Synthesis and Testing of New Decontaminant “bio - chem“
Based on Oxidizing Compounds

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“Bio-chem” intentional or unintentional contamination, is a real risk, to which must have effective management to prevent and combat. Decontamination, subsequent chemical and/or biological attacks, terrorist attacks or industrial accidents with “bio-chem” agents is the first measure to be applied to protect personnel and the environment, in order to reduce the number of victims and the liquidation of the event. The products used in microbial decontamination are numerous, but very few meet all the requirements conditions of a good decontaminant. Among these, there are a series of new “bio-chem” decontaminant obtained from oxidizing compounds. The most effective substances, selected after making tests in different experimental conditions, can be produced and used for decontamination, in accordance with legal procedures.

Keywords: “bio-chem” contamination, decontaminants, oxidizing compounds, chemical synthesis

Biological contamination is related to bacteria, viruses and toxins, which can cause various diseases such as anthrax, plague, pox, botulism etc. In terms of chemical structure the range of products used in microbial remediation is wide but very few meet all the conditions for a good decontaminant.

In the present study were taken into account compounds with organic peracids type oxidizing action (especially peracetic acid) produced by the oxidation of polyvinyl alcohol in the presence of acetic acid and hydrogen peroxide in aqueous medium.

The oxidation with strong oxidizing agents (solutions peroxide, potassium permanganate, calcium hypochlorite) resulted in the destruction of the cellular structure of the microorganisms and toxic compounds or, in the case of molecules containing sulfur or nitrogen atoms, formation of corresponding sulfonates and N-oxides.

Preparation of bio-chem decontaminant based on oxidizing compounds
Preparation of the products based on oxidizing compounds

This method is based on the oxidation of polyvinyl alcohol in the presence of acetic acid and hydrogen peroxide in the aqueous medium or hydrogen peroxide and sulphuric acid in the aqueous medium.

The concentrated suspension is prepared by mixing polyvinyl alcohol with stirring to the polyvinyl alcohol powder with the heated water at a temperature of 40-45°C. Stirring was continued and to the reaction vessel concentrated acetic acid and hydrogen peroxide was added.

The reaction mixture was stirred for 1 h at a temperature of 82-86°C, after which the reaction product is stirred until cooling at 40°C.

After cooling to room temperature the reaction product is removed from the vessel through siphoning and it is analyzed. The product is presented as a clear, colorless liquid with a pungent characteristic odor of acetic acid, having a density at 20°C = 1.04 to 1.05; pH = 2-3; carboxyl = 14-16%.

Physico-chemical characterization of prepared products
Product Code DC - 1 The aqueous solution of organic acids produced by the oxidation reaction (conc. 26-27%), density (20°C) from 1.03 to 1.05, pKa 2-3, COOH,14-16%, refractive index 1.3590;

Product Code DC - 1 The aqueous solution of organic acids and peracids produced by the oxidation reaction (conc. 20%), density (20°C) 1.027, pH 2-3;

Product code DC - 1b The aqueous solution of organic acids and peracids produced by the oxidation reaction (conc. 10%), density (20°C) 1.012, pH 2-3;

Product code DC - 14 The aqueous solution of organic peracids produced by the oxidation reaction of the polyhydric alcohols (conc.20%), density (20°C) 1.02 pH 3.0 -3.5, carboxyl-7 to 7.5%;

Product code DC - 15 The aqueous solution of organic acids produced by the oxidation reaction of the polyhydric alcohols (conc. 20%), density (20°C) 1.03, pH 0.5;

Conditioning of synthesized compounds for testing
All decontaminants synthesized and conditioned in various forms have been designed in accordance with national law and the European Union regarding the ecology and environment.

Decontaminating substances must have a large action spectrum and a penetrating ability in order to be able to penetrate and destroy contaminants. This quality can be enhanced with a surfactant, which reduces surface tension to facilitate wetting, foaming, adherence and therefore contact between the microbial cell and compound decontaminant.

Choosing the active substance or decontaminant product is based on antimicrobial qualities, its purpose and the conditions under which it may be used. The contact time in which the destructive action of the decontaminant on biological contaminants becomes effective on is an important moment of decontamination.

Each substance or decontaminant product has a certain contact time that needs to be studied and known via experimental measurements. The concentration of
decontaminating solutions is another factor which influences the effectiveness of the decontamination. The quantity of solution used per unit area determines the effectiveness of the decontamination. The application of decontaminant agent influences its effect. Thus, it is required that working solutions to be finely dispersed in inaccessible spaces.

