

Synthesis and Testing of New Decontaminant "bio-chem" Based on Quaternary Ammonium Compounds

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Decontamination after biological attacks and / or chemical terrorist attacks or industrial accidents with "bio-chem" agents must be fast and efficient in order to reduce the number of victims and to erase the traces of the event. The decontamination of living or non-living biological agents and toxic chemicals can be achieved by hydrolysis in the different experimental conditions, in particular in an alkaline medium, reaction with amines or ammonia, alcohols and phenols, etc.. and transformation into less toxic degradation products. Intentional or unintentional "bio-chem" contamination, is a real risk, to which must have effective management to prevent and combat. Decontamination is an essential measure to protect the personnel and the environment. The synthesis and testing of new "bio-chem" decontaminant agents, based on quaternary ammonium salts, completes the arsenal of protection from chemical and biological agents. The synthesized substances that were tested by us for "bio-chem" decontamination are effective due to their specific action, less toxicity, easy to prepare and use and low costs. The most efficient substances that were selected can be produced and used for decontamination, in accordance with the law.

Keywords: "bio-chem" contamination, decontamination, disinfectants, quaternary ammonium salts, synthetic chemical, microbiological testing.

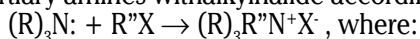
Decontamination after biological attacks and / or chemical terrorist attacks or industrial accidents with "bio-chem" agents must be fast and efficient in order to reduce the number of victims and to erase the traces of the event. The decontamination of living biological agents (bacteria, viruses, fungi, parasites) or nonliving (toxins, regulators) and toxic chemical products can be achieved by hydrolysis reactions in different experimental conditions (especially in alkaline environment), reactions with amines or ammonia, with alcoholates and phenolates, etc. and the transformation of degradation products less toxic.

Biological contamination refers to bacteria or viruses which can cause various diseases such as anthrax, plague, smallpox, botulism, etc. In terms of chemical structure the range of products used in microbial remediation is wide but very few meet the conditions for a good decontaminant. In the present work, quaternary ammonium salts obtained by treating tertiary amines with alkylhalide (alkylchlorides in particular or benzylchloride), were taken into consideration. These compounds are the most numerous and important agents with biocide action.

Preparation of "bio-chem" decontaminants based on quaternary ammonium salts

Preparation of quaternary ammonium salts

Quaternary ammonium salts are obtained by treating tertiary amines with alkylhalide according to the reaction:



R or R' can be alkyl radicals C₁ – C₂

R'' can be alkyl radical C₁ – C₈, C₁₈ – C₂₀, benzyl

X = halogen (chlorine is preferred)

Benzylchloride can be used as alkylation agent for the quaternizing reaction instead of the alkyl chloride. The reaction is applicable even when the nitrogen atom is part of the cycle of pyridine, quinoline, morpholine, piperazine.

Synthesis of tetramethyl ammonium chloride

In a two-necked reaction flask equipped with thermometer and dropping funnel were introduced 1 mol tertiary amine (trimethylamine, triethylamine) alkyl chloride and 1.5 mole of freshly distilled. A homogeneous solution is obtained which is left for 20-25 h at room temperature. The quaternizing reaction of the tertiary amine with alkyl chloride takes place in time, when the formation of crystal of a quaternary ammonium compound is observed. The crystals was filtered in a closed system as they are very hygroscopic and collect water from the atmosphere, forming a viscous liquid. The reaction products have low melting point 40-60°C very hard to determine.

Synthesis of N-alkyl salts-piridiniu

In a reaction vessel equipped with mechanical stirring, ascending refrigerant thermometers and insert 1 mol derivative of pyridine and 1.1 moles halogenated derivative in an appropriate solvent (acetone, alcohol, methylethylketon content etc.). The reaction mixture warms stirring for 10-15 h, depending on the reactivity of the reaction. After that remove the solvent by distillation and reaction product is recrystallized from a suitable solvent or mixture of solvents. Yields in the finished product are 80-90%. The products of the reaction are characterized by their melting points and for halogen determinations (including chlorine).

Physico-chemical characterization of products prepared

Product Code DC-3 aqueous solution of tetraalkyl-ammonium chloride (conc. 15%), density (20°C) 1.06 – 1.08; pH 9 – 9.5; Cl 10.5-11 %, the refractive index of 1.3777;

Product Code DC-7 Similar to DC 3;

Product Code DC-16 aqueous solution with quaternary ammonium salts and organic acids (conc. 25%), density (20°C) pH 3,0, -3.5; the refractive index of 1.025

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No.	Species	Collection code	Observations
1	<i>Staphylococcus aureus</i>	Oxford CIP 53 154(1) (ATCC9 144)	aerobic and gram-positive bacteria
2	<i>Bacillus anthracis</i>	34F2 Sterne	aerobic and gram-positive bacteria
3	<i>Escherichia coli</i>	CIP54 127(1) (ATCC 10536)	aerobic and gram-negative bacteria
4	<i>Pseudomonas aeruginosa</i>	CIPA 22(1)	aerobic and gram-negative bacteria
5	<i>Vibrio cholerae</i>	El Tor	aerobic and gram-negative bacteria

Table 1
BACTERIAL SPECIES USED
FOR TESTING

No.	Substance	<i>S. aureus</i> mm	<i>B. ant.</i> mm	<i>E. coli</i> mm	<i>Ps. aer.</i> mm	<i>V. hol.</i> mm	Mixture mm	Total mm	Media mm	Observations
1	DC3	0	6.6	0	10.1	0	4.5	21.2	3.5	low
2	DC7	22.5 MR	27.5 MR	24.1	30.0	31.3	28.3	173.7	28.9	very good , synergistically with oxidizing compounds

Table 2
SCREENING TEST RESULTS

Note: observation of drug-resistant mutant was denoted by MR.

