NATO NORTH ATLANTIC COUNCIL
OTAN CONSEIL DE L'ATLANTIQUE NORD

NATO UNCLASSIFIED

5 December 2005

DOCUMENT
AC/225-D(2005)0002-REV1

NATO ARMY ARMAMENTS GROUP

NATO STAFF REQUIREMENT (NSR) ON NON-CORROSIVE, BIOTECHNOLOGY-BASED DECONTAMINANTS FOR CHEMICAL AND BIOLOGICAL WARFARE AGENTS

Note by the Secretary

1. The NATO Staff Requirement on Non-Corrosive, Biotechnology-based Decontaminants for Chemical and Biological Warfare Agents has been developed by Project Group 31.

2. After closure of the PG/31, the custodianship of this document is now transferred to Land Group 7, Hazard Management Sub-Group.

3. This issue is a revised version, issued upon comments by a nation, to replace and supersede the initial version (AC/225-D(2005)0002).

4. The approval of this document will be sought at the NAAG meeting on 13-14 December, where the Chairman of the PG/31 will submit his final report.

(signed) O. TASMAN

1 Annex

Action Officer: O. Tasman
Original: English
G:\Docum\AC225\Ac225\Documents\AC225\AC_225-(2005)0002REV1.doc

NATO STAFF REQUIREMENT (NSR)

"NON-CORROSIVE, BIOTECHNOLOGY-BASED DECONTAMINANTS FOR CHEMICAL AND BIOLOGICAL WARFARE AGENTS"

1.0 INTRODUCTION

Recent events confirm that while the large scale chemical-biological (CB) threat may have decreased, it has now changed into a more localized problem. Although this document addresses only the military threat that might be managed by NATO, the global threat posed by CB agents extends to the entire civilian sector and the environment. Advances in biotechnology processes and the availability of this information have potentially enabled countries or groups to produce significant quantities of biological agents with relative ease. Chemical and biological molecules can be engineered to a desired potency, toxicity and efficiency. Moreover, they can be weaponized and applied under various field conditions. A number of nations with state-of-the-art technology are not signatories to the chemical, biological and toxin weapons conventions.

Decontamination is needed to make personnel, resources, or areas safe by absorbing, destroying, neutralizing, making harmless, or removing chemical or biological agents or by removing radiological materials; this includes both decontaminants and their means of application. Current decontamination materials are corrosive, potentially toxic, and non-specific. While they decontaminate the battlefield and equipment, they can damage sensitive equipment such as electronics and pose environmental and health hazards. Biotechnology offers a cleaner, non-corrosive, and a more specific alternative to chemical decontamination. By using enzymes, biosurfactants and other biological materials, personnel, equipment and environments can be decontaminated in a more efficient manner. In order to optimize the decontaminating action on the battlefield, a decontamination system composed of enzymes, certain environmentally benign chemicals, and emulsifiers is proposed. This biological decontamination system is designed to kill BW agents and break down CW agents into harmless components without causing additional environmental hazards, as in the case with some chemical decontaminants. Decontamination solution can be sprayed from a spray tank or a container vehicle and be effective under normal field conditions. Similar biological systems are developed and used in agriculture and the food industry. They can also be used for the decontamination of sensitive equipment (that can tolerate exposure to water).

2.0 MISSION NEED

2.1 ASSESSMENT OF NEED

Chemical and biological warfare agents as well as toxic industrial chemicals (TIC's) pose serious threats to military personnel, equipment, and facilities. A new generation of decontaminants is required that are effective, safe for personnel, equipment and the environment, and concentrated to reduce the logistical burden of mobile units or storage requirements for fixed facilities. Levels of efficacy need to meet those described in NATO STANAG 4653 on Decontamination of Chemical Warfare Agents and in the Biological Agent Decontamination Annex.

2.2 REQUIRED OPERATIONAL CAPABILITIES

2.2.1 Mobility and Transportation:

- Will not impact on current transportation requirements.
- Will not require special handling during transportation.
- Needs to be easily handled by personnel in PPE.

2.2.2 Manpower:

- Will not generate new manpower requirements.
- Will be usable by any soldier regardless of specialty/gender.