Testing of prepared products

Screening testing

The tests were performed at the Laboratory of Microbiology and Epidemiology, in the laboratory for biological agents diagnostic, with the biosafety level P2+, for microorganisms pathogenic. For screening of potentially decontaminating have been testing conducted bacterial sensitivity by the antibiogram disc diffusion method, on multiple lots. Bacteria were grown on Mueller-Hinton solid culture medium, incubated aerobically for 72 h at 37°C, reading everyday. The measurement was carried out with precision of 0.1 mm (with calipers under a magnifying glass) on multiple tests to calculate the arithmetic average of each substance in each species. The tests were made on the following bacterial species: Staphylococcus aureus, Bacillus anthracis, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae (table 1). In parallel we also performed sensitivity tests on anaerobic bacteria (Clostridium botulinum), on mixture of bacteria, on bacterial spores (Bacillus anthracis) and mixture of micettes (fungi and yeasts), the culture media and their specific conditions, but the results are not satisfactory and were not processed statistically and are not present in this paper.

Decontaminated DC1, DC1a, DC1b tested, presented promising activity, with the specification that has a narrow spectrum of activity (table 2). The possible synergic effect of quaternary ammonium compounds was also tested in order to achieve possible decontaminant mixture.

Testing was carried out in view to the bactericidal effect of the substances from the standard set of bacteria (gram-positive and gram-negative, anaerobic and bacterial spores) and other pathogenic microorganisms. Bacterial sensitivity results were read after 24 and 48 h of incubation and after keeping at room temperature for 48 h to follow up the effect in time.

The quantification of antimicrobial effect was performed by calculating the arithmetic mean diameters bacterial inhibition zones (measured in millimeters) to test substances. Bactericidal concentration (g/L) is calculated by the quotient of the amount of substance used respectively 20 mg and average volume (cylinder volume formula) in which diffusion antimicrobial certainly had placed. The results obtained are shown in table 3.

Toxicological testing

In the laboratory screening tests, the toxicology screening for acute toxicity were performed, by intraperitoneal injection of 0.5 mL of working solution in experience mice, young adults with a weight of approx. 20 g, where morbidity and mortality was monitored for three days. After we have had confirmation that the substances are not very toxic, therefore there is no danger to the operators, we started the tests under minipolygon conditions.

Experimentation of decontaminating products

After screening test and the selection of the most effective products in terms of antimicrobial activity, these were experimented in natural conditions (outdoors), in minipolygon experimentation, which was specially arranged. The experimental contamination and

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### Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Collection cod</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>Oxford CIP 53 154(1)</td>
<td>aerobic bacteria, gram-positive</td>
</tr>
<tr>
<td>2</td>
<td>Bacillus anthracis</td>
<td>34F2 Sterne</td>
<td>aerobic bacteria, gram-positive</td>
</tr>
<tr>
<td>3</td>
<td>Escherichia coli</td>
<td>CIP54 127(1)</td>
<td>aerobic bacteria, gram-positive</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas aeruginosa</td>
<td>CIPA 22(1)</td>
<td>aerobic bacteria, gram-negative</td>
</tr>
<tr>
<td>5</td>
<td>Vibrio cholerae</td>
<td>El Tor</td>
<td>aerobic bacteria, gram-negative</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Substance</th>
<th>S. aureus</th>
<th>B. ant.</th>
<th>E. coli</th>
<th>Ps. aer.</th>
<th>V. hol.</th>
<th>Mixture</th>
<th>Total mm</th>
<th>Score mm</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>DC1</td>
<td>5.7</td>
<td>10.5 MR</td>
<td>21.5 MR</td>
<td>8.0</td>
<td>15.0</td>
<td>18.7 MR</td>
<td>79.4</td>
<td>13.2</td>
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</tr>
<tr>
<td>2</td>
<td>DC1a</td>
<td>8.5</td>
<td>7.0 MR</td>
<td>12.6 MR</td>
<td>0</td>
<td>15.0</td>
<td>11.3 MR</td>
<td>54.4</td>
<td>9.1</td>
<td>synergy with quaternary ammonium compounds</td>
</tr>
<tr>
<td>3</td>
<td>DC1b</td>
<td>5.5</td>
<td>0</td>
<td>10.0 MR</td>
<td>0</td>
<td>11.0</td>
<td>9.0 MR</td>
<td>35.5</td>
<td>5.0</td>
<td>low</td>
</tr>
</tbody>
</table>

Note: observation of resistant mutants was noted with MR.

