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New quaternaryammonium salts baseddecontaminants

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Abstract: *Decontamination after terrorist attacks or industrial accidents with biological and/or chemical agents („bio-chem") must be fast and efficient, in order to reduce the number of victims and to eliminate the consequent damages. The decontamination of*
living biological agents (bacteria, viruses) or nonliving ones (toxins, regulators) and toxic chemicals could be accomplished by reactions of hydrolysis in various experimental conditions, in particular in alkaline medium, reactions with amines or ammonia, alcohols, phenols etc. and by their transformation into less toxic degradation products.
"Bio-chem" intentional or unintentional contamination is a real risk, towards which an effective management must be available to prevent and control it. Decontamination is an essential measure to protect the personnel and the environment.
Synthesis and testing of new „bio-chem" decontaminants, based on quaternary ammonium salts, complete the arsenal of protection against chemical and biological agents.
The most effective selected substances could be produced and used for decontamination in accordance with legal procedures.

Keywords: *„bio-chem" contamination, decontaminants, disinfectant, quaternary ammonium salts, chemical synthesis*

DECONTAMINATION

After terrorist attacks, biologicaland/or chemical attacks or industrial hazardswith bio-chem agents, the decontaminationmust be prompt and efficient, in order to reducethe number of victims and to eliminate the consequentdamages. The decontamination of the livingbiological agents (bacteria, viruses, fungus, andparasites) or of the nonliving agents (toxins, regulators)and of the toxic chemicals could be accomplishedby hydrolytic reactions in various experimentalconditions (especially in alkaline medium),reactions with amines or ammonia, with alcoholsand phenols etc. or by their transformation in lesstoxic degradation products.

The biological contamination refers to bacteriaand viruses generating diseases as anthrax,plague, smallpox, botulism etc. From the chemicalstructure point of view, the range of the productsused in microbial decontamination is wide butvery few fulfill the conditions of a good decontaminant.

In this paper there are taken into accountthe quaternary ammonia salts obtained by treatingthe tertiary amines with alkyl halogen, particularlyalkyl chlorides or benzyl chlorides. These compoundsare

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the most important agents having abiocide action.

Synthesis and conditioning decontaminant byMechanical Engineering and Research Institute,Bucharest (ICTCM) in different forms with thechemical structure of quaternary ammonium saltsoluble in water was carried out in accordance withnational and European Union legislation related toecology and environmental protection.

Figure 1. Microbiological testing of decontaminating substances, in vitro, in CCSMM microbiology laboratory

Figure 2. Microbiological testing of decontaminating substances in various materials, CCSMM minipolygon biological testing

Spectrum of activity and penetrating ability ofdecontaminating substance is enhanced by a surfactantthat is designed to reduce surface tensionthus facilitating contact between the microbiancell and decontaminant compound.

Decontaminant qualities of each product aredetermined by the choice of the active substanceand should take account of its antimicrobial qualities,the purpose, and the conditions that will beused in.

Decontamination efficiency is determinedby the contact time of each decontaminant product,decontaminating solution concentration, theamount of solution used per unit area and, not thelast, by the decontaminant agent application mode.

SCREENING TEST

Screening of potential decontaminatingsubstances

was performed in the Laboratoryof Microbiology-Epidemiology, in diagnosislaboratory for biological agents, ranked by P2+biosafety level for the pathogen microorganisms.

Bacterial sensitivity assays were performed (theantibiotic disc diffusion method on several lots),according to the following parameters: cultivationon solid culture medium Mueller-Hinton, 72hours aerobic incubation at 37°C, with daily reading.

The following bacterial species were tested:Staphylococcusaureas, Bacillus anthracis, Escherichiacoli, Pseudomonas aeruginosa, Vibrio cholerae.

Results were quantified by measuring with 0.1mmof accuracy (caliper and magnifier), comparatively,on many tests, to calculate the average of each substanceon each species.

Product code DC-3 and code DC-7 was carriedout on quaternary ammonium salts and Nalkylpyridiniumbasis. Their testing revealed thatthe best microbiological activity was recorded inthe product code DC-7. The possible synergisticeffect with oxidizing compounds was tested in orderto achieve possible decontaminating mixtures.

The bactericidal effect of the substance wastracked for a set of standard bacterial (gram-positiveand gram-negative, anaerobic bacterial and spores)and other pathogenic microorganisms; bacterialsensitivity results were read after 24 and 48 hours ofincubation and then, after storage at room temperature48 hours, to watch the effect in time.

The antimicrobial effect was quantified by calculatingthe average diameter of bacterial inhibitionzone and bactericidal concentration (g/liter),calculated as the quantity of substance (20 mg)divided by the volume in which the antimicrobialdiffusion was effective. (figure 1, 2).

Figure 3. Microbiological testing of decontaminating substances in various materials, CCSMM minipolygon biological testing

TOXICOLOGICAL TESTING

Toxicological screeningfor acute toxicity aimed to confirm that thesesubstances are not highly toxic and are not dangerousto operators.

To demonstrate, 0.5 ml ofeach substance was injected in mice (approx. 20gweight, young adult), tracking morbidity and mortalityfor three days. The test results have enabledexperiment achievements under conditions of aspecially arranged experimental minipolygon.

