection limit after one minute.

With existing organic loads a reduction by 2.28 log_{10} can be reached. The reduction can be increased to 2.60 log_{10} when left up until 180 minutes.

An increase of the Ecasol concentration to 100 ppm for the same length of time leads to a slightly better result – 2.38 log_{10} steps after one minute and 2.68 log_{10} steps after 180 minutes (Fig. 1.).

3.2 Ecasol in closed circuit system

In order to examine the efficacy of the biocide substance Ecasol with regards to fighting of biofilm, higher concentrations of Ecasol (100 and 200 ppm) were used in the closed circuit system (see 2.2.1). Here the efficacy was examined at differing temperatures (22℃ and 37℃) and time exposures (180 minutes and 360 minutes). The biofilms were three years old and were generated in the silicone hose model of the Institute for Hygiene and Public Health Bonn. Silicone tubes with a length of 30 cm were used. The start-up germ count was 6 x 10^7 KBE/cm^2, the flow speed was 400 ml/min.

A reduction of 3.15 log_{10} steps was noticed after treatment with an Ecasol concentration of 100 ppm at 22℃ after 180 minutes. After a treatment time of 360 minutes the reduction of 3.78 log_{10} steps was a bit higher.

A temperature increase to 37℃ lead to a reduction of 4.59 log_{10} steps after 180 minutes. After the treatment time of 360 minutes no KBE could be proven.

After treatment time of 180 minutes at an Ecasol concentration of 200 ppm at 22℃ no KBE could be proven. The procedure at 37℃ showed the same result (Fig. 2).

When the temperature was increased during continuous flow and increased concentration the efficacy of the biocide under investigation became stronger.

3. Discussion

The liquid biocide Ecasol produced by the Trustwater-Ecaflo procedure has an antibacterial effect. The examinations showed that bacteria that are present in a homogeneous solution are already completely inactivated within one minute (Fig. 1). As soon as an organic load is present (0.03% BSA in the examinations) Ecasol is being consumed. However, the bacteria could be reduced by 2.68 log_{10} steps in the quantitative suspension trials (Fig. 1).

The efficacy of Ecasol to remove drinking water biofilm was examined in the closed circuit system and under continuous addition of fresh water. The biofilms were generated in the silicone hose model of the Institute for Hygiene and Public Health Bonn. In the circuit system it could be shown that after treatment with an Ecasol concentration of 200 ppm and after 180 minutes the germ count in the biofilm lay below the HPC detection limit; this was irrespective of the temperature (22°C and 37°C). At an Ecasol concentration of 100 ppm the results were temperature dependent. At 22°C no germs could be detected after 360 minutes, at 37°C this was already the case after 180 minutes. During continuous flow it was noticed that Ecaflo is temperature dependent.

A germ-reducing efficacy of the Trustwater Ecaflo procedure without an organic load can be confirmed based on the examinations performed. Sufficient efficacy of the Trustwater Ecaflo procedure at short-term treatment of drinking water biofilm can be certified based on the examinations performed.

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