NORTH ATLANTIC TREATY ORGANISATION

RESEARCH AND TECHNOLOGY ORGANISATION

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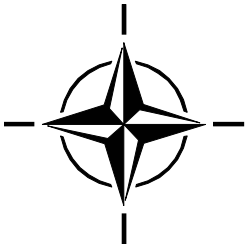
RTO TECHNICAL REPORT **TR-HFM-041**

Prophylaxis and Therapy Against Chemical Agents

(Prophylaxie et thérapie contre les agents chimiques)

Final Report of the HFM-041/TG-004 for the Period 1999 to 2005.



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Final Report of the HFM-041/TG-004 for the Period 1999 to 2005.

The final report of the HFM-041 includes the complete scientific proceedings of meetings sponsored under this task group and held in 1999, 2000, 2002, 2003 and 2005. The report also includes a summary report on bioscavengers as a new pre-treatment for nerve agent poisoning.

The Research and Technology Organisation (RTO) of NATO

RTO is the single focus in NATO for Defence Research and Technology activities. Its mission is to conduct and promote co-operative research and information exchange. The objective is to support the development and effective use of national defence research and technology and to meet the military needs of the Alliance, to maintain a technological lead, and to provide advice to NATO and national decision makers. The RTO performs its mission with the support of an extensive network of national experts. It also ensures effective co-ordination with other NATO bodies involved in R&T activities.

RTO reports both to the Military Committee of NATO and to the Conference of National Armament Directors. It comprises a Research and Technology Board (RTB) as the highest level of national representation and the Research and Technology Agency (RTA), a dedicated staff with its headquarters in Neuilly, near Paris, France. In order to facilitate contacts with the military users and other NATO activities, a small part of the RTA staff is located in NATO Headquarters in Brussels. The Brussels staff also co-ordinates RTO's co-operation with nations in Middle and Eastern Europe, to which RTO attaches particular importance especially as working together in the field of research is one of the more promising areas of co-operation.

The total spectrum of R&T activities is covered by the following 7 bodies:

- AVT Applied Vehicle Technology Panel
- HFM Human Factors and Medicine Panel
- IST Information Systems Technology Panel
- NMSG NATO Modelling and Simulation Group
- SAS System Analysis and Studies Panel
- SCI Systems Concepts and Integration Panel
- SET Sensors and Electronics Technology Panel

These bodies are made up of national representatives as well as generally recognised 'world class' scientists. They also provide a communication link to military users and other NATO bodies. RTO's scientific and technological work is carried out by Technical Teams, created for specific activities and with a specific duration. Such Technical Teams can organise workshops, symposia, field trials, lecture series and training courses. An important function of these Technical Teams is to ensure the continuity of the expert networks.

RTO builds upon earlier co-operation in defence research and technology as set-up under the Advisory Group for Aerospace Research and Development (AGARD) and the Defence Research Group (DRG). AGARD and the DRG share common roots in that they were both established at the initiative of Dr Theodore von Kármán, a leading aerospace scientist, who early on recognised the importance of scientific support for the Allied Armed Forces. RTO is capitalising on these common roots in order to provide the Alliance and the NATO nations with a strong scientific and technological basis that will guarantee a solid base for the future.

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Prophylaxis and Therapy Against Chemical Agents

(RTO-TR-HFM-041)

Executive Summary

HFM-041 TG-004 served as a forum for the presentation of basic scientific research concerning the toxic mechanism of action and the effectiveness of experimental and actual clinical therapies against known or potential chemical warfare (CW) agents including toxins. Member countries explored new innovative concepts and developed new strategies for medical chemical defence. Therapies or prophylactic measures were aimed at the development of drugs, often antagonists, to intervene in the pathological processes involved. The crossover of research results and ensuing theories on agent actions among the nerve agents, vesicants, midspectrum toxins and neuromodulators optimizes the utilization of scientific resources.

This report focuses on the mutual efforts of the member countries to identify compounds and therapeutic interventions that could be developed into new drugs or treatments, approved by their respective medical approval agencies, e.g., the US Food and Drug Administration (USFDA) in the United States, that would provide enhanced protection against chemical warfare threats faced by the NATO forces. These threats have included chemical warfare nerve agents such as sarin, soman or tabun, vesicating agents such as sulfur mustard or lewisite, lung damaging agents such as phosgene and, to a much lesser extent, chlorine, blood agents such as cyanide and toxins such as botulinum toxin.

The participating member states were Canada, Germany, France, the Netherlands, Belgium (until 2000), the United Kingdom, Norway, the United States, the Czech Republic (since 2000) and Sweden. Representatives from Poland, Hungary and Italy were corresponding members but did not actively participate in the research exchange meetings.

In order to maintain close cooperative efforts in the mutual goals of identifying new antidotes for chemical warfare poisoning worthy of advanced development and ultimately licensure by the appropriate regulatory agencies, e.g., the USFDA, Health Canada, technical working meetings were held periodically at 18 to 24 month intervals. These meetings were usually 4 to 4½ days in length during which time scientists from the member countries made oral presentations of their latest scientific advances in identifying potential antidotes. In addition, time was spent in formal discussions centering on which approaches would best allow for advanced development of new drugs that could be standardized for overall NATO use.

Prophylaxie et thérapie contre les agents chimiques

(RTO-TR-HFM-041)

Synthèse

Le HFM-041 TG-004 a servi de tribune à la présentation des recherches scientifiques fondamentales relatives au mécanisme toxique de l'action des agents de guerre chimique (CW) connus ou potentiels – y compris les toxines – et à l'efficacité des thérapies cliniques expérimentales et appliquées. Les nations membres ont exploré des concepts novateurs et développé de nouvelles stratégies en matière de défense médicale contre les agents chimiques. Les mesures prophylactiques ou thérapeutiques concernées visaient à développer des médicaments, souvent antagonistes, destinés à intervenir dans les processus pathologiques impliqués. Le croisement des résultats de recherche et les théories qui en ont découlé sur l'action des agents – agents neurotoxiques, agents vésicants, toxines à mi-spectre et neuromodulateurs – permettent d'optimiser l'utilisation des ressources scientifiques.

Le présent rapport s'intéresse aux efforts mutuels des nations membres en vue d'identifier les composés et les interventions thérapeutiques qui pourraient être développés et aboutir à de nouveaux médicaments ou traitements approuvés par leurs organismes respectifs d'homologation médicale – par exemple, la US Food and Drug Administration (USFDA) aux Etats-Unis – et qui fourniraient une protection accrue contre les menaces de guerre chimique auxquelles les forces de l'OTAN sont confrontées. Ces menaces comprennent des agents de guerre chimique neurotoxiques tels que le sarin, le soman ou le tabun ; des agents vésicants tels que l'ypérite au soufre ou la lewisite ; des agents suffocants tels que le phosgène ou, dans une moindre mesure, le chlore ; des agents hémotoxiques tels que le cyanure ; et des toxines telles que la toxine botulinique.

La liste des états membres participants comprenait le Canada, l'Allemagne, la France, les Pays-Bas, la Belgique (jusqu'en 2000), le Royaume-Uni, la Norvège, les Etats-Unis, la République tchèque (depuis 2000) et la Suède. Des représentants de la Pologne, de la Hongrie et de l'Italie ont joué le rôle de correspondants, mais n'ont pas pris une part active dans les réunions de recherche coopérative.

Afin de continuer à collaborer étroitement à cet objectif mutuel – l'identification de nouveaux antidotes à l'empoisonnement par des agents de guerre chimique, antidotes susceptibles d'être développés de manière avancée et finalement homologués par les organismes de réglementation concernés (l'USFDA ou Santé Canada, par exemple) – des réunions de travail techniques ont été organisées périodiquement, à 18 ou 24 mois d'intervalle. Ces réunions duraient généralement de 4 à 4 jours ½, au cours desquels les scientifiques des nations membres présentaient oralement les derniers progrès qu'ils avaient accomplis en matière d'identification d'antidotes potentiels. En outre, un certain temps fut consacré à des débats formels sur les approches qui favoriseraient au mieux les développements avancés de nouveaux médicaments pouvant être standardisés en vue d'un usage général par l'OTAN.

Chapter 1 – SUMMARY OF THE MEETING HELD IN BRUSSELS (BEL) 22-26 MARCH 1999

The meeting was held in Brussels, Belgium at the Queen Astrid Military Hospital. Attending countries were the United States, Belgium, United Kingdom, France, The Netherlands, Canada, Germany, Norway and Sweden. The topics discussed were: the new organization of the TG-004, vesicants (both sulfur mustard and Lewisite), oximes, anticonvulsants, biological scavengers, centrally acting drugs as pre-treatments for nerve agent poisoning, approval for medical chemical defence pharmaceutical products by national agencies, UNSCOM inspections, and management of either civilian or military chemical casualties. The meeting offered the singular opportunity to obtain information that was new, unique and, often, unpublished regarding medical chemical defence.

There was a discussion of the new organizational structure, which has replaced the RSG-III under Technical Panel 8. The group is now the Technical Group (TG) – 004 under the Human Factors and Medical (HFM) panel (Dr. Johnson-Winegar is the US representative in the Human Protection Area) of the NATO Research and Technology Board (RTO). The discussion focused on the actions of the HFM and the role of the TG-004. This will be particularly helpful in future discussions of the integration of the member nation programs within the TG-004 mission.

There were three presentations on the drug approval process within the European Union (EU) as well as within individual NATO countries. These presentations were quite valuable given the current efforts in the US to get FDA approval of the pyridostigmine NDA. Many of the NATO representatives feel that if the US can get approval, then approval within the EU for pyridostigmine will be much easier. That will certainly enhance not only US but also NATO effectiveness. Drs. McDonough and Smith of the US delegation felt that the subject was also germane for the upcoming milestone 1 and milestone 0 deadlines for anticonvulsants and vesicants respectively.

The numerous presentations dealing with oximes other than the standard US oxime 2-PAM, such as HI-6 and HLö-7, most unpublished and hence not obtainable elsewhere, addressed the value of these compounds as treatments for nerve agent poisoning. Given the absence of an active program in this area in the US, the data were particularly useful given the current US program in the area of novel threat agents, some of which may be resistant to current therapeutic approaches.

During the day and a half devoted to vesicant research the presentations on sulfur mustard (HD) toxicokinetics in the hairless guinea pig, the HD skin penetration studies from the UK and the comparison of percutaneous vs. respiratory exposure to HD presented by The Netherlands as well as the UK and the Lewisite studies of the UK gave valuable information on an aspect of medical chemical defence that is currently not under investigation in the US. During the discussion of the UK data on human skin absorption of HD in vitro, several members of the US delegation identified the importance of Dr. Rice's presentation to the US topical skin protectant (TSP) program and the reactive TSP program. Dr. Rice's data will probably be available only through meetings such as the TG-004 which serves to emphasize the value of face to face exchanges on these subjects as much of the details were discussed informally during breaks and after hours.

The biological scavenger program, which has been largely a US effort for several years, has expanded as evidenced by one presentation from the French laboratory and one joint French/US effort. The ability of the participating counties to leverage this program through the NATO partners comes as a direct result of prior TG-004 meetings in which collaborative efforts were identified and agreed upon.