Experiencing decontaminating products

Representative microbial strains were used for

themain groups of pathogenic bacteria: gram-positivecocci: Staphylococcus aureus, Streptococcuspneumoniae, Enterococcus fecalis; gram-positivebacilli: Bacillus anthracis (vaccine strain), Bacilluscereus, Bacillus subtilis; gram-negative bacilli:Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae,Pseudomonas aeruginosa; vibrio: Vibriocholerae.

The experimental contamination and decontaminationhave been carried out on an out ofservice military vehicle, representing the "target"(figure 3, 4) marked with numbered areas of approximately0.1 square meters on which have beenimplemented operating procedures for CBRN(chemical, biological, radiological and nuclear)contamination control.

Areas were chosen as follows: vertical paintedmetal sheet, for aqueous solution decontaminants;glass window for aqueous solution decontaminants;rubber tires for aqueous solution decontaminantsand horizontal painted metal sheet for suppliedpowder decontaminants as a positive control.

Microbial contamination was done by sprayingthe allocated surface, separately, with every microbial strain and with a mixture of them also. Amicrobial suspension culture in a liquid mediumwas used, with approximately 1 million live bacteriaper ml. Also, it was sprayed 1 ml of suspensionper square decimeter, enough to create a uniformcontaminant film. For liquid decontaminatingsubstances, the aqueous solution (conc.10%) wassprayed to cover the contaminated area until theexcess liquid begun to tear, about, 10ml/sqdm onsmooth surfaces (glass, metal sheets) and about20ml/sqdm on rough surfaces (peeling metalsheet, tires).

Figure 4. Microbiological testing of decontaminating substances on combat vehicle with Biological Mobile Intervention Team of CCSMM in CBRN training area from Campulung

Microbiological samples were collected bymeans of hygienic-sanitary pad, as follows:

0. before contamination (to establish a baselinelevel of natural contamination);

1. immediately after contamination (tocheck the level of contamination experiment);

2. after decontamination for each decontaminantupon each biological agent within 10 min(as required for military using decontaminant);

3. at every 45 min (as for general householddisinfectant).

In addition, at the end of the experiment,samples were collected from different parts of theoperator's protective equipment and from the environmentto detect any residual contamination. Allsamples were immediately transported under biosafetyconditions and tested in the microbiologyP2+ laboratory.

Decontamination achieved resultsafter 45 min are shown in Table 1.

Table 1. Contamination level after decontamination by 10% aqueous solution

No.	Decontaminant	Biological agent	Surface	Time (min.)	Growth (tube)	Growth (pane)	Remarks
1	DC 17 ICTCM	Mixture	Glass	45	-	++hem.	Contaminated
2	DC 17 ICTCM	B. cereus	Glass	45	-	-	Uncontaminated
3	DC 17 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
4	DC 17 ICTCM	B. cereus	vertical metal sheet	45	+/-	-	Uncontaminated
5	DC 18 ICTCM	Mixture	Glass	45	-	-	Uncontaminated
6	DC 18 ICTCM	B. cereus	Glass	45	+/-	-	Uncontaminated
7	DC 18 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
8	DC 18 ICTCM	B. cereus	vertical metal sheet	45	+dep.	-	Contaminated
9	DC 19 ICTCM	Mixture	Glass	45	-	-	Uncontaminated
10	DC 19 ICTCM	B. cereus	Glass	45	+/-	-	Uncontaminated
11	DC 19 ICTCM	Mixture	vertical metal sheet	45	+dep.	-	Contaminated
12	DC 19 ICTCM	B. cereus	vertical metal sheet	45	+veil, dep.	+	Contaminated

- or +/-: no microbial growth; dep.: bacterial deposit at medium bottom;
veil: bacterial population at medium top; hem.: hemolytic colonies.

COMMENTS

The antimicrobial effect remains and is moreobvious after 45 min, suggesting that these productscan be proposed as potential disinfectants formedical or hygienic-sanitary use, according withDrug Law.

The decontamination with powder is less effectivein all cases, compared with the laboratorycontrol sample, because of weak contact betweenmicro-organism and decontaminant, so a residualcontamination remains on surfaces.

In all cases, the final stage of decontaminationshould be washing with water because the substancesused can be corrosive upon certain materialsor irritating for personnel.

After the final washingaction, a microbiological analyze was madeto determine residual contamination of surfaces,waste water or protection equipment, but a significantcontamination was not recorded, so it can beconcluded that there is no risk to the environment.

CONCLUSIONS

A number of potential decontaminating substanceswere synthesized and tested, eight of whichwere selected and recommended as decontaminatingagents because these can be characterized byhigh chemical stability. These products are solublein water and various organic solvents. These biocidalproducts have bactericidal and fungicidal capabilities,with a large spectrum of usability. Thesubstances are characterized by low toxicity onanimals.

Theoretical and experimental scientific researchhas been made to design and implementpolyvalent “bio-chem” decontaminants for destructionof biological and toxic chemical agents,hazardous to health. Technologies have been developedfor obtaining polyvalent decontaminants,selected by efficiency assays, in laboratory. The experimentalpolyvalent

model for chemical and biologicaldecontamination with decontaminants ofquaternary ammonium salts types was developed.

Among the tested products, the best antimicrobialactivity is achieved by products based onmixture of oxidizing compounds and quaternaryammonium salts, which also have a synergic effectwhen used, expanding the action spectrum.

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