2.2.3 Performance Characteristics:

- Agents to be targeted: all classical nerve and vesicant agents (neat and thickened), and BW agents. Optional enhancements would include toxic industrial chemicals (TICs) or materials (TIMs).
- Temperature range during operation: -5 to 55°C is believed to be a reasonable threshold capability. Eventual goal would be for -32 to 55°C.
- Speed of reaction: should complete reaction in 15 minutes or less.
- Application methods: should be compatible with all current and planned decontamination equipment.
- Safety: no health or environmental hazard during storage, transportation, use or disposal. No negative impact on personnel or the environment. No toxic by-products should be produced during use and effluent should not require any special handling.
- Stability: 2 – 5 years shelf life with no special storage requirements. Longer shelf life may be obtained by refrigeration.
- Reconstitution: concentrated material will be compatible with all potential water sources (fresh, sea, potable).
- Will not degrade the operation of any vehicle or equipment.

2.2.4 Logistics:

- Lightweight decontaminant components supplied in lightweight packages
- Packaged in sizes for use by individual soldier up to large unit systems

2.2.5 Training:

- Can be used in training operations
- Can be used with agents or simulants during training. Depending on the simulants used, additional simulant-specific enzymes may be required if killing or degradation of the simulant is desired.

2.2.6 Standardization:

- Should be usable by all NATO and PfP forces
- Will be compatible with current/future decontamination and detection systems

2.3 FORMULATION CONCEPT

The basic system will consist of a mixture of catalytic enzymes and other components (such as cofactors, trace metals, etc.) in the form of dry solids (freeze-dried, spray-dried or granulated). If feasible, all the dry components of the will be contained in a single package that would be added to an aqueous system just prior to use. These systems could consist of water alone, fire-fighting foams and sprays, aircraft deicing fluids, aqueous degreasers or detergents, specialized microemulsions, etc. Once prepared, the decontaminant would be employed with conventional decontamination equipment or other spray or foam systems that may be available.

Separate packaging of the enzymes for different classes of agents could optimize activity and reduce cost. Based on the type of agent encountered, only the appropriate enzymes are used. For example, three separate packets of enzymes could be for nerve agents, blister agents, and BW agents. The solids are combined with buffering materials and added to an aqueous or semi-aqueous system that is available to the operational unit and compatible with the enzymes. The actual method of decontamination would be the same or similar to methods already in use for such equipment, facilities or environments. The only significant change may be the reduced need for rinsing after application of the decontaminant.

2.4 ENVIRONMENT

Other than the need to remain liquid, the decontaminant will not be limited in the type of geographic area or meteorological conditions that may be faced. In order to meet low temperature requirements, some type of antifreeze may be required. In addition, enzymes with lower optimum temperatures may need to be included. In areas of extreme dryness, additional quantities or repeated applications of the decontaminant may be required in case of rapid drying. It should be capable of use on land or shipboard.

Enzymes function primarily in an aqueous environment. While some application systems may have reduced water levels, a significant amount will still be required. For that reason, any material being decontaminated will need to be able to tolerate exposure to water or water/solvent mixtures for the time required for decontamination.

Enzymatic formulations would require less water than older legacy systems because of reduced requirement for pre-wash and rinse steps.

3.0 TECHNICAL CONSIDERATIONS

3.1 SYSTEM REQUIREMENTS

Each enzyme employed in the system will need to be catalytic against its target agent/substrate. It should have a pH optimum in the range of non-corrosive conditions in order to avoid material compatibility problems. It also needs to be compatible with the other enzymes in the system. In dry form, the enzyme should be stable under normal storage conditions for a minimum of 2 - 5 years. Once reconstituted with water, the enzyme should retain sufficient activity to meet efficacy requirements for at least six hours (threshold objective). Eventual goal is to meet AEP-58 guidelines. For each of the major classes of chemical or biological agents, the system will contain at least one and preferably several different enzymes with overlapping or complementary activity.

More detailed descriptions of the enzymes for each agent class are provided in the Annexes to this document.