In the area of anticonvulsant research the French reported on investigations on NMDA antagonists. In subsequent discussions between Dr. McDonough and one of the French representatives it was indicated that they are awaiting approval of their lead compound, which is in phase II or III clinical trails for spinal cord injury, for human use before they expend additional effort. That revelation indicates that the US will retain the lead in NATO for development of a diazepam replacement.

1.1 COOPERATION

One important result of the meeting was the identification of a variety of areas where there are opportunities for joint NATO allies research efforts. These include US/UK efforts on anticonvulsants (since the UK has an excellent behavioral toxicology program), US/French efforts on anticonvulsants and bioscavengers, and US/ Netherlands efforts on HD. The opportunity for the scientists from individual countries to meet and discuss these international research plans has led to reduced duplication of efforts with its attendant cost savings as well as the ability to gain access to information that will not appear in any other forum.

Chapter 2 – SUMMARY OF THE MEETING HELD IN THE HAGUE (NLD) 1-5 OCTBER 2000

A meeting of the TG-004 was held at The Netherlands Defence Academy, The Hague, The Netherlands 1-5 October 2000. There were a total of 51 participants from 10 countries who made 49 oral or poster presentation encompassing the chemical warfare agents covered under the TOR of TG-004. These agents include the nerve agents, sulfur mustard, lewisite, botulinum toxin, ricin and phosgene. Representatives of all the active TG-004 countries were present with the exception of Belgium, Poland and Hungary. A representative from Italy was present as was an invited guest from Sweden.

In the area of developing effective treatment against injuries caused by the vesicating agents sulfur mustard and Lewisite the focus was on immediate treatment or on efforts to understand the mechanism of sulfur mustard skin injury so that effective pharmacological interventions could be developed. With respect to Lewisite, the use of physical dermabrasion by either mechanical means (surgical dermabrasion) or by laser resulted in more rapid recovery in comparison to the rate of spontaneous recovery. The conclusion was made to recommend the use of dermabrasion as the new standard of care for Lewisite injury and to use surgical dermabrasion in the field where appropriate lasers may not be available. It was noted that dermabrasion was not as effective against Lewisite injury as it is for treatment of sulfur mustard, but it represents a marked advantage for treatment of injuries from either source of vesicating agent. In the area of pharmacological interventions for treatment of sulfur mustard injury, several models using different cell types to assess drugs in early development were described. In addition, the potential role of matrix metalloproteases in preventing blister formation after sulfur mustard exposure were discussed.

A variety of the presentations addressed very practical approaches to providing protection against chemical agent exposures. Among these were the proposed use of sponges that had cholinesterase immobilized in the sponge matrix as active moiety for decontaminating skin after exposure to VX. A second approach was the use of RSDL (reactive skin decontaminate lotion), as a topical treatment to decontaminate skin following VX exposure. There was a min-symposium on drug development centered on pyridostigmine bromide as a pre-treatment for nerve agent poisoning and a clinical study on the effectiveness of obidoxime and the issues involved in the need for international cooperation in gaining drug approval for drugs to replace currently fielded antidotes.

The numerous efforts by the member laboratories with respect to medical protection against nerve agent poisoning covered a wide range of efforts. The feasibility to use naturally occurring human enzymes such as butyrylcholinesterase or paraoxonase to rapidly hydrolyze nerve agents in vivo was demonstrated. The concerns about in vivo toxic effects following low dose chronic exposure to nerve agents were addressed in several papers. These efforts were complimented by work on the ability to determine the actual levels of toxicant in the blood after low dose exposures. The continued efforts to address the complicating neurologic effects after nerve agent poisoning were exemplified in the numerous papers on the use of anticonvulsants, the relationship of stress to neurologic function and the overall role of cholinergic neuropharmacology. These efforts were all directed on finding new therapeutic interventions using existing centrally active drugs or in identifying the properties needed in new drugs under development

The member laboratories maintain continued, but low level efforts in the areas of toxin poisoning and in the areas of lung injury following exposure to phosgene or other lung oedemagens.

The information presented demonstrated the continued emphasis by the panel members to identify and develop new antidotes for chemical warfare poisoning. The increased attention to the issues involved in transition of

drugs to advanced development in the foreseeable future was identified as a new area for increased cooperation in the efforts to produce new and more treatments for the NATO soldier.

2.1 COOPERATION

The mini-symposium of drug development was specifically designed to bring attention to the need for cooperation between the member research efforts. Given the expense of gaining regulatory approval for any drug, by any country, the need to cost share the effort through cooperative research efforts was agreed to be an effort to continue to pursue. Likewise the need for neurological interventions is such a complicated scientific problem that the participants agreed that collaborative efforts in that arena was also an area that would yield great collective benefits.

Chapter 3 – SUMMARY OF THE MEETING HELD IN OSLO (NOR) 4-7 NOVEMBER 2002

A meeting of the TG-004 was held at the Norwegian Defence College, Oslo, Norway, 4-7 November 2002. There was a total of 41 participants who made 46 oral or poster presentation encompassing the chemical warfare agents covered under the TOR of TG-004. These agents include the nerve agents, sulfur mustard, lewisite, botulinum toxin, ricin and phosgene. Representatives of all the active TG-004 countries were present with the exception of Belgium, Poland and Hungary. A representative from Italy was present as was an invited guest from Sweden.

In the area of developing effective treatment against sulfur mustard injuries, two main approaches were discussed. One involved the ability to treat mustard wounds, primarily by debridement using laser technology. Research in this area has demonstrated enhanced healing times in animal models suggesting that laser therapy is more effective than previously used surgical techniques. This represents information that could be rapidly translated into alternative clinical approaches for physicians to use in the treatment of sulfur mustard chemical wounds. The second major area of research discussed dealt with advances in identifying drugs that could be used to treat a sulfur mustard injury. It is expected that the current research efforts will result in the down-selection of a drug(s) for transition to advanced development within the next 12 – 15 months.

Studies on antidotes for nerve agent poisoning demonstrated the continued emphasis on the development of a replacement anticonvulsant for diazepam. The interactions between anticonvulsant drugs and atropine in affording improved neuroprotection as well as anticonvulsant protection suggested there might be some beneficial synergistic interactions when these drugs are used in combination. Work on bioscavengers identified human butyrylcholinesterase as the enzyme most likely to be chosen to transition to advance development. Research on oximes received particular attention both with respect to new oxime development and with respect to the process of gaining regulatory approval of HI-6 in the near future. Pyridostigmine bromide pre-treatment was identified as a necessary adjunct to any alteration in oxime therapy.

Pharmacological approaches to the treatment of botulinum poisoning and the development of a ricin vaccine suggested that it might be possible to offer several new ways of post exposure treatment to toxin poisoning.

The information presented demonstrated an increased emphasis by the panel members to identify and develop new antidotes for chemical warfare poisoning. The improved likelihood of transition of several drugs to advanced development in the foreseeable future increases the hope that more effective treatments will become available for the NATO soldier.

3.1 COOPERATION

A focused discussion on the joint effort of several panel members' nations to field HI-6 as a replacement oxime led to an expanded discussion of how to work jointly to field an antidote. The topics discussed included the dose of the new material in an auto-injector, how a new oxime might affect the dose of atropine to be used, and the dose of oxime that should be available at a medical facility. The resistance of sulfur mustard injury to treatment continues to be a major concern. The work in this area on wound healing by several panel members and the identification of several classes of drugs as candidates for possible advanced development represents a more encouraging position with respect medical treatment for the sulfur mustard threat. The work on diagnosis, which incorporates the ability to identify histologically the location and extent of injury, has allowed for a better

understanding of the mechanism of sulfur mustard injury. That work, coupled with biochemical studies, comprises the joint efforts to identify specific classes of drugs with potential for sulfur mustard antidotal efficacy. Work on the synergistic effect of anticonvulsants and anticholinergics to provide enhanced neuroprotection suggested a new avenue of research for improved therapy against nerve agent poisoning.

Chapter 4 – SUMMARY OF THE MEETING HELD IN MEDICINE HAT (CAN) 29 SEPTEMBER – 3 OCTOBER 2003

A meeting of the TG-004 was held at the Medicine Hat Lodge, Medicine Hat, CA, 29 September – 3 October 2003. There was a total of 48 participants who made 50 oral or poster presentation encompassing the chemical warfare agents covered under the TOR of TG-004. These agents include the nerve agents, sulfur mustard, lewisite, ricin and phosgene. Representatives of all the active TG-004 countries were present with the exception of Belgium, Italy, Poland and Hungary. Two representatives were present as invited guests from Sweden.

In the area of developing effective treatment against sulfur mustard injuries the presentations focused on expanding the knowledge base of the mechanism of action of sulfur mustard and reporting on the effectiveness of anti-inflammatory drugs as potential therapeutic compounds. In the former area, presentations on the role of apoptosis indicated that while cells exposed to sulfur mustard begin to undergo apoptosis, they convert to a necrotic death cycle as cellular energy stores are depleted. These results suggest that early intervention with drugs that can prevent or reverse the apoptotic cycle may result in reduced sulfur mustard injury. Other presentations focused on the role of anti-inflammatory drugs in reducing the severity of sulfur mustard skin injury. Several drugs in this category were identified as being candidates for advanced development as therapeutics for sulfur mustard skin injury. The promise of potential therapeutic for sulfur mustard would be a major advance in the medical treatment of this injury. The role of decontamination and the use of an FDA approved barrier cream for prevention of exposure was also discussed.

Oxime research efforts remained primarily directed toward the development of HI-6 as a next generation oxime, although the utility of other, newer oximes was also discussed. Studies on the mechanism of induction of seizures following exposure to nerve agents demonstrated the continued emphasis on the development of a replacement anticonvulsant for diazepam. It also underscored the joint concern with being able to reverse the effects of seizure activity as well as simply stopping seizures once they had begun. This concern represents a more sophisticated approach to seizure control and will probably require considerable joint effort over the next 3 –5 years. Results with the human butyrylcholinesterase demonstrated that this enzyme would provide protection against multiple chemical warfare agents at exposure levels as high as 5 x LD50. Pharmacokinetic data in guinea pigs and cynomolgus monkeys demonstrated an in vivo half-life of the material in excess of 3-4 days, in contrast to the rapid clearance of the standard therapeutic drugs currently fielded.

While the discussion on ricin was of a forensic nature, very interesting and practical data on the interactions resulting from the administration of vaccines and pyridostigmine were presented. Carefully designed studies showed that no negative interactions resulted with respect to physical or cognitive variables following co-administration of vaccines or pyridostigmine. The results were of particular clinical value to medical personnel who must be concerned about the overall health of the troops in the field.

As was noted at the last TG-004 meeting, the information presented demonstrated a continued emphasis by the panel members to identify and develop new antidotes for chemical warfare poisoning. The identification of a Canadian developed product, RDSL, that has been granted FDA approval as a medical device for skin decontamination, a FDA approved skin barrier cream, SERPACWA, developed in the US, both of which can provide protection against sulfur mustard, as well as the recent FDA approval in the US for the use of pyridostigmine as a pre-treatment for soman were all positive outcomes. Those results suggest that it will be possible to transition several additional drugs to advanced development in the foreseeable future thereby increasing the number of effective treatments that will be available for the NATO soldier.