Product Code DC-17 aqueous solution based of piridinium salt and tensioactivă cationic surfactant (conc. 25%), density (20°C), pH 2.5 -3.0; the refractive index of 1.03

Product Code DC-18 Solution based of quinolinium salt and cationic surfactant (substance conc. 20%), density (20°C) pH 1.05-1. 2;

Product Code DC-19 Solution based of heterocarbon derived with nitrogen (conc. 20%), density (20°C) 1.04, pH 3.0 -3.5;

Product Code DC-20 Aqueous solution based of quaternary ammonium compound (conc. 20%), density (20°C), pH 1.0 1.07.

Product Code DC - 21 similar to DC 20.

Conditioning of synthesized compounds for testing

The synthesis and conditioning of decontaminants, made by us in various forms, with the chemical structure of quaternary ammonium salt-soluble in water, have been carried out in accordance with the national legislation and the European Union legislation, concerning the ecology and environment protection.

The spectrum of action and the penetrative ability of decontaminants is intensified by a surfactant that is designed to reduce surface tension, thus facilitating contact between microbial and cell compound decontaminant.

The qualities of each product are determined by the choice of active substance of each decontaminant and should take account of its antimicrobial qualities, of its purpose and of the conditions under which it will be used.

The efficiency of decontamination is determined by the time of contact of each product, the concentration of decontaminant solutions, the quantity of the solution used on the surface and lastly of the way of the application of the decontaminant agent.

Testing of the prepared products

Screening test

Screening of potential decontaminant products was performed at the Microbiology-Epidemiology laboratory, in a diagnostic laboratory for biological agents BSL2 +, for the pathogenic microorganisms. Bacterial sensitivity tests were performed on multiple lots (the antibiotic disc diffusion method), while respecting the following conditions: cultivation on solid Müller-Hinton medium, 72 h aerobic incubation at 37°C, with daily reading. The following bacterial species were tested: *Staphylococcus aureus*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio cholerae* (table 1). The results were quantified by measuring the diameters of inhibition with an accuracy of 0.1 mm (under a microscope with calipers) compared on several tests in order to calculate the arithmetic mean of the individual substances for each species.

DC3 and DC7 code products were made on the basis of quaternary ammonium salts and N-alkylpyridinium. Their testing showed that the best microbiological activity has been registered on DC7 product (table 2). The possible synergistic effect with oxidizing compounds was tested in order to achieve possible decontaminant mixture.

Bactericidal effect of substances was tracked against a standard set of bacteria (gram-positive, aerobic and anaerobic, gram-negative bacteria and microbial spores) and other pathogen, and bacterial susceptibility results were read after 24 and 48 h of incubation and then after keeping at room temperature 48 h, to track the effect over time.

Antimicrobial effect was quantified by calculating the arithmetic mean of the diameters of the zones of inhibition of bacterial and bactericidal concentration (g/L) was calculated by the quotient between the quantity of the substance used (20 mg) and the volume of the medium diffusion antimicrobial effect had it (table 3).

No.	Substance	MΦ mm <i>S. aureus</i>	MΦ mm <i>E. coli</i>	MΦ mm <i>Ps. aer.</i>	MΦ mm <i>B. ant.</i>	MΦ mm <i>V. hol.</i>	Media mm	Conc g/l	Observations
1	DC-17	30.0	30.0	25.0	30.0	30.0	29.0	6.34	good
2	DC-18	25.0	30.0	25.0	30.0	30.0	28.0	6.80	good
3	DC-19	25.0	25.0	17.0	25.0	30.0	24.4	9.26	good
4	DC-20	26.5	20.0	21.0	22.0	25.0	22.9	11.0	well
5	DC-21	26.5	22.0	24.5	25.0	22.0	24.0	9.20	good