3.2 SYSTEM CHARACTERISTICS

The system will consist of some or all of the following components in one or more sealed containers

Required:

- Enzymes – dry solids
- Buffering materials (to control pH)
- Required trace metals or co-factors – dry solids

Optional (dependent on decontamination system and situation – all need to be enzyme compatible):

- Foam concentrate (for retention on surfaces and as a vapor barrier) – liquid or solid
- Organic components for emulsion (for agent/thickener solubilization and extraction from surfaces) – liquid
- Aqueous degreaser (for agent/thickener solubilization and extraction from surfaces) – liquid or solid
- Aircraft deicing solution
- Disinfectant, sporicide or biocide (for enhanced BW efficacy) – liquid or solid

In preparing the formulation, the components are added to water or a mixed solvent system in a manner that maximizes enzyme efficacy and stability. Once prepared, the decontaminant should be used within six hours.

By packaging the components separately, new or improved enzymes and other materials can be quickly incorporated into the system. In addition, an operator can use only the enzymes required for a decontamination operation and not waste the others.

Test and evaluation criteria for determining the efficacy of the decontaminant will be according to NATO STANAG 4653 or other appropriate standardized Test Operating Procedures.

3.3 LOGISTICS, TRAINING AND INFRASTRUCTURE

Because the decontaminant contains no hazardous materials, it can be stored under standard conditions. An expiration date will be on the packaging. An inventory system will be required to track the expiration dates for long-term storage and replenishment needs. If there is any evidence that the enzyme component has been improperly sealed, torn open or otherwise exposed to the environment, it should be assumed inactive and disposed of properly. Other than this, it will require no special surveillance or maintenance.

The enzyme-based decontaminant is intended to be employed and used with the same equipment and procedures as current decontaminants. No special operational requirements are anticipated. A simulant-based test system should be developed to ensure that the decontaminant has been mixed properly and is still effective.

The decontaminant will be useful for training purposes. It should result in reduced logistics for training as well as eliminate most disposal problems faced with current decontaminants. Depending on the situation and location, training is feasible with either actual agents or appropriate simulants. Because of the specificity of enzymes, simulants used may be different from those currently in use.

4.0 ECONOMIC/MANAGEMENT CONSIDERATIONS

4.1 COST ANALYSIS

The goal is a cost per unit volume of prepared (ready to use) decontaminant at or below that of currently available decontaminants (5-10 €/liter). Initially, because of small production runs, the enzyme costs will be quite high (1000-2000 €/kg) although only small quantities are used in the final product. As the scale of enzyme production increases and with improved bioprocessing methods, the costs should decrease considerably – one to two orders of magnitude. It is not possible at this time to predict the final costs of the decontaminant since it will be dependent on the number of enzymes involved and the other, non-biological components of the system (ex. buffers, trace metals, detergents, and foams).

4.2 ECONOMIC CONSIDERATIONS

Biotechnology-based decontamination systems using many of the same enzymes have much broader potential uses in the civilian sector for dealing with terrorist incident response, accidental chemical spills, and in agricultural and industrial applications. As these products multiply and production quantities increase, military specific systems should benefit and the cost decrease substantially.

4.3 MANAGEMENT APPROACH

For the Alliance to acquire a biotechnology-based decontamination capability via the Phased Armaments Programming System (PAPS) process, the following phases will need to take place:

a. Request by Land Group 7 (LG/7) for CNAD to designate the project as a NATO Project. Establishment of the corresponding NATO Project Steering Committee.

b. Milestone 5: NATO Design and Development Objective (NADDO). Participating nations prepare and approve MOU.

b. Milestone 6: NATO Production Objective (NAPO). May be national or co-operative.

c. Milestone 7: NATO In-Service Goals (NISEG). Configuration management of the deployed systems must be co-operative to maintain standardisation.

d. Milestone 8: National Disengagement Intention (NADI).

National developments may produce products ahead of the NATO timeframe that may be used to fill the identified capability gap. As part of the management approach, national developments will be monitored closely.

Overall requirements will be developed that deal with such issues as the quantities to be developed/produced, production rates, and which configurations/formulations will be required with what equipment,.