4.1 COOPERATION

The session illustrated how the cooperative research efforts of the NATO counties in the TG-004 have lead to potential therapies for nerve agent poisoning. The session further illustrated that these cooperative efforts have also resulted in recently approved barrier creams and decontamination devices to prevent or reduce the severity of sulfur mustard injury. Much of the data that supported the new drug approval applications for all three products was generated as a result of close cooperation between the NATO member laboratories. That led to more rapid product approval at lower cost.

The joint effort of several member nations to field HI-6 as a replacement oxime continued to be an important topic. There was considerable informal discussion of how to address the requirements of a regulatory body to gain approval of such a product. Concomitantly efforts to develop an improved anticonvulsant were covered in several presentations and a variety of posters. All the national attendees agreed on the need for the rapid development of a new anticonvulsant. The ability to stop or reverse the effects of seizures after nerve agent poisoning was viewed as a critical need. The utility of bioscavengers as a prophylactic for preventing nerve agent poisoning was demonstrated, thereby providing proof of concept or that approach. Workers from the UK approached the problem from the perspective of using currently available drugs, physostigmine and hyoscine as a potential pre-treatment combination to provide protection against nerve agent poisoning. The resistance of sulfur mustard injury to treatment continues to be a major concern. Despite the approval of products that decontaminated exposed sites or barrier creams that can protect against topical exposure, once a service member sustains a vesicant injury the lack of therapeutic drugs presents a continuing problem. The continued effort on understanding the mechanism of vesicant skin injury emphasized the concern about this unresolved problem. The continued work on the synergistic effect of anticonvulsants and anticholinergics to provide enhanced neuroprotection as well as the demonstrated ability of a bioscavenger to provide protection without the need of post exposure therapy against soman and VX suggested new avenues of research that could lead to improved protection against nerve agent poisoning.

Chapter 5 – SUMMARY OF THE MEETING HELD IN HRADEC KRÁLOVÉ (CZE) 23-26 MAY 2005

A meeting of the TG-004 was held at the Faculty of Military Health Sciences, Hradec Králové, CZ, on 23-26 May 2005. There were a total of 54 participants from 9 countries who made 69 oral or poster presentation encompassing the chemical warfare agents covered under the TOR of TG-004. These agents include the nerve agents, sulfur mustard, lewisite, ricin and phosgene. Representatives of all the active TG-004 countries were present with the exception of Belgium, Italy, Poland and Hungary. One representative was present as an invited guest from Sweden.

The meeting began with two lectures each addressing a topic of wide interest with respect to many aspects of medical chemical defence. The first of these was given by COL Gennady Platoff of the US who described recently developed software modules that allowed for the virtually reality treatment of nerve agent intoxication. The software package is designed to allow a health care provider to choose, or design, a nerve agent attack scenario and then establish a medical response to the threat at the level of designing patient care strategies. This software package allows the various health care providers to treat a virtual patient in 'real time' and get immediate feed-back on their therapeutic actions. It holds great promise as a training tool for military medical service members as well as civilian medical responders and represents a new approach to addressing the emerging asymmetric threats that the NATO countries face, i.e., finding NATO military forces under attack by terrorist groups. The second presentation by Dr. Doctor (US) dealt with a new approach to the determination of choinesterase activity in blood or a variety of tissue homogenates. The technique allows for the use of very small (> 10 μL) sample sizes and allows for the simultaneous determination of both acetylcholinesterase and butyrylcholinestrase. Given that assays of this type are so widely used for monitoring of potential exposures or for evaluating drugs that reverse nerve agent poisoning, the ability to monitor cholinesterase activities in small samples will be reflected in enhanced precision in measurements and a reduced need for large samples from small animals.

The area of developing effective treatments against sulfur mustard injuries comprised the largest session of the meeting devoted to a single topic. This amount of effort and interest reflects the level of concern of the TG-004 countries with respect to the seriousness of the vesicant threat. The presentations addressed the progress that has been made over the past five years in the identification of drugs available for advanced development both for skin injury and for ocular injury as well. The promise of potential for therapeutic drugs for sulfur mustard, approved by regulatory agencies such as the US Food and Drug Administration (USFDA) would be a major advance in the medical treatment of this injury. The seeming disinterest in the advanced development community in the NATO countries to pursue these compounds was the focus of a round table discussion and will be included in a status report on vesicant medical countermeasures to be prepared by a committee comprised of representatives from theTG-004 member countries.

The interest in providing medical care for sulfur mustard injury has expanded over the past three to four years to include new methods in enhancing wound healing following sulfur mustard injury. These techniques were shown to enhance the rate of wound healing and to minimize the tissue scarring that inevitability accompanies sulfur mustard injuries. Reports of the effects of sulfur mustard at the cellular level provided basic science information critical to the continued development of new therapeutic compounds. Work on the genomic analysis of sulfur mustard toxicity exemplified how Toxicogenomics could be used for both diagnostic tests as well as a tool to evaluate emerging therapeutic drugs early in the development cycle. Lastly, the value of this discipline in predicting consistency of mechanism of action of a therapeutic drug across species holds great promise for strengthening licensure applications to drug approval agencies.

Progress in the area of protection against nerve agent poisoning was divided into five separate sessions dealing with bioscavengers, prophylaxis, oximes, treatment and central nervous system (CNS) effects. The bioscavenger topic area focused on the recent progress in the area of human butyrylcholinesterase as a prophylactic for nerve agent poisoning. This material has been used to demonstrate efficacy against several nerve agents in guinea pigs and cynomolgus monkeys. In addition stability studies and pharmacokinetic studies have been carried out to support its selection for advanced development by the US. Progress in this area is the result of joint research efforts in France, the Netherlands, the Czech Republic and the USA. Related approaches for prophylaxis against nerve agent poisoning to include other drugs or proteins that could afford protection against a broad spectrum of nerve agents were also discussed. Interest in the development of an oxime to replace 2-PAM (or P2S) remains an area of considerable activity in many of the member nations of the TG-004. It is widely recognized that a new oxime is needed given the asymmetric threats facing NATO forces. Presentations by the Germans, the US and the Czech Republic focused on several oximes that are approaching advanced stages of development and may be sent for review by various regulatory agencies for licensure for human use. Other presentations on the optimal dose of atropine and progress in neuroprotective drugs further emphasized the common efforts by the TG-004 countries to provide advanced protection for NATO forces.

5.1 COOPERATION

The fact that this meeting was held in the Czech Republic for the first time is a most positive illustration of the expanded efforts of the TG-004. The inclusion of the Czech scientific program in medical chemical defence arena of NATO has added considerable depth to the ongoing efforts in the areas of new oxime development and bioscavengers. With respect to oxime development the Czech military has deployed MMB-4, a lead oxime in the US program and the Czech experience with that material will prove to be very valuable to the US efforts to go forward with advanced development of MMB-4 to the US FDA. Those efforts coupled with the continuing work in Germany on new oxime development has led to an expanded multinational effort to identify and ultimately license a replacement for 2-PAM.

The work in bioscavengers is following a similar pattern of expanded involvement by more of the member nations of theTG-004. The US and the Netherlands have a highly integrated effort in this area. The continued efforts by France on the 3-dimensional structure of butyrylcholinestrase are providing fundamental information critical to understanding the in vivo pharmacological results that have been obtained to date. The new efforts by the Czech scientists in studying the efficacy of bioscavengers with other nerve agent threats will expand the body of data available for demonstrating the broad spectrum ability of bioscavengers to provide protection against nerve agent poisoning. Given the fact that NATO forces will be facing both conventional and asymmetric threats in the future, the progress being made in oxime development and bioscavenger development holds great promise for providing improved protection for NATO forces in a changing threat environment.

The mutual, and almost unanimous, concern about the threat posed by sulfur mustard by the TG-004 member nations cannot be stressed too strongly. The joint opinion that the threat posed by sulfur mustard still requires a medical treatment will be addressed by a ‘white paper’ to be jointly authored by at least three of the member nations’ scientists for submission to the HFM panel. The recent progress made toward identifying both therapeutic drugs for advanced development and the progress in developing techniques to enhance wound healing led to the conclusion that additional efforts in the area of sulfur mustard treatment are needed. The potential value and likelihood of a practical therapeutic drug resulting needs to be communicated to the NATO decision making bodies as soon and as strongly as possible.

Chapter 6 – SUMMARY OF ACCOMPLISHMENTS

Over the course of the period 1999 to 2005, the TG-004 was highly productive in initiating joint research efforts on a variety of tasks that are now leading to improved medical products for protecting the NATO forces against chemical warfare weapons. In addition to new product development, the joint efforts of the TG-004 member nations have identified the need for continued cooperation in drug development, particularly with respect to joint licensure in both the United States and Europe. Over the last six years, the recognition of the difficulty in getting approval from the NATO partner governmental regulatory agencies for a new antidote has clearly defined the need for continuation of the TG-004 mission in the years to come. The fact that the drug approval process takes on average ten to twelve years and must go through a more complicated regulatory approval path than that associated with hardware acquisition speaks decisively to the value of a medical group under the HFM that has a long range mission and a long range mandate to accomplish it.

A short summary of the accomplishments of the TG-004 demonstrates the breadth of its scope and its ability to carry out Translational Research that will benefit NATO forces both on the battlefield and under asymmetric threat situations. The group prepared and submitted an updated paper to the HFM on the current status of Bioscavengers as a new prophylactic approach to nerve agent poisoning. This is currently available on the NATO web site in draft format and the final version is attached in appendix **X**. The concept has now moved to advanced development in the US as of 1 October 2004. Currently the TG-004 pioneering efforts have contributed to the decision to production of human butyrylcholinestrase under good manufacturing practice (GMP) conditions for Phase 1 human clinical trials in the United States. The TG-004 member nations remain engaged in joint research efforts to identify a 'next generation' catalytic bioscavenger. Efforts in that area have led to a formal collaboration between France and Germany on paraoxonase-{Daniel Rochu (CRSSA) / Franz Worek (IPT Munich)}. In addition, informal collaborative efforts between those groups and the US continue unabated.

The UK and Norway have been engaged in a joint effort (including visits), where Norway in late 2004/early 2005 initiated a study in the UK on management of late seizures following soman intoxication. The work started in the summer of 2004 and has reached the discussion phase regarding the evaluation of the results so far and how to address possible follow-up studies. This would be a potential effort for any follow-on technical group under the HFM. In a scientific exchange project a French scientist from CRESSA was in residence for one year at DRDC Suffield for collaborative work on EEG and the effect of high dose atropine therapy on clinical response in organophosphate poisoned pigs. Joint projects arising from that visit, which were planned for expansion to include participation by German scientists, are set to focus on the study of the effect of oxime dose in poisoned pigs on the demand for atropine. This will include studies on the neuromuscular junction in pigs to determine whether clinical signs may predict clinical outcome and the need for additional oximes. Initial plans are also underway for the visit of Canadian scientists to visit French labs (CRESSA). Additional examples of joint efforts between TG-004 member nations included:

1) Lt. Daniel Jun (Hradec Kralove) spent two weeks in CRSSA in 2004, working on transcutaneous penetration of OPs, using Franz cells as ex vivo model;
2) Visits by German scientists to France to discuss areas of mutual interest and coordinate projects;
3) Joint efforts by US and German scientists to develop broad-spectrum oximes and enzymes for enhanced protection against nerve agent poisoning;
4) Visit by German scientists to Bulgaria to discuss areas of mutual interest and plan future cooperation;

5) Contribution by Dutch scientists (TNO) to the USA to provide input to the Neuroprotection Collaboration Review, organized by US Army Medical Research and Materiel Command, September 2004; and

6) Contribution by Dutch scientists (TNO) to the 'Force Health Protection' conference in Albuquerque (NM), August 2004.

