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Ref: Biocide action

Validation of biocide action of Eoxide LQ.

The biocide efficacy of any chemical reagent, including chloride dioxide, is generally evaluated by testing its ability to block the growth of microorganisms or inactivate the toxicity of a given substance, e.g. proteins.

It is noted that the test on proteins is equivalent to the test on viruses, since proteins are covering them, as for the case of CV-19 and the SPIKE protein.

We have performed bacteriological and toxicity tests on surface treated with a solution containing 50 ppm of ClO_2 with a purity better than 99,99%, namely the **Eoxide LQ**, to evaluate its efficacy in killing bacteria and inactivation of proteins.

For the ability to kill microorganisms we have put on two glass slides (1.5 x 1.5 cm) 50 μl of a solution containing 3000 cfu (colony formation unit) of E. coli bacteria and let evaporate the majority of solution at room temperature (completely dry cells undergo to severe stress ending in death). One glass was treated with ClO_2 whereas the second glass kept away in another room. After the treatment both glasses have been immerged in 5 ml of distilled water. The water was used to remove (elute) from the glass slides bacteria. 1 ml of elution solution was used to inoculate 10 ml of E. coli growth medium and incubated at 37 °C in agitation for 7 days. After the incubation period, from the glass slide treated with ClO_2 we did not observed any growth of E. coli bacteria, whereas from the glass untreated after 7 days the E. coli bacteria where growing properly. The absence of any bacterial growth in the elute from the glass slide treated ClO_2 confirmed the biocide potential since all viable cells present on the glass have been killed by this reagent.

For the ability to inactivate isolate proteins, like toxin, we used toxicity test and a protein commonly used to kill ex-vivo human cells, TRAIL. As above, on top of two glass slides we put 50 μl containing 5 μg of TRAIL protein. For both slide the solvent was evaporated almost completely and one of the two slides was treated with 50 ppm of ClO_2. After the treatment both slides have been immerged in culture plates containing human colon cells. The cells have been incubated together with slide for 48 hours, in this period of time the TRAIL protein was free dissolve in the cell culture medium and eventually kill the cells. We observed that the glass treated with ClO_2 did not affected at all the in vitro growth of the cell line, whereas the untreated glass slide caused more than 70% of cell death. This demonstrated that up treatment with ClO_2 TRAIL protein was not able to exerts its toxic activity because it was inactivated.

Similar experiments have been performed using the gel form of Eoxide LQ, illustrating similar results. This gel, which is produced according to the Category 2 of the Commission Regulation (EC) No 104/2005 of 13 June, Official Journal of the European Union L 178/1 , Publication date 9.7.2005, is appropriate for the disinfection of surfaces, of inner spaces.

Sincerely

Evangelos V Hristoforou