5.0 STANDARDIZATION

Biotechnology-based decontaminants must be compatible with current decontamination systems as well as civilian (first responder) systems that could be used as mission-expedient equipment. They must be compatible with the materials of construction of NATO equipment and vehicles on which they are used. Reference should be made to related STANAG or APs and to other standards that may need to be used or developed.

6.0 FUTURE INTENTIONS

Further actions on the development of biotechnology-based decontaminants should be coordinated through the Hazard Management Subgroup (HMSG) of LG/7.

APPENDIX: A

ENZYMES FOR G-TYPE NERVE AGENTS

1.0 NAME

Squid Diisopropylfluorophosphatase

1.1 SYNONYMS

DFPase, EC 3.1.8.2

1.2 ORIGINAL SOURCE

Loligo vulgaris head ganglia

Also detected in the head ganglia of several cephalopods (*L. opalescens, L. pealei, Octopus vulgaris,* and *Todarodes pacificus steenstrup*)

1.3 CHARACTERISTICS

1.3.1 Structure

The DFPase from *L. vulgaris* consists of 314 amino acids with a molecular weight of 35 kDa and appears to exist as a single polypeptide chain. It contains high- and low-affinity calcium binding sites. Based on X-ray crystallographic analysis, the structure resembles a six-bladed β propeller. The blades are arranged around a central water-filled tunnel that contains two calcium ions.

1.3.2 Activity

The DFPase from *L. vulgaris* has maximum activity 35°C and pH 8.0. Under these standard conditions, it has the following activity:

Substrate	k_{cat} (sec^{-1})
DFP	208
Sarin (GB)	109
Soman (GD)	77
Tabun (GA)	37
VX	0

k_{cat} is the turnover number of the enzyme ≡ number of substrate molecules reacted with by each enzyme molecule per second.

1.3.3 Natural Function

The natural substrate and function of this enzyme is unknown.

1.4 AVAILABILITY

The squid DFPase is not available commercially. Prof. Rüterjans should be contacted about the most current information on availability as he holds a patent on the enzyme.

1.5 REFERENCES

- Hoskin, F.C.G. and Roush, A.H. (1982) Hydrolysis of Nerve Gas by Squid-Type Diisopropyl Phosphorofluoridate Hydrolyzing Enzyme on Agarose Resin. *Science* **215**, 1255-1257.
- Wang, F., Xiao, M., and Mu, S. (1993) Purification and Properties of a Diisopropyl-Fluorophosphatase from Squid *Todarodes pacificus steenstrup. J. Biochem. Toxicol.* **8**, 161-166.
- Rüterjans, H. and Dierl, S. (1999) Diisopropylfluorophosphatase sowie deren Verwendung und Herstellung. German Pat. No. DE19808192 A 19990909.
- Hartleib, J., Geschwindner, S., Scharff, E.I., and Rüterjans, H. (2001) Role of Calcium Ions in the Structure and Function of the Di-isopropylfluorophatase from *Loligo vulgaris. Biochem. J.* **353**, 579-589
- Hartleib, J. and Rüterjans, H. (2001) High-Yield Expression, Purification, and Characterization of the Recombinant Diisopropylfluorophosphatase from *Loligo vulgaris*. *Protein Expression and Purification* **21**, 210-219.
- Hartleib, J. and Rüterjans, H. (2001) Insights into the Reaction Mechanism of the Diisopropyl Fluorophosphatase from *Loligo vulgaris* by Means of Kinetic Studies, Chemical Modification and Site-Directed Mutagenesis. *Biochimica et Biophysica Acta* **1546**, 312-324.
- Koepke, J., Scharff, E.I., Lücke, C., Rüterjans, H. and Fritzsch, G. (2002) Atomic resolution crystal structure of squid ganglion DFPase. *Acta Cryst.* **D58**, 1757-1759.