Finally, there have been other less formal interactions between the scientists in the TRG-004 member countries to discuss how to move forward in a cooperative manner toward the development and licensing of a new oxime and a new anticonvulsant. Many of these critical efforts are now in jeopardy of not going forward without the formal umbrella of the TG-004, or its successor group to provide the member nations an easy venue for joint research projects. The people most affected will, of course, be the NATO member nation fighting forces.

In short, the TG-004 continued to be a very active technical group that showed remarkable vitality, creativity and cooperation and no signs of becoming obsolete. We certainly appreciate all the support the HFM has given the TG-004 over the years and look forward to providing continued value to the HFM in the area of medical defence against chemical warfare threats.

Chapter 7 – TERMS OF REFERENCE

I. GENERAL AIM AND PURPOSE OF TG-004

TG-004 should serve as a forum for the presentation of basic scientific research concerning the toxic mechanism of action and the effectiveness of experimental and actual clinical therapies against known or potential chemical warfare (CW) agents including toxins. Member countries will explore new innovative concepts and develop new strategies for medical chemical defence. Therapies or prophylactic measures may result in the development of drugs, often antagonists, to intervene in the pathological processes involved. The crossover of research results and ensuing theories on agent actions among the nerve agents, vesicants, midspectrum toxins and neuromodulators will optimize the utilization of scientific resources.

II. ORIGIN

In recent years, the efforts of biomedical science to provide therapy and prophylaxis against injuries caused by "classical" chemical weapons have shifted in emphasis. While the classical military threat of the use of CW agents on the battlefield remains, there has arisen an asymmetric threat for troops, often in peacekeeping roles, by terrorist actions with CW agents. This kind of threat will be characterized by a higher degree of surprise attacks and thereby lower protection level and higher number of casualties.

The fielding of a nerve agent pre-treatment (pyridostigmine) coupled with an effective treatment regimen (atropine and oxime) has reduced the probability of mortality in casualties exposed to nerve agent poisoning. Considerable work remains, however, to allow the rapid recovery of the poisoned soldier's combat efficiency. Such a restoration is expected to result from the development of improved pre-treatment regimens, human recombinant bioscavengers, elimination enhancers, new oximes and better anticonvulsants for the prevention of nerve agent induced brain injury.

Vesicating agents (such as sulfur mustard (HD)) have emerged for many nations as the focus of more immediate concern. The medical care burden of mass vesicant casualties would be overwhelming. The symptoms and rate of blister development, as well as lung and eye damage caused by HD are well known. Information concerning the mechanism(s) of blister formation and the damage caused by HD at the ultra structural, cellular and organ level is still incomplete. Nonetheless, research within this group has developed pharmacological strategies that are being tested in different laboratories. These strategies have identified prototypical compounds that show significant protection against HD toxicity in model systems. This work is of utmost importance for development of future vesicant treatment(s), clinical diagnostics, and strategies for prevention and treatment or amelioration of late effects of vesicants on lungs and eyes, which could become a major burden for the health care system.

The Iran-Iraq war demonstrated that nerve agents and vesicants can be produced in quantity by any country with even minimal chemical and industrial capabilities. Technically advanced nations, can, however be tempted to use agents produced through more sophisticated chemical or bioengineering processes which may not be agents of the classical chemical inventories. These new or emerging agents are referred to by some NATO countries as "midspectrum" agents. These agents include toxins and neuromodulators acting on ionic channels, neurotransmitter systems, or which have other mechanisms of action. Some can be produced in large quantities by biotechnological methods. There is an increasing medical defence concern for these groups of compounds since there are, presently, only minimal medical countermeasures available.

The accelerated development of improved medical therapies against all forms of CW exposures will benefit from further research regarding the mechanism of action of the current or any newly identified potential

threats. Basic knowledge regarding the ensuing toxicology will expedite the application of recent pharmacological advances in the design of new antidotes. With respect to obtaining rapid approval by the respective national regulatory agencies, and with the difficulty of obtaining appropriate human efficacy estimates, TG-004 should agree on joint efforts to clearly identify the mechanism of action of new antidotes in (at least) two appropriate species. In addition, joint efforts should be undertaken to identify surrogate markers that are verifiable and defensible to these regulatory agencies and that can predict human efficacy. It is important that these efforts be performed in conjunction with human safety and pharmacokinetic studies. Data exchange for the preparation of cooperative drug licensing applications should be a priority.

NATO countries have been concerned about mitigating the lethal effects of acute, high level exposure to chemical warfare agents; however, concerns about the health effects of possible agent exposures below the levels causing acute signs of toxicity have been raised. Scientific investigations are needed to address these concerns about low-level exposures by providing data pertinent to:

1) The systemic effects of low dose exposures of varying durations to CW agents (CWA);
2) Persistent changes/effects/pathology due to low level exposure to CWA; and
3) The efficacy of existing CWA therapeutics/prophylactics against low level exposure.

Durations of exposure should be considered that are relevant to both medicine and the battlefield and have been classically defined in Casarett & Doull's Toxicology: The Basic Science of Poisons as 14 days for subacute, 28 days for subchronic and >90 days for chronic.

All experiences gained through the treatment of chemical casualties are obviously of utmost significance. There is now clinical experience with the treatment of mustard casualties in NATO countries. Some experience related to nerve agent casualties is also found in hospitals treating organophosphorus insecticide poisoning.

Although ratified in many countries, the Chemical Warfare Convention will support but not supplant the deterrence provided by continued scientific research to ensure that effective medical chemical defensive countermeasures are available to NATO countries. The panel should continue its efforts to uncover and confirm human exposure to CW agents.

III. OBJECTIVES

From the standpoint of potential medical scientific advances that would satisfy militarily significant knowledge gaps, TG-004 will focus its work and cooperative scientific dialogue on the following topic areas:

a) Vesicants (e.g., mustard and lewisite);
b) Nerve agents (e.g., G- and V- agents);
c) Selected toxins and neuromodulators (e.g., botulinum toxin and tetrodotoxin);
d) Edemagenic agents (e.g., phosgene); and
e) New or emerging threats.

The specific goal of TG-004 is to facilitate the communication and coordination of medical chemical defence research among the participating NATO countries. The TG-004 will encourage research incorporating emerging advances in biomedical technology, which may facilitate and speed the delivery of novel medical countermeasures.

This goal will be realized through periodic workshops and symposia conducted by TG-004 and the exchange among all participants of appropriate technical research results. Specific technical reports will be prepared by TG-004 on topics coordinated between the Chairperson, TG-004 and AC/225 (Panel VIII).

In addition to the objectives in 11 and 12 above, the TG should actively encourage the exchange of personnel through the auspices of NATO travel awards. Such exchanges will allow excellent opportunities for complementary training and education of NATO scientists in areas of mutual interest in national laboratories having significant expertise in the specific areas (e.g., behaviour, detection/analysis, neuropathology). One exchange each year for periods up to 6 months should be decided during the Executive session and sent to HFM for approval.

The TG-004 is authorized to conduct its actions under these Terms of Reference from October 2003 until October 2006.

IV. RESOURCES

Medical chemical defence research is conducted in participating NATO countries. Representation to TG-004 by participating countries should be made by physicians, biochemists, chemists, biologists, neuroscientists, toxicologists, pharmacists and other scientific researchers having experience in the toxicology or treatment of chemical agent poisoning**.** Special needs of TG-004 should be limited to the use of appropriate secure facilities for the conduct of executive and scientific meetings.

V. SECURITY LEVEL

TG-004 information exchanges may be conducted up to and including the NATO SECRET level.

VI. LIAISON

Liaison by members of TG-004 may be conducted with the N13C Medical Working Party and, regarding work on diagnosis and dosimetry, with relevant study groups under AC/225 (Panel VII) (e.g., the Sampling and Identification of Biological, Chemical and Radiological Agents (SIBCRA).

Annex A – TASK GROUP ON PROPHYLAXIS AND THERAPY AGAINST CHEMICAL AGENTS

A.1 MEETING OF TG-004 – 22-26 MARCH 1999, BRUSSELS, BELGIUM

A.1.1 Program

Monday

10:40 – 11:00 **Administration and Welcome**
Welcome by the Major General M. De Coninck, MD
Chief of Staff, Medical Service of the BE Armed Forces

Welcome and Outline of the Meeting
Dr. M. Zizi and Pha Henry

11:00 **RTA-RTO: New Structures…Streamlining of our Scientific Co-operation**
Med. Col. R. Van Hoof, Director of the Queen Astrid Military Hospital, BE representative to RTA

OP Poisoning Chair: Dr. Lenz

11:20 – 12:20 **Diagnosis and Dosimetry of Exposure of Rhesus Monkeys to Nerve Agents Based on Phosphorylated Cholinesterases**
M. Polhuijs, et al.

Intravenous Toxicokinetics and Metabolism of (±)-VX in the Atropinized Hairless Guinea Pig
M. Van der Schans, et al.

The Effects of an Acutely Administered Low Dose of Sarin on Cognitive Behaviour and the Electroencephalogram in the Common Marmoset
P.C. Pearce, et al.

12:20 *Recess*

Drug Certification within the EU Chair: Dr. Van Xanten

13:40 – 15:10 **Presentation of the Approval Procedures of the European Medical Agency**
N. Wathion, PhD, EMA, Head of Section Regulatory Affairs & Pharmacovigilance

Drug and Vaccine Approval, Situation within the BE MOD
Med. Maj. D. Bruggeman, MST-AMG, BE

Approval of Antidotes in GE
C. Stephen, MD, Head of GastroEnterology & Metabolism, Federal Institute for Drugs

15:10 *Refreshments*

OP Poisoning Oximes (Part 1) Chair: Dr. Szinicz

15:20 – 16:40 **The Effects of Oximes on the Cholinergic Nerve Terminal**
P. Aas

Physostigmine as a Pretreatment Against Organophosphate Intoxication
I.H.C.H.M. Philippens

Dissolution Properties and Stability of HI 6 Dichloride and HI 6 Dimethanosulfonate
H. Thiermann, P. Eyer and L. Szinicz

Therapeutic Effects of the Oxime HI 6 and the Anticonvulsant Diazepam on Soman-Poisoned Guinea Pigs
R. Busker, et al.

16:40 **Discussion**

Tuesday

OP Poisoning and Anticonvulsants Chair: Dr. Lallement

09:00 – 10:20 **Diazepam Dosage Necessary to Terminate Soman-Induced Seizures in a Rhesus Monkey Model**
J.H. McDonough, et al.