Table 3
QUANTIFICATION OF
ANTIMICROBIAL EFFECT

No.	Decontaminants	Biological agent	Surface	Time min.	Growth tube	Growth plate	Observations
1	DC 17 ICTCM	Mixt	glass	45	-	++ hem	contaminated
2	DC 17 ICTCM	<i>B.cereus</i>	glass	45	-	-	uncontaminated
3	DC 17 ICTCM	Mixt	vertical tin	45	+ dep	-	contaminated
4	DC 17 ICTCM	<i>B.cereus</i>	vertical tin	45	+/-	-	uncontaminated
5	DC 18 ICTCM	Mixt	glass	45	-	-	uncontaminated
6	DC 18 ICTCM	<i>B.cereus</i>	glass	45	+/-	-	uncontaminated
7	DC 18 ICTCM	Mixt	vertical tin	45	+ dep	-	contaminated
8	DC 18 ICTCM	<i>B.cereus</i>	vertical tin	45	+ dep	-	contaminated
9	DC 19 ICTCM	Mixt	glass	45	-	-	uncontaminated
10	DC 19 ICTCM	<i>B.cereus</i>	glass	45	+/-	-	uncontaminated
11	DC 19 ICTCM	Mixt	vertical tin	45	+ dep	-	contaminated
12	DC 19 ICTCM	<i>B.cereus</i>	vertical tin	45	+ veil dep	+	contaminated

Legend: + or ++ = increase microbial, - or +/- = lack of microbial growth;
dep = bacteria grow at the bottom of the tube as a deposit, to grow bacteria = veil surface;
hem. = hemolytic colonies.

Table 4
LEVEL OF CONTAMINATION
AFTER DECONTAMINATING
SOLUTION 10%

Toxicological testing

Toxicological screening for acute toxicity pursued confirmation that these substances are not highly toxic and present no danger to the operators. Therefore tests were performed by injecting 0.5 mL of experience working solution in mice (young adults), weighing approx. 20 g with tracking morbidity and mortality for three days. The test results have enabled experiments under minipoligon specially arranged.

Experimenting decontaminant products

Microbial strains were used as the main representative of the group of pathogenic bacteria: Gram-positive cocci: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*; gram-positive bacteria: *Bacillus anthracis* (vaccine strain), *Bacillus cereus*, *Bacillus subtilis*; gram-negative bacilli: *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*; vibroni: *Vibrio cholerae*.

The experimental contamination and decontamination was made on a military vehicle which was out of service, representing, the "target". It was marked with numbered area of approximately 0.1 square meters on which have been applied operating procedures for controlling CBRN contamination (chemical, biological, radiological and nuclear). Areas were chosen as follows: a vertical metal sheets, for aqueous solutions decontaminant; window glass for aqueous solutions decontaminant; rubber tires for aqueous solutions decontaminant and painted sheet horizontally for powder decontaminant supplied as a positive control.

Microbial contamination was done by spraying the surface allocated separately to each strain and with a mixture resulting from their concoction. The microbial suspension used was cultured in a liquid medium, with approximately 1 million live bacteria per mL, respectively 1 mL/dmp, enough to create a uniform film contamination. For decontaminant liquid spraying the aqueous solution was performed up to cover 10% of the contaminated area and commence the excess liquid, on average about 10 mL/dmp on smooth surfaces (glass, metal sheets) and about 20 mL/dmp rough surfaces (sheet peeling, tires).

Microbiological samples were collected through hygiene and sanitary buffer method, as follows:

- before contamination (in order to establish the initial level of contamination);
- immediately after contamination (to check the level of contamination);
- after each decontamination with each biological agent decontaminant, every 10 min (according to requirement for military use);
- every 45 min (as for disinfectants of general use).

In addition, at the end of the experiment were collected samples of various pieces of equipment for protection of the environment and operators to detect any residual contamination. All samples collected were transported immediately under conditions of biosafety and tested in the Microbiology laboratory level P2 +. The results obtained after 45 min decontamination are shown in table 4.

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

It is noted that for the laboratory witness powder our decontamination powder is less effective in all cases, the areas remain a degree of residual contamination because it is made intimate contact between the microorganism and decontaminant.

In all cases, the final stage of the decontamination must be washing with water, because the materials used may be corrosive or irritant for some materials for personal use. Were carried out and eventual contamination microbiological analysis for residual areas (after washing), waste water and the protective equipment used by operators, but there were no significant contamination, so there is no risk to the environment.

It was synthesized and tested a number of substances potentially decontaminants, of which eight were selected and featured as decontaminant agents, since they are characterized by their high chemical stability. The products are soluble in water and in various organic solvents. These are products with biocides, fungicides, bactericidal action which have multiple uses. The substances are characterized by low toxicity for animals.

There have been conducted theoretical and experimental scientific research aimed at the development and execution of "bio-chem" decontaminant polyvalents

for the destruction of toxic chemical agents and biological agents, dangerous for health. The technologies were developed for laboratory professionals selected decontaminant as a result of efficiency tests carried out. There has been three-experimental model of chemical and biological decontamination for decontaminant, type quaternary ammonium salts.

Of the products tested the best antimicrobial activity shows the products based on the mixture of compounds, oxidants and quaternary ammonium salts that have a synergistic effect on use, expanding the spectrum of action.

Conclusions

Synthesis and testing of new substances decontaminants "bio-chem" based on quaternary ammonium salts, complement the arsenal of protection from chemical and biological agents.

The new substances synthesized and tested by us for decontamination "bio-chem" (DC17, DC18, DC7, DC19 and DC21) are effective through specific action, least toxic, easy to prepare and use, with low costs.

Synergistic effect of the compound DC7 with oxidants compounds can be noted.

Most effective substances selected can be manufactured and used for decontamination, in compliance with the legal provisions.

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