1.6 POINTS OF CONTACT

Prof. Heinz Rüterjans
Institute of Biophysical Chemistry
Johann Wolfgang Goethe University of Frankfurt/M.
Marie-Curie-Strasse 9
60439 Frankfurt/M
Germany
Tel: 49-69-7982-9630
Fax: 49-69-7982-9632
E-mail: hruet@bpc.uni-frankfurt.de

2.0 NAME

Bacterial Organophosphorus Acid Anhydrolase

2.1 SYNONYMS

OPAA, DFPase (EC 3.1.8.2)
Prolidase, Xaa-Pro Dipeptidase (EC 3.4.13.9)

2.2 ORIGINAL SOURCE

Alteromonas sp. JD6.5 – a halophilic bacterial isolate

OPAA enzymes with activity against organophosphorus (OP) compounds were also detected in other *Alteromonas* species (*haloplanktis* and *undina*), *E. coli,* hog kidney and humans. It is believed that this type of dipeptidase enzyme is ubiquitous to all living organisms, but differences between species determine whether they will have activity against OP compounds.

2.3 CHARACTERISTICS

2.3.1 Structure

The OPAA from *A.* sp. JD6.5 consists of 517 amino acids with a molecular weight of 58.5 kDa and appears to generally exist as a single polypeptide chain. However, there is some evidence from its crystal structure that it can form a trimer. The OPAA's from *A. haloplanktis* and *A. undina* have a high degree of homology with the JD6.5 enzyme, but are 7 to 10 kDa smaller. This difference lies in the length of the C-terminus of the protein that does not appear to be involved in enzyme activity. The OPAA's and prolidases/dipeptidases in general use Manganese in the active site, but in some cases Cobalt will substitute without loss in activity.

2.3.2 Activity

The pH optimum of the three *Alteromonas* OPAA's is between 7.5-8.5 and the temperature optimum between 40-55°C.

Substrate	k_{cat} (sec^{-1})		
	A. sp. JD6.5	*A. haloplanktis*	*A. undina*
DFP	1820	575	1239
Cyclosarin (GF)	1654	269	1586
Paraoxon	124	-	-
Sarin (GB)	611	257	376
Soman (GD)	3145	1389	2496
Tabun (GA)	85	113	292
VX	0	0	0

k_{cat} is the turnover number of the enzyme ≡ number of substrate molecules reacted with by each enzyme molecule per second.

2.3.3 Natural Function

The natural substrates for this type of enzyme are dipeptides with a proline at the C-terminal position. The function of this enzyme most likely deals with the recycling of intracellular proteins and peptides. Through the action of this enzyme and a variety of proteases and peptidases, proteins are hydrolyzed to free amino acids that can then be used in the synthesis of new proteins. Hydrolysis of OP compounds is not a natural function of this enzyme.

2.4 AVAILABILITY

The U.S. Army has licensed two patents on the OPAA from *Alteromonas* sp. JD6.5 to Genencor International for the large-scale production of the enzyme.

2.5 REFERENCES

- DeFrank, J.J. and Cheng, T.-c., (1991) Purification and Properties of an Organophosphorus Acid Anhydrase from a Halophilic Bacterial Isolate. *J. Bacteriol.*, **173**, 1938-1943.
- Cheng, T.-c., Harvey, S.P., and Stroup, A.D. (1993) Purification and Properties of a Highly Active Organophosphorus Acid Anhydrolase from *Alteromonas undina*. *Appl. Environ. Microbiol.* **59**, 3138-3140.
- DeFrank, J.J., Beaudry, W.T., Cheng, T.-c., Harvey, S.P., Szafraniec, L.L., and Stroup, A.N. (1993) Screening of Halophilic Bacteria and *Alteromonas* sp. for Organophosphorus Hydrolyzing Enzyme Activity. *Chem.-Biol. Interactions* **87**, 141-148.
- Cheng, T-c. and Calomiris, J.J. (1996) A Cloned Bacterial Enzyme for Nerve Agent Decontamination. *Enzyme Microb. Technol.* **18**, 597-601.
- Cheng, T-c., Harvey, S.P., and Chen, G.L. (1996) Cloning and Expression of a Gene Encoding a Bacterial Enzyme for Decontamination of Organophosphorus Nerve Agents and Nucleotide Sequence of the Enzyme. *Appl. Environ. Microbiol*. **62**, 1636-1641.
- Hoskin, F.C.G. and Walker, J.E. (1998) A Closer Look at the Natural Substraate for a Nerve-Gas Hydrolyzing Enzyme in Squid Nerve. *Biol. Bull.* **195**, 197-198.
- Cheng, T.-c., Liu, L., Wang, B., Wu, J., DeFrank, J.J., Anderson, D., and Hamilton, A.B. (1997) Nucleotide Sequence of a Gene Encoding an Organophosphorus Nerve Agent Degrading Enzyme from *Alteromonas haloplanktis*. *J. Ind. Microbiol. & Biotech.* **18**, 49-55.
- Cheng, T.-c. and DeFrank, J.J. (1999) Enzymatic Detoxification of Organophosphorus Compounds. U.S. Patent number 5,928,927.
- Cheng, T.-c., DeFrank, J.J., and Rastogi, V.K., "*Alteromonas* Prolidase for Organophosphorus G-Agent Decontamination," (1999), Chem.-Biol. Interactions, 119-120:455-462.
- Cheng, T.-c. and DeFrank, J.J. (2000) Enzymatic Detoxification of Organophosphorus Compounds," U.S. Patent number 6,080,566.
- Hill, C.M., Wu, FY, Cheng, T-c., DeFrank, J.J., and Raushel, F.M. (2000) Substrate and Stereochemical Specificity of the Organophosphorus Acid Anhydrolase from *Alteromonas* sp. JD6.5 Toward *p*-Nitrophenyl Phosphotriesters. *Bioorg. Med. Chem. Letters* **10**, 1285-1288.