Efficacy of Biperiden and Atropine as Anticonvulsant Treatment for Organophosphorus Nerve Agent Intoxication
T.-M. Shih and J.H. McDonough

Neuroprotective Effects of HU-211 on Brain Damage Resulting from Soman-Induced Seizures
M.G. Filbert, J. Forster and G. Ballough

Value of Gacyclidine (GK-11) as Adjuvant Medication to Conventional Treatments of OP Poisoning: Primate Experiments Mimicking Various Scenarii of Military or Terrorist Attacks by Soman
G. Lallement

10:20 *Break*

10:40 – 12:00 **Adverse Interaction among Organophosphates and Drugs Used in Anesthetic Induction and Maintenance**
T. Hamilton, P. Lundy, T.W. Sawyer and J.D. Conley

Time-Related Changes of Intracellular Calcium Activity, Reactive Oxygen Species Production and Mitochondrial Depolarization after Sustained Glutamate Receptor Activation in Primary Cerebrocortical Cultures
T. Gillessen, C. Grasshoff, H. Thiermann and L. Szinicz

Modulation and Endogenous GABA Release from Superfused Rat Striatal Slices under Physiological and Pathophysiological Conditions
C. Grasshoff, H. Thiermann, L. Szinicz and T. Gillessen

Efficacy of Atropine and CEB-1957 in Preventing Soman-Induced Seizures, EEG, Behavioral and Neuropathological Sequelae
Y. Manunta, et al.

12:00 *Recess*

Scavengers Chair: Dr. Benschop

13:40 – 14:40 **Carboxylesterase: Specificity and Spontaneous Reactivation of an Endogenous Scavenger for Organophosphorous Compounds**
D.M. Maxwell, et al.

Site-Directed Mutagenesis of Human Carboxylesterase
S. Kirby and C.A. Broomfield

Paraoxonase as a Protective Scavenger Against Poisoning by Nerve Agents
D. Josse

14:40 **Discussion**

15:00 – 15:20 **Kinetics of Nerve Agent Hydrolysis by a Human OPAH Hydrolase**
C.A. Broomfield, B. Morris, D. Josse and P. Masson

BE and UNSCOM Chair: Dr. Zizi

15:20 – 16:00 **Chemical Inspection**
L. Sempoux

Biological Monitoring and Inspection: What is Possible and What is Not
M. Zizi

16:00 **Discussion**

Wednesday

OP Poisoning and Oximes (Part 2) Chair: Dr. Szinicz

09:00 – 10:00 **Pharmacokinetics of HI 6 and Atropine in Human Volunteers after Administration by a Binary Autoinjector – Outlines of a Study**
S. Persson

Time-Dependent Treatment of Soman Poisoning with HI 6 and Atropine: Cerebral Blood Flow and Seizure Strokes
G. Cassel, et al.

The Effects of Oximes when Combined with Atropine and Avizafone Against Nerve Agent Poisoning in Unpretreated Marmosets
P. Pearce, et al.

10:00 *Break*

Chemical Dismantling in Poelkappelle, BE Chair: Dr. Bellanger*
(* To be confirmed)

10:20 – 11:00 **Biological Monitoring of the Exposure to Carcinogens During the Dismantling of Chemical Weapons**
C. Carton

Medical Intern and Extern Contingency Plan for the Dismantling Installation of Poelkappelle, BE
D. Dons, et al.

Vesicants – Systemic Effects (Part 1) Chair: Dr. Hurst

11:00 – 12:00 **Human Skin Absorption of Sulphur Mustard In Vitro**
R.P. Chilcott, W. Carrick and J. Jenner

Non-Invasive Biophysical Quantification of Skin Injuries Resulting from Exposure to Vesicating Agents
R.P. Chilcott, R.F.R. Brown and P. Rice

Respiratory and Percutaneous Toxokinetics of Sulphur Mustard and its DNA Adducts in the Hairless Guinea Pig
J.P. Langenberg, Presented by H.P. Benschop

12:00 *Recess*

13:40 ***Brussels: Past, Present***
Guided tour of the City interrupted by relaxing strolls (Weather permitting). Friends and spouses are welcome to join us. A bus will pick you up at the Hotel Arenberg (Schedule to be announced). At the end of the afternoon, you will be brought back to the hotels… to leisurely prepare yourself to…

Brussels, the Other City of Food *(Hosted dinner with Partners, Formal)*

19:10 *The Shuttle will depart from the Arenberg*

*Arrival at "**Le Pavillon LOUIS XV**" at 19:30.*
Cocktails
Dinner at 20:00
You could expect being back at the hotels around 22:30 – 23:00.

Thursday

Vesicants – Systemic Effects (Part 2) Chair: Dr. Lundy

09:00 – 10:00 **Acute Pulmonary Effects of Sulphur Mustard Following Nose-Only or Intratracheal Exposure: Therapeutic Efficacy of Exogenous Lung Surfactant and the Bronchodilatator Salbutamol**
H.P.M. Van Helden

Domestic Swine Model for the Assessment of Chemical Warfare Agent-Anesthetic Interactions. Some Effects of Sulphur Mustard
J.D. Conley, K. Grychowski, P. Lundy, T. Hamilton and T.W. Sawyer

The Development of Lewisite Vapour-Induced Lesions in the Domestic White Pig
P. Rice and R.F.R. Brown

10:00 *Break*

10:20 – 11:20 **In Vitro Human Skin Absorption of 2-Chlorovinyl Dichloroarsine (Lewisite)**
Z. Ashley, et al.

Gelatinase Activity in Sulphur Mustard Induced Acute Airway Injury in Guinea Pig
J.-H. Clavet, et al.

Comparative Toxicity of Sulphur Mustard and Nitrogen Mustard on Tracheal Epithelial Cells in Primary Cultures
J.-H. Clavet, et al.

HD – Molecular and Cellular Effects (Part 1) Chair: Dr. Smith

11:20 – 12:20 **In Vitro Screening of Candidate Medical Countermeasures Against Vesicant Agents**
W. Smith, et al.

Effects of Sulphur Mustard on Cytokine Release in Cell Cultures
G. Krebs, et al.

Temporal Keratinocytes Gene Expression Following Sulphur Mustard Exposure
J. Schlager

12:20 *Recess*

HD – Molecular and Cellular Effects (Part 2) Chair: Dr. Rice

13:40 – 14:40 **Incision-Excision DNA Repair Mechanism in Sulphur Mustard-Exposed Normal Human Epidermal Keratinocytes**
K.R. Bhatt, B.J. Benton and R. Ray

On the Mechanism of Protection Against Sulphur Mustard Injury by Inhibition of Poly(ADP-Ribose) Polymerase
R. Ray, et al.

Studies of Cellular Biochemical Changes Induced in Human Cells by Sulphur Mustard
W. Smith

14:40 **Discussion**

15:00 – 15:40 **The Involvement of Matrix Metalloproteases and Serine Proteases in the Onset of Sulphur Mustard-Induced Blister Formation**
M.A.E. Mol, et al.

Multi-Site Interruption of HD-Induced Cytotoxicity
T.W. Sawyer

HD Detection Chair: Dr. Benschop

15:40 – 16:20 **Immunochemical Detection of Sulphur Mustard Adducts with DNA and Proteins. Explanatory Research on Adducts with Proteins**
G. Van der Schans, et al.

An Improved Method for Diagnosis of Exposure to Sulphur Mustard: Mass Spectrometric Analysis of Adducts to Human Serum Albumin
D. Noort, et al.

16:20 **Discussion**

Friday

HD Protection Chair: Dr. Rice

09:00 – 09:40 **In Vitro Evaluation of a Barrier Cream Against Sulphur Mustard**
R.P. Chilcott and J. Jenner

Comparison of the In Vitro Efficacy of Four Skin Decontaminants Towards Sulphur Mustard on Pig Ear Skin to Prevent Sulphur Mustard-DNA Adduct Formation
G. Van der Schans, et al.

Efficacy of RSDL and Fuller's Earth in Preventing HD-Induced Skin Injuries in the Hairless Guinea Pig
I. Delamanche, G. Guillot, H. Cocher, M. Pommies, P. Mery, S. Morio, A. Crolbois, J.-C. Bizot

10:00 **General Discussion**

10:30 *Coffee Break*

10:40 **Administrative Session for the POCs** Chair: Dr. Lundy

Free rolling…if desired lunch will be served, if not BE will call it a wrap!
We wish you then an enjoyable time during this sunny Friday, there is quite a lot to see and to do on our few square miles ☺. Have a safe trip home.

A.2 MEETING OF TG-004 – 1-5 OCTOBER 2000, THE HAGUE, NETHERLANDS

A.2.1 Program

Sunday Evening

20:00 – 22:00 **Informal Get-Together, Registration** (Hotel Mercure, The Hague)

Monday Morning

09:00 **Welcome and Domestic Business**

09:20 **Opening Address**
Brig.Gen. E.G. van Ankum M.D., Director of the Military Medical Service Agency

Nerve Agents Chairperson: Dr. Bhupendra Doctor

09:40 **Inhibition of Acetylcholinesterase Enhances Depolarisation Induced GABA Release in Rat Striatal Slices via M-Cholinoreceptors**
C. Grasshoff, T. Gillessen, H. Thiermann and **L. Szinicz**

10:00 **IL-1b in Rat Brain after Soman Intoxication**
I. Svensson, L. Waara, A. Bucht, L. Johansson and **G. Cassel**

10:20 – 10:50 Coffee Break

10:50 **Presynaptic Inhibition of Central Acetylcholine Release with A1 Ligands: Prevention of Cholinergic Crisis**
T.J.H. Bueters, B. Groen, M. Dankof, A.P. Ijzerman and H.P.M. van Helden

11:10 **Differences in the Orientation of Organophosphate and Organosphosphonate Moieties in the Active-Site Gorge of Cholinesterases Revealed by Reactivation Studies with Oximes**
C. Luo, B.P. Doctor and **A. Saxena**

11:30 **Resolution of the Three-Dimensional Structure of Two OP-Reacting Human Enzymes (Paraoxonase and Butyrylcholinesterase): A Big Step Toward the Rational Design of Efficient OPA Hydrolases**
P. Masson, F. Nachon, D. Josse, F. Renault, N. Viguié, O. Lockridge, J.C. Fontecilla-Camps, E. Chabrière and R. Moralès

11:50 **Hydrolysis of VX by Human Paraoxonase**
C.A. Broomfield, R. Anderson, B.N. LaDu, D. Josse and P. Masson

12:10 **Generation of Genetically Modified Mice with Low Serum Carboxylesterase Activity**
D.M. Cerasoli, D.M. Maxwell and **D.E. Lenz**

12:30 – 14:00 Lunch

Nerve Agents (continued) Chairperson: Dr. Paul Rice

14:00 **Toxicokinetics of (±)-VX and Results of an Exploratory Study on the In Vitro Metabolism of (±)-VX**
M.J. van der Schans, B.J. Lander, G.W.H. Moes, H.J. van der Wiel, J.P. Langenberg and H.P. Benschop