### Table 3

<table>
<thead>
<tr>
<th>No.</th>
<th>Substance</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>Ps. aer.</th>
<th>B. ant.</th>
<th>V. hol.</th>
<th>Score mm</th>
<th>Conc. g/l</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DC-14</td>
<td>24.0</td>
<td>20.0</td>
<td>20.0</td>
<td>25.0</td>
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</tr>
<tr>
<td>2</td>
<td>DC 15</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>unsatisfying</td>
<td></td>
</tr>
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</table>
decontamination was made on a military vehicle which was out of service, representing, the "target" on which the operating procedures for contamination control CBRN (chemical, biological, radiological and nuclear) were applied. Microbial strains used represent the main groups of pathogenic bacteria: gram-positive cocci: Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus fecalis; gram-positive bacilli: Bacillus anthracis (vaccine strain), Bacillus cereus, Bacillus subtilis; gram-negative bacilli: Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa; Vibrio: Vibrio cholerae.

The exterior of the target vehicle was marked with numbered areas about 0.1 square meters for contamination and decontamination. Areas were chosen as such: a vertical painted sheet metal, for aqueous solution decontaminants; glass window for aqueous solution decontaminants; rubber tires for aqueous solutions decontaminants and painted sheet horizontally for powder decontaminating supplied, as a positive control.

The microbial contamination was done by sprinkling on the surface allocated separately with each strain and the resulting mixture from their mixture. The microbial suspension used was cultured in a liquid medium, with approximately 1 million live bacteria per mL, the dmp 1 mL, enough to create a uniform film contamination. For decontaminating liquid sprinkling on the surface decontaminated with an appropriate buffer. Harvesting times were: - before contamination (to establish a baseline level of natural contamination); - immediately after contamination (to check the contamination experiment); - after decontamination for each decontaminant with each biological agent, at every 10 min (as required for military use for decontaminants); - at intervals of 45 min (as for for general use disinfectant); - moreover, at the end of the experiment samples were collected from different parts of the protective equipment of the operators and the environment for detecting any residual contamination.

All samples were immediately transported in biosafety conditions, tested in the microbiology laboratory at P2 +. Decontamination results obtained after 45 min are shown in table 4.

The antimicrobial effect is preserved and obvious after 45 min, suggesting that these products can be proposed as potential disinfectants for medical or hygienic-sanitary according to Medicines Act.

To compare the high effectiveness of the decontamination products listed, we tested a powder compound (control) from the laboratory. It was observed that the powder blank for decontamination is less effective in all cases, and the remaining surfaces remain with a degree of residual contamination, because it does not provide an intimate contact between the microorganism and the decontaminant. To correct this deficiency, the powder should be applied to wet, pressed and rubbed into the surface decontaminated with an appropriate buffer.

In all cases, the final stage of decontamination should be washed with water as the substances used can be corrosive or irritant for some materials or the personal. Microbiological analyzes were also performed for any residual contamination of surfaces (after the final wash), wastewater and protective equipment used by operators, but there was no significant contamination, so there is was no risk to the environment.

Were synthesized and tested a series of potential decontaminating substances, of which five substances were selected and recommended as decontaminating agents. These substances are characterized by a high chemical stability. The products are soluble in water and various organic solvents. These products are biocides with bactericidal and fungicides action which have multiple uses. The substances are characterized by low toxicity to animals.

There has been theoretical and experimental scientific research to design and implementation of "bio-chem" decontaminated polyvalent for destruction of toxic chemical and biological agents, hazardous to health. Laboratory technologies were developed in order to obtain the selected decontaminants as a result of efficiency tests carried out. There was developed polyvalent experimental model of chemical and biological decontamination to decontaminate type oxidizing compounds.

Conclusions

Intentional or unintentional “bio-chem” contamination, is a real risk, to which we must have an effective management to prevent and combat.

Decontamination is an essential measure to protect both the personal and the environment.

Synthesis and testing of new “bio-chem” decontaminating substances from oxidizing compounds complements the arsenal of protection against biological and chemical agents.

Synthesized and tested substances by us for “bio-chem” decontamination are efficient in the specific action, less toxic, easy to prepare and use, and have low costs.
Synergistic effect of DC1 and DC1a compounds with quaternary ammonium salts can be noted. The most effective substances selected (DC1, DC1a and DC14) can be produced and used for decontamination observing the legal provisions.

References
7. JACKSON J.B., Decontaminating solution, Brevet SUA nr.3.079.346, 1963
8. *** Antiseptiques et desinfectants, AFNOR, ed. 2, AFNOR 1989

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