- Hill, C.M., Li, W.-S., Cheng, T.-c., DeFrank, J.J., and Raushel, F.M. (2001) Stereochemical Specificity of Organophosphorus Acid Anhydrolase Toward *p*-Nitrophenyl Analogs of Soman and Sarin. *Bioorg. Chem.* **29**, 27-35.

2.6 POINTS OF CONTACT

For basic information:

Dr. Joseph J. DeFrank
U.S. Army Edgewood Chemical Biological Center
AMSRD-ECB-RT-BP (Bldg. E3549)
Aberdeen Proving Ground
Maryland 21010-5424
United States
Tel: 1-410-436-3749
Fax: 1-410-436-8661
E-mail: joseph.defrank@us.army.mil

For commercial availability information:

Mr. Christopher S. Penet
Director, Specialties Enzymes Business
Genencor International, Inc.
200 Meridian Centre Blvd.
Rochester
New York 14618
United States
Tel: 1-585-256-5262
Fax: 1-585-256-5286
E-mail: cpenet@genencor.com

3.0 NAME

Bacterial Organophosphorus Hydrolase

3.1 SYNONYMS

OPH (EC 3.1.8.1)
Parathion Hydrolase
Phosphotriesterase (PTE)

3.2 ORIGINAL SOURCE

Pseudomonas diminuta
Flavobacterium sp.

Although found in bacteria from opposite sides of the world and with genes on totally different plasmids, the enzymes are essentially identical. Several related OPH's have been described from other bacterial isolates. All are very similar to the original enzymes.

3.3 CHARACTERISTICS

3.3.1 Structure

OPH initially consisted of a 365 amino acid precursor. A 29 amino acid leader sequence was removed to generate a mature enzyme with a molecular weight of 36 kDa. It is believed to generally exist as a dimer. The native enzyme has two Zinc ions in the active site; however, a variety of other divalent metal ions can be substituted. The Cobalt enzyme has the greatest activity on substrates with P-F and P-S bonds.

3.3.2 Activity

The pH optimum of OPH is fairly broad between 8 and 10 with a temperature optimum of ~50°C. Preferred substrates are the organophosphorus pesticides with P-O bonds, followed by P-F bonds and finally P-S bonds. This is the currently the only well characterized enzyme with activity against V-agents.

Substrate	k_{cat} (sec^{-1})
Acephate	2.8
Demeton-S	1.25
DFP	465
Paraoxon	3170
Parathion	630
Sarin (GB)	56
Soman (GD)	5
VX	0.3

k_{cat} is the turnover number of the enzyme ≡ number of substrate molecules reacted with by each enzyme molecule per second.