14:20 **Development of an Animal Dosing Model to Study the Effects of Low Dose Chronic Exposure (LDCE) to Chemical Warfare Nerve Agents (CWNA)**
C.R. Atchison, C. Holmes, S. Akers, C.R. Clark, S. Duniho, K. Armstrong and T.-M. Shih

14:40 **The Effects of Acutely Administered, Low Dose Sarin (GB) on Sleep in the Common Marmoset**
P.C. Pearce, N.G. Muggleton, H.C. Crofts and E.A.M. Scott

15:00 **Diagnosis and Verification of Exposure to Organophosphorus Compounds**
M.J. van der Schans, C.E.A.M. Degenhardt, M. Polhuijs, J.P. Langenberg and H.P. Benschop

15:20 – 15:50 Tea Break

15:50 **Immunochemical Detection of Sulfur Mustard-Adducts with DNA and Proteins; Exploratory Research on Adducts with Proteins**
G.P. van der Schans, R.H. Mars-Groenendijk, D. Noort, L.P.A. de Jong, P.L.B. Bruijnzeel and H.P. Benschop

Lung Oedemagens

16:10 **In Vitro Adduct Formation of Phosgene with Albumin and Hemoglobin in Human Blood**
D. Noort, A.G. Hulst, A. Fidder, R.A. van Gurp, L.P.A. de Jong and H.P. Benschop

16:30 **Lung Injury Caused by Lung Oedemagens: Treatment with Surfactant and Anti-Inflammatory Drugs**
D. van de Meent, J.P. Oostdijk, M.J.A. Joosen, W.C. Kuijpers and **H.P.M. van Helden**

Tuesday Morning

Nerve Agents (continued) Chairperson: Dr. Murray Hamilton

09:00 **The Interference of Stress on Physostigmine Pretreatment Against Soman Intoxication in Guinea Pigs**
I.H.C.H.M. Philippens, M.J.A. Joosen, B. Groen and R.A.P. Vanwersch

09:20 **Effects of Miosis and Ciliary Spasm on Aspects of Performance in Human Volunteers**
S. Chamberlain and S.C. Bowditch

09:40 **The Effect of Stress on the Medical Treatment of Soman Poisoning in the Brain**
P. Aas and K. Wangen Rustad

10:00 **The Pretreatment and Therapy of Nerve Agent Poisoning: An Overview of UK Data**
E.A.M. Scott (paper not presented)

10:20 – 10:50 Coffee Break

10:50 **Toxicity and Treatment of Russian V-Agent (VR) Intoxication in Guinea Pigs**
I. Koplovitz, M. Shutz, S. Schulz and R. Railer

11:10 **New Aspects on the Reactivation by Oximes of Organophosphate-Inhibited Human Acetylcholinesterase In Vitro**
F. Worek, P. Eyer, P. Littig, R. Widmann and **L. Szinicz**

Drug Approval Chairperson: Dr. David Lenz

11:30 **A Clinical Study on Effects of Obidozime in Organophosphorus Compound Poisoning**
L. Szinicz, H. Thiermann, T.R. Zilker, M. Haberkorn, N. Felgenhauer and P. Eyer

11:50 **Pyridostigmine Bromide: Developing Strategies for Obtaining Regulatory Approval**
D.E. Lenz, M. Adler and R.E. Clawson

12:05 **Discussion on International Cooperation in Drug Approval**

13:00 – 14:00 Lunch

Tuesday Afternoon

14:00 – 16:00 **Poster Session** Chairperson: Dr. Ladislaus Szinicz

16:00 – 17:00 **Discussion on Poster Presentations**

Wednesday Morning

Seizures Chairperson: Dr. Patrick Masson

09:00 **Electrographic Correlates of Neuroprotection Following Soman-Induced Status Epilepticus**
G.P.H. Ballough and M.G. Filbert

09:20 **The Experimental and Programmatic Process of Development of an Advanced Anticonvulsant for the Treatment of Nerve Agent Induced Seizures**
J.H. McDonough

09:40 **Protection Against Nerve Agent-Induced Seizures is Critical for Neuroprotection and Survival**
T.-M. Shih, S.M. Duniho and J.H. McDonough, Jr.

10:00 **Magnetic Resonance Imaging Predicts Soman-Induced Neuropathology in the Rodent**
Y.A. Bhagat, A. Obenaus, M.G. Hamilton and E.J. Kendall

10:20 – 11:00 Coffee Break

Toxins

11:00 **Discovery and Design of Novel Inhibitors of Botulinus Neurotoxin A: Targeted "Hinge" Peptide Libraries**
J. Hayden, J. Pires, S. Roy, M.G. Hamilton and G.J. Moore

11:20 **Generic Treatment of Gram-Negative Infections by Catalytic Antibody Mediated Cleavage of Lipid A: A Preliminary Study**
R.J.B.H.N. van den Berg, D. Noort, E.S. Milder-Enacache, G.A. van der Marel, J.H. van Boom and H.P. Benschop

Lunch, Excursion and Dinner

Thursday Morning

Skin Protection and Decontamination Chairperson: Dr. Jan Langenberg

09:00 **Polyurethane Sponges and Immobilized Cholinesterases for Decontamination and Detoxification of Nerve Agents on Skin**
R.K. Gordon, A.T. Gunduz, S.R. Feaster, B.P. Doctor, D.M. Maxwell, R.C. Macalalag, D.E. Lenz, E.D. Clarkson and J.P. Skvorak

09:20 **Efficacy of a Novel Barrier Cream (AG-7) Against Liquid Challenges of VX and GD In Vitro**
Z. Ashley, R.P. Chilcott and J. Jenner

09:40 **Toxicological Evaluation of the Contact Risk from (de)Contaminated Painted Surfaces**
J.P. Langenberg, M. Husson, J.A. Cordia and H.P. Benschop

10:00 **Percutaneous Poisoning by VX: Effect of Solvents, Location and RSDL**
M.G. Hamilton, P.M. Lundy and J.D. Conley

10:20 – 10:50 Coffee Break

Vesicants Chairperson: Dr. Pal Aas

10:50 **Regulatory Effects of Vitamin D3 Analogues on Interleukins 6 and 8 Secretion Induced by Sulfur Mustard in Normal Human Keratinocytes**
C.M. Arroyo, R.J. Schafer, D.W. Kahler and **C.A. Broomfield**

11:10 **Effects of Low Dose Sulfur Mustard on Growth and DNA Damage in Human Cells in Culture**
W.J. Smith, B.S. Toliver, O.E. Clark III, J. Moser, E.W. Nealley, J.J. Guzman and C.L. Gross

11:30 **Protection of Human Melanoma Cells Against Sulphur Mustard**
C.N. Smith and C.D. Lindsay

11:50 **Comparative Protection from Cytotoxic Effects Induced by Sulfur Mustard and Nitrogen Mustard on Bronchial Epithelial Cells In Vitro**
S. Rappeneau, A. Baeza-Squiban, F. Marano and **J.-H. Calvet**

12:10 **Prevention of Epidermal-Dermal Separation by Inhibitors of Matrix Metalloproteases**
M.A.E. Mol, S.W. Alblas, A. Hammer and H.P. Benschop

12:30 **The Role of Dermabrasion in Lewisite-Induced Skin Injury**
P. Rice, N.J. Bennett, D.G.K. Lam and R.F.R. Brown

12:50 **Closure**

13:00 – 14:00 Lunch

Thursday Afternoon

14:00 **Management Meeting**

A.3 MEETING OF TG-004 – 4-7 NOVEMBER 2002, OSLO, NORWAY

A.3.1 Program

Sunday Evening, November 3

19:00 – 21:00 **Informal Get-Together, Registration** (Scandic Hotel St. Olav, Oslo)

Monday Morning, November 4

09:00 **Welcome**

09:10 **Opening Address**
Sergeant General, Dr. Leif Rosén, Medical Division, HQ Defence Command Norway

09:25 **Welcome**
Director of Science, Dr. Bjørn A. Johnsen, Norwegian Defence Research Establishment

Anticonvulsants/Neuroprotection Chairperson:

09:40 **A Viable Neuroprotection Strategy following Soman-Induced Status Epilepticus**
G. Ballough[2], J. Jaworski[2], B. Hontz[2], D. Fath[2], M. Walters[2] and **M.G. Filbert**[1]
[1] USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground, MD 21010-5400 (USA)
[2] La Salle University, Department of Biology, Philadelphia, PA, 19141-1199 (USA)

10:00 **Studies on Anticonvulsants against Nerve Agent-Induced Seizures**
J.H. McDonough, J. McMonagle, T. Rowland, M. Gonzales and T.-M. Shih
Pharmacology Division, USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground, MD 21010-5400 (USA)

10:20 – 10:50 Coffee Break

10:50 **The UK Strategies for Assessing Anticonvulsant Drugs**
H. Mumford, J.R. Wetherell and **E.A.H. Scott**
Biomedical Sciences, Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ (GBR)

11:10 **Therapeutic Efficacy in Organophosphate Poisoning by Inhibiting Central Release of Acetylcholine**
T.J.H. Bueters[1], B. Groen[1], P.K. Harrison[2], J.E.H. Tattersall[2], A.P. IJzerman[3], M. Danhof[4] and **H.P.M. van Helden[1]**
[1] Medical Countermeasures, TNO Prins Maurits Laboratory, Rijswijk (NLD)
[2] Dstl Chemical & Biological Sciences, Porton Down (GBR)
[3] Medicinal Chemistry, LACDR, Leiden University, Leiden (NLD)
[4] Pharmacology, LACDR, Leiden University, Leiden (NLD)

11:30 **Soman-Induced Seizures in Rats: Possible Treatments and Prophylaxis**
T. Myhrer, S. Enger and P. Aas
Norwegian Defence Research Establishment, Division for Protection and Materiel,
P.O. Box 25, NO-2027 Kjeller (NOR)

11:50 **Alternative Methods to Study CNS Effects of OP: Cell Lines, Brain Slices and Capillaries**
I. Swensson, B. Karlsson, L. Johansson, A. Göransson-Nyberg and **G. Cassel**
Department of Medical Countermeasures, FOI NBC defence, Swedish Defence Research Agency, Cementvägen 20, SE-90182 Umeå (SWE)

12:10 **The Effect of Procyclidin and Huperzine Inhibition of NMDA Receptors in Cerebellar Granule Cells**
A. Ring, R. Tansø and P. Aas
Norwegian Defence Research Establishment, Division for Protection and Materiel,
P.O. Box 25, NO-2027 Kjeller (NOR)

12:30 – 13:30 Lunch Break

Monday Afternoon, November 4

Pyridostigmine Bromide Chairperson:

13:30 **Pyridostigmine Bromide: Dose Dependent Biochemical and Physiological Responses**
D.E. Lenz[1], M. Adler[1], I. Koplovitz[2], S. Feaster[3] and R.E. Clawson[4]
[1] Pharmacology Division, USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground, MD 21010-5400 (USA)
[2] Drug Assessment Division, US Army Medical Research Institute of Chemical Defence, Aberdeen Proving Ground, MD 21010 (USA)
[3] Walter Reid Army Institute of Research, Department of Biochemistry, 503 Robert Grant Road, Silver Spring, MD 20910 (USA)
[4] U.S. Army Medical Materiel Development Activity, Ft. Detrick, MD 21702-5009 (USA)

13:50 **Cholinergic-Immune Interactions: The Effect of Pyridostigmine Bromide on Immune Activation**
L. Wilkinson[1], V. Worrall[1], D. Cook[1], C. Green[1], G. Telford[2], D. Hooi[1], D. Pritchard[2] and **G.D. Griffiths[1]**
[1] Biomedical Sciences, Dstl, Porton Down, Salisbury, Wiltshire SP4 0JQ (GBR)
[2] Immune Modulation Laboratory, School of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD (GBR)

General Topics on Nerve Agents Chairperson:

14:10 **Present Antidotal Means against Chemical Warfare Agents in the Czech Army**
J. Fusek, J. Bajgar, J. Kassa and J. Vachek
Purkyně Military Medical Academy, Dept. of Toxicology, Hradec Králové (CZE)

14:30 **Animal Models of Nerve Agent Intoxication and Treatment of Human Nerve Agent Casualties: Identification of Key Variables for Immediate Therapy**
J.H. McDonough
Pharmacology Division, USAMRICD, 3100 Ricketts Point Rd, E-3100,
Aberdeen Proving Ground, MD 21010-5400 (USA)

14:50 – 15:20 Coffee Break

15:20 **Metabolic Intervention in Organophosphate Poisoning. What are the Possibilities?**
B. Hassel
Norwegian Defence Research Establishment, Division for Protection and Materiel,
P.O. Box 25, NO-2027 Kjeller (NOR)

15:40 **Decontamination of GD, VX and HD on Guinea Pig Skin by Polyurethane Immobilized Enzymes**
R.K. Gordon[1], A.T. Gunduz[1], B.P. Doctor[1], D.E. Lenz[2], D.M. Maxwell[2], E.D. Clarkson[2], J.P. Skvorak[3] and M.C. Ross[3]
[1] Division of Biochemistry, Walter Reed Army Institute of Research, Division of Biochemistry, Dept. of Biochemical Pharmacology, 503 Robert Grant Road, Silver Spring, MD 20910-7500 (USA)
[2] United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010 (USA)
[3] USA Medical Research Materiel Command, Fort Detrick, MD 21701-5912 (USA)

16:00 **End of Session**

Tuesday Morning, November 5

Scavengers Chairperson:

09:00 **Designing Genetically Modified Mice with Low Serum Carboxylesterase Activity**
D.M. Cerasoli, S.M. Chrest, K.J. Skyorak, S.M. Maxwell and **D.E. Lenz**
USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground,
MD 21010-5400 (USA)

09:20 **X-Ray Structure of Native and Soman-Aged Human Butyrylcholinesterase**
F. Nachon[1,2], O. Lockridge[2], Y. Nicolet[3], J.-C. Fontecilla-Camps[3] and **P. Masson**[1]
[1] CRSSA, Département de toxicologie, Unité d'enzymologie, BP 87,
38702 La Tronche Cedex (FRA)
[2] University of Nebraska Medical Center, Eppley Institute, Omaha, NE 68198-6805 (USA)
[3] Institut de Biologie Structurale, LCCP, (CEA, CNRS, UJF), 38027 Grenoble Cedex (FRA)

09:40 **Effect of Pretreatment with Human Butyrylcholinesterase Scavengers on the Toxicokinetics and Binding of Nerve Agents in Guinea Pigs**
M.J. van der Schans, D. Noort, H.J. van der Wiel, K. Pleijsier, H. Spruit, L.P.A. de Jong, H.P. Benschop and J.P. Langenberg
Division of Chemical and Biological Protection, TNO Prins Maurits Laboratory, P.O. Box 45, 2280 AA Rijswijk (NLD)

10:00 – 10:30 Coffee Break

Oximes Chairperson:

10:30 **Alternative Oximes for Treatment of Nerve Agent Poisoning**
I. Koplovitz, S. Schulz, R. Railer, K. Newkirk and M. Shutz
USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground, MD 21010-5400 (USA)

10:50 **HI 6 Dichloride should be Substituted by HI 6 Dimethanesulfonate**
H. Thiermann[1], F. Worek[1], L. Szinicz[1], W. Hartwich[2], S. Krummer[2] and P. Eyer[2]
[1] German Armed Forces Institute of Pharmacology and Toxicology, Neuherbergstr. 11, 80937 Munich (DEU)
[2] Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University, Nussbaumstr. 26, 80336 Munich (DEU)

11:10 **Acetylcholinesterase Activity and Muscle Function in Mouse Diaphragm Preparation and Organophosphate Poisoned Patients**
H. Thiermann[1], F. Worek[1], L. Szinicz[1], T. Zilker[2] and P. Eyer[3]
[1] German Armed Forces Institute of Pharmacology and Toxicology, Neuherbergstr. 11, 80937 Munich (DEU)
[2] Toxicological Department of II. Medical Clinic, Technical University, Ismaninger Str. 22, 81664 Munich (DEU)
[3] Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University, Nussbaumstr. 26, 80336 Munich (DEU)

11:30 **In Vitro Models for the Assessment of Oxime Efficacy in Nerve Agent Poisoning: Reactivation Kinetics of Various Oximes in Different Species**
F. Worek, H. Thiermann, P. Littig, K. Aumüller, I. Lindemann and L. Szinicz
German Armed Forces Institute of Pharmacology and Toxicology, Neuherbergstrasse 11, D-80937 Munich (DEU)

12:00 – 13:00 Lunch Break

13:00 **Photo Session**

Tuesday Afternoon, November 5

13:10 **Discussion of Fielding New Oximes** Chairperson: Dr. Paul Lundy

14:30 – 15:00 *Coffee Break*

15:00 **Posters and Poster Discussion**

16:30 **End of Session**

Wednesday Morning, November 6

Mustard and Lung Injuries Chairperson:

09:00 **Efficacy of Laser Debridement with Autologous Split-Thickness Skin Grafting in Promoting Improved Healing of Deep Cutaneous Sulfur Mustard Burns**
J.S. Graham[1], K.T. Schomacker[2], R.D. Glatter[2], C.M. Briscoe[1], E.H. Braue, Jr.[3] and K.S. Squibb[4]
[1] Comparative Pathology Branch, Comparative Medicine Division, USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground, MD 21010-5400 (USA)
[2] Wellman Laboratories of Photomedicine, Massachusetts General Hospital, Boston, MA 02114 (USA)
[3] Advanced Assessment Branch, Drug Assessment Division, US Army Medical Research Institute of Chemical Defence, Aberdeen Proving Ground, MD 21010 (USA)
[4] Department of Epidemiology and Preventative Medicine, Division of Environmental Epidemiology and Toxicology, University of Maryland at Baltimore, Baltimore, MD 21201 (USA)

09:20 **The Treatment of Sulphur Mustard Burns with Laser Debridement**
D. Evison, R.F.R Brown and P. Rice (Presented by Chris Dalton)
Biomedical Sciences, Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ (GBR)

09:40 **Development of Immunochemical Assays for Detection of Sulfur Mustard-Adducts with Proteins**
G.P. van der Schans, R.H. Mars-Groenendijk, D. Noort and J.P. Langenberg
TNO Prins Maurits Laboratory, P.O. Box 45, 2280 AA Rijswijk (NLD)

10:00 – 10:30 *Coffee Break*

10:30 **A Pilot Study on the Protective Effect of Compound X against Sulfur Mustard Vapor Skin Injury**
G.P. van der Schans, R.H. Mars-Groenendijk and J.P. Langenberg
TNO Prins Maurits Laboratory, P.O. Box 45, 2280 AA Rijswijk (NLD)

10:50 **Human Epidermal Keratinocytes Exposed In Vitro to the Vesicating Agent Sulfur Mustard Express Markers of Apoptosis and Inflammation**
W.J. Smith, E.W. Nealley, O.E. Clark and F.M. Cowan
Pharmacology Division, USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground, MD 21010-5400 (USA)

11:10 **Clinical Drug Treatment of Edemagenic Gas-Induced Lung Injury**
A.M. Sciuto, T.S. Moran and H.H. Hurt
USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground,
MD 21010-5400 (USA)

11:30 – 12:30 Lunch Break

13:00 – 17:00 Excursion

19:00 – 23:00 Dinner

Thursday Morning, November 7

Analytical Methods Chairperson:

09:00 **WRAIR Protocols for Soldier Status and Readiness to Organophosphate Exposure: Unprocessed Whole Blood Cholinesterase and Pyridostigmine Bromide Quantification**
G. Garcia[1], S.R. Feaster[1], D.R. Moorad[1], B.P. Doctor[1], R.K. Gordon[1], C.R. Clark[2], R.E. Reitstetter[2], J. R. Smith[2] and B.J. Lukey[2]
[1] Walter Reed Army Institute of Research, Division of Biochemistry, Dept. of Biochemical Pharmacology, 503 Robert Grant Road, Silver Spring, MD 20910-7500 (USA)
[2] U.S. Army Medical Research Institute of Chemical Defense, U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD 21010 (USA)

09:20 **Retrospective Detection of Exposure to Organophosphorus Anti-Cholinesterases: Mass Spectrometric Analysis of Phosphylated Human Butyrylcholinesterase**
D. Noort, A. Fidder, A.G. Hulst, **M.J. van der Schans**, H.P. Benschop and J.P. Langenberg
Division Chemical & Biological Protection, TNO Prins Maurits Laboratory, P.O. Box 45,
2280 AA Rijswijk (NLD)

Low Dose Exposure to Nerve Agents Chairperson:

09:40 **Studies of Altered Sensitivities to Neurotoxic Substances following Exposure to Low Doses of Soman**
K.P. Lehre and B. Hassel
Norwegian Defence Research Establishment, Division for Protection and Materiel,
P.O. Box 25, NO-2027 Kjeller (NOR)

10:00 – 10:30 Coffee Break

10:30 **Effects of Miosis and Ciliary Spasm on Operation of the Rapier Air Defence Weapon System**
S. Chamberlain, E.H. Dyson, S.C. Bowditch, R. Cock, S. Stokes and R.W. Hargreaves
(Presented by S. Fairhall)
Biomedical Sciences, Dstl, Porton Down, Salisbury, Wiltshire SP4 0JQ (GBR)

10:50 **Sarin and Vision in Primates**
S.J. Fairhall[1], P.C. Pearce[1], R.J. Jones[2], C.A. Dickson[1], M. Maidment[1] and J. Scawin[1]
[1] Biomedical Sciences, Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ (GBR)
[2] QinetiQ, Farnborough (GBR)

Toxins Chairperson:

11:10 **Evaluation of Active Site Inhibitors of Botulinum Neurotoxin B Light Chain using Capillary Electrophoresis**
M. Adler, H. Shafer, J.D. Nicholson, J.E. Keller, J. Powers and B.E. Hackley, Jr.
USAMRICD, 3100 Ricketts Point Rd, E-3100, Aberdeen Proving Ground,
MD 21010-5400 (USA)