Site-directed mutagenesis of OPH has resulted in modification of its activity on a variety of substrates. Both the catalytic activity (k_{cat}) and substrate specificity (k_{cat}/K_m) of OPH has been changed as well as its stereospecificity against chiral substrates. Increases in activity of 20-40 fold have been achieved with soman and to a lesser extent with DFP, VX and acephate.

3.3.3 Natural Function

The natural substrate and function of this enzyme is unknown

3.4 AVAILABILITY

The U.S. Army has licensed a patent on a modified wild type OPH to Genencor International for the large-scale production of the enzyme.

3.5 REFERENCES

- Sethanathan, N. and Yoshida, T. (1973) A *Flavobacterium* that Degrades Diazinon and Parathion. *Can. J. Microbiol.* **19**, 873-875.
- Munnecke, D.M. (1976) Enzymatic Hydrolysis of Organophosphate Insecticides, a Possible Pesticide Disposal Method. *Appl. Environ. Microbiol.* **32**, 7-13.
- Serdar, C.M. and Gibson, D.T. (1985) Enzymatic Hydrolysis of Organophosphates: Cloning and Expression of a Parathion Hydrolase Gene from *Pseudomonas diminuta*. *Bio/Technol.* **2**, 567-571.
- Serdar, C.M., Murdock, D.C., and Rohde, M.F. (1989) Parathion Hydrolase Gene from *Pseudomonas diminuta* MG: Subcloning, Complete Nucleotide Sequence, and Expression of the Mature Portion of the Enzyme in *Escherichia coli*. *Bio/Technol.* **7**, 1151-1155.
- Benning, M.m., Kuo, J.M., Raushel, F.M., and Holden, H.M. (1995) Three-Dimensional Structure of the Binuclear Metal Center of Phosphotriesterase. *Biochemistry* **34**, 7973-7978.
- Lai, K., Grimsley, J.K., Kuhlmann, B.D., Scapozza, L., Harvey, S.P., DeFrank, J.J., Kolakowski, J.E., and Wild, J.R. (1996) Rational Enzyme Design: Computer Modeling and Site-Directed Mutagenesis for the Modification of Catalytic Specificity in Organophosphorus Hydrolase. *Chimia* **50**, 430-431.
- Kolakowski, J.E., DeFrank, J.J., Harvey, S.P., Szafraniec, L.L., Beaudry, W.T., Lai, K., and Wild, J.R. (1997) Enzymatic Hydrolysis of the Chemical Warfare Agent VX and its Neurotoxic Analogues by Organophosphorus Hydrolase. *Biocat. Biotrans.* **15**, 297-312.
- Watkins, L.M., Mahoney, H.J., McCulloch, J.K., and Raushel, F.M. (1997) Augmented Hydrolysis of Diisopropyl Fluorophosphate in Engineered Mutants of Phosphotriesterase. *J. Biol. Chem.* **272**, 25596-25601.

3.6 POINTS OF CONTACT

For basic information:

Dr. Vipin K. Rastogi
U.S. Army Edgewood Chemical Biological Center

AMSRD-ECB-RT-BP (Bldg. E3150)
Aberdeen Proving Ground
Maryland 21010-5424
United States
Tel: 1-410-436-4856
Fax: 1-410-436-2081
E-mail: vipin.rastogi@us.army.mil

For commercial availability information:

Mr. Christopher S. Penet
Director, Specialties Enzymes Business
Genencor International, Inc.
200 Meridian Centre Blvd.
Rochester
New York 14618
United States
Tel: 1-585-256-5262
Fax: 1-585-256-5286
E-mail: cpenet@genencor.com

APPENDIX: B

ENZYMES FOR V-TYPE NERVE AGENTS

1.0 NAME

Bacterial Organophosphorus Hydrolase (see APPENDIX A; Section 3.0)

APPENDIX: C

ENZYMES FOR BLISTER AGENTS

1.0 NAME

APPENDIX: D

ENZYMES FOR BIOLOGICAL AGENTS

1.0 NAME

APPENDIX: E

ENZYMES FOR CARBAMATE PESTICIDES

1.0 NAME

APPENDIX: F

ENZYMES FOR PROTEIN TOXINS

1.0 NAME