11:30 **An Assessment of the Toxicities of Several Ricin Variants against Human Pulmonary Cell Lines**
G.J. Phillips, S.J. Knight, P. Rice and **G.D. Griffiths**
Biomedical Sciences, Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ (GBR)

11:50 **Closing of the Meeting**

12:00 – 13:00 Lunch Break

Thursday Afternoon, November 7

13:15 – 15:00 **Business Meeting**

A.3.2 Posters

Nerve Agents

1) **Neurogenesis Induced by Mobilization and Engraftment of Neural or Bone-Marrow Stem Cells in Soman Poisoned Mice**
J.M. Collombet[1], F. Mourcin[2], E. Four[1]; D. Bernabé[3], P. Filliat[1], N. Grenier[2], D. Baubichon[1], C. Masqueliez[1], F. Hérodin[2] and G. Lallement[1] (Presented by P. Masson)
CRSSA 24, avenue des Maquis du Grésivaudan, BP 87, 38702 La Tronche Cedex (FRA)
[1] Neuropharmacology unit
[2] Experimental radiohaematology unit
[3] Microscopy and Imaging unit

2) **Scavenger Protection against Organophosphate Agents by Human Serum Butyrylcholinesterase**
A. Saxena[1], C. Luo[1], R. Bansal[1], W. Sun[1], M. Clark[1], **D.E. Lenz**[2], Yacov Ashani[3] and B.P. Doctor[1]
[1] Walter Reed Army Institute of Research, Silver Spring, MD 20910 (USA)
[2] US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010 (USA)
[3] Israel Institute for Biological Research, P.O. Box 19, Ness-Ziona (ISR)

3) **Edrophonium is a Reactivator of Organophosphate-Inhibited Human Butyrylcholinesterase**
A. Saxena[1], D. McKissic[1], O. Lockridge[2], B.P. Doctor[1] and Chunyuan Luo[1] (Presented by D. Lenz)
[1] Division of Biochemistry, Walter Reed Army Institute of Research, Silver Spring, MD 20910 (USA)
[2] Eppley Cancer Institute, University of Nebraska Medical Center, Omaha, NE 68198 (USA)

4) **A Theoretical Expression for the Protection Associated with Stoichiometric and Scavengers in a Cingle Compartment Model of Organophosphorus Poisoning**
R.E. Sweeney[1] and D.M. Maxwell[2] (Presented by D. Lenz)
[1] RESECO, Research Engineering Consultants, P.O. Box 2311, Upper Darby PA 19082 (USA)
[2] U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010 (USA)

5) **Next Generation Medical Countermeasures against Nerve Agent Poisoning**
J.R. Wetherell, M.E. Price, H. Mumford and S.J. Armstrong (Presented by L. Scott)
Biomedical Sciences, Dstl Porton Down, Salisbury, SP4 OJQ (GBR)

6) **Measurement and Analysis of EEG Signals in a Guinea Pig Model of Nerve Agent-Induced Seizure**
H. Mumford and J.R. Wetherell (Presented by L. Scott)
Biomedical Sciences, Dstl Porton Down, Salisbury, Wilts, SP4 0JQ (GBR)

7) **Novel Adenosine A1 Ligands Inhibit Sarin-Induced Epileptiform Activity in the Guinea Pig Hippocampal Slice**
P.K. Harrison[1], T.J.H. Bueters[2], A.P. IJzerman[3], H.P.M. van Helden[2] and J.E.H. Tattersall[1] (Presented by L. Scott)
[1] Biomedical Sciences, Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ (GBR)
[2] Research Group Medical Countermeasures, TNO Prins Maurits Laboratory, Lange Kleiweg 137, P.O. Box 45, 2280 AA Rijswijk (NLD)
[3] Department of Medicinal Chemistry, Leiden/Amsterdam Centre for Drug Research, Leiden University, Einsteinweg 55, P.O. Box 9502, 2300 RA Leiden (NLD)

8) **The Effect of Toxogonin, HI-6, HLö-7, PS2 and 2-PAM on the Release of Acetylcholine from Rat Hippocampus Slices during Soman Intoxication**
P. Aas
Norwegian Defence Research Establishment, Division for Protection and Materiel, P.O. Box 25, NO-2027 Kjeller (NOR)

Mustard and Lung Injuries

9) **Progress in Developing an Active Topical Skin Protectant**
E.H. Braue Jr., S.T. Hobson, E.K. Lehnert, N. Lewis, C.R. Nalls-Braue, B.F. Doxzon, R.T. Simons, P.A. Doxzon, R.T. Simons, P.A. DiLeonardi, T.L. Nohe and J.S. Graham (Presented by D. Lenz)
U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MA 21010-5400 (USA)

10) **Protection Against Acute Lung Injury by Vitamin E**
D. Rocksen, B. Ekstrand-Hammarström, L. Johansson and **A. Bucht**
Department of Medical Countermeasures, FOI NBC Defence, Swedish Defence Research Agency, SE-901 82 Umeå, (SWE)

11) **Respiratory Pathophysiological Changes Induced by Phosgene in the Anaesthetised Pig**
R.F.R. Brown, F.M.J. Harban, B.J.A. Jugg, Z. Ashley, J. Platt, C.E. Kenward and J. Jenner (Presented by C. Dalton)
Biomedical Sciences, Dstl Porton Down, Salisbury, SP4 OJQ (GBR)

A.4 MEETING OF TG-004 – 29 SEPTEMBER – 3 OCTOBER 2003, MEDICINE HAT, ALBERTA, CANADA

Presentations, Poster Sessions, Coffee Breaks and the Thursday evening Dinner take place at the 'Saamis A' Ballroom of the Medicine Hat Lodge.

A.4.1 Program

Monday

08:30 – 09:00 **Registration**

09:00 – 09:30 **Opening Remarks**

Vesicants Chairs: Masson (FRA) and Braue (USA)

09:30 – 10:30 **Mustard-Stimulated Proteases in Human Epidermal Keratinocytes (HEK): Involvement in Vesicant Mechanism and Intervention**
P. Ray, X. Jin, G. Xu and R. Ray

Vesicant Intervention Via DNA Repair and Apoptosis: A Worthwhile Concept
R. Ray, K.R. Bhat, D.S. Rosenthal, B.J. Benton, D.R. Anderson, W. Holmes, J.P. Petrali, T. Hamilton, P. **Ray** and W.J. Smith

Therapeutic Efficacy of Anti-inflammatory and Scavenger Compounds as Post Exposure Treatments in a Mouse Ear Vesicant Model
C. Bossone, E. Clarkson, R. Railer, K. Newkirk, S. Schulz, M. Shutz, N. Washington, C. Kelleher, T. Allen, I. Koplovitz and W. Smith

10:30 – 10:45 Coffee Break

Vesicants Chairs: Masson (FRA) and Braue (USA)

10:45 – 12:00 **Gene Expression after Exposure to Sulfur Mustard**
B.N. Ford, T.W. Sawyer and Y. Shei

Proteomics as a Strategy to Study the Mechanistic Toxicology of Sulfur Mustard
M.A.E. Mol, R.M. van den Berg, C. van Dijk and A.L. de Jong

TNF-α Expression Patterns as Potential Molecular Biomarker for Human Skin Cells Exposed to Vesicant Chemical Warfare Agents: Sulfur Mustard (HD) and Lewisite (L)
C.M. Arroyo, D.L. Burman, D.W. Kahler, M.A. Smith, M.R. Nelson, C.M. Corun, J.J. Guzman, B.E. Hackley, Jr., S.-D. Soni and C.A. Broomfield

12:00 – 13:30 Lunch Break

CNS Session Chairs: Worek (DEU) and Rice (GBR)

13:30 – 15:00 **Alterations in Map-2 Immunostaining in Relation to Fluoro-Jade Histofluorescence Following Acute Soman Intoxication**
R.K. Kan, C.M. Pleva, T.A. Hamilton, G.P.H. Ballough, S.J. Estep, M.M. Brown and J.P. Petrali

Place of Ketamine in the Delayed Treatment of Soman-Induced Status Epilepticus
F. Dorandeu, D. Baubichon, E. Four, P. Carpentier and G. Lallement

Prophylactic Drugs against Soman-Induced Seizures and Cognitive Side Effects in Rats
T. Myhrer, J.M. Andersen, N.H.T. Nguyen, S. Enger and P. Aas

Acute Effects of Neuroactive Steroids against the Convulsive and Lethal Effects of Soman Intoxication in Rat: Comparison With Diazepam
G. Cassel, V. Heldestadt, I. Svensson and A. Goransson-Nyberg

15:00 – 15:15 Coffee Break

General Topics Chairs: **Lundy** (CAN) & Fusek (CZE)

15:15 – 16:30 **Subacute Low Dose Nerve Agent Exposure Causes DNA Fragmentation in Guinea Pig Leukocytes**
J.R. Dave, J.R. Moffett, S.M. Anderson, M.L. Sipos, A.V. Moran, S.M. DeFord and F.C. Tortella

Update on the New French Two-Compartment Autoinjector
Col. P. Clair, Ltc. G. Lallement and Col. D. Zabe

Use of Analgesics and Anaesthetics in Large Scale Law Enforcement – Medical Aspects
Sven-Ake Persson

Tuesday

General Topics Chairs: Lundy (CAN) & Fusek (CZE)

09:00 – 10:30 **Protective Ventilation Strategies in the Management of Chemical Induced Lung Injury in an Anaesthetized Pig Model**
R.F.R. Brown, B.J.A. Jugg, J. Platt, C.E. Kenward, A.J. Smith, J. Jenner and P. Rice

Gender Issues in CB Defense
L.A. Gay, E. Gosden, S. Tattersall and S. Bowditch

Development of Active Barrier Skin Creams That Protect Against Chemical Warfare Agents
E.H. Braue, Jr., J.S. Graham, T.H. Snider, B.F. Doxzon, E.C. Miller, H.L. Lumpkin, R.L. Hall, R.S. Stevenson and S.T. Hobson

Real Life Experiences Using the WRAIR Whole Blood Cholinesterase and Pyridostigmine Assays
R.K. Gordon, J.R. Haigh, S.R. Feaster, B.P. Doctor, G.E. Garcia, D.E. Lenz, M.A. Riel, R.E. Clawson and P.S. Aisen

10:30 – 10:45 Coffee Break

Toxins Session Chairs: Hackley (USA) and Cassel (SWE)

10:45 – 12:00 **Retrospective Identification of Ricin Exposure**
G.D. Griffiths

New Insights into the Mechanisms of Action of Clostridium Perfringens Epsilon Toxin In Vitro
A.C. Green and R.D. Heal

12:00 – 13:30 Lunch Break

Oximes/Nerve Agents Chairs: Doctor (USA) & Mol (NLD)

13:30 – 14:30 **Use of a Fully Instrumented Swine Model to Estimate the Effect of Anesthetic Techniques on Survival Following Nerve Agent Poisoning**
I.H. Hill, K.M. Wetherby, C. Davidson and P.M. Lundy

The Pharmacokinetics and Pharmacodynamics of Two HI-6 Salts in Pigs and Efficacy of HI-6 Dimethanesulfonate Against Nerve Agents in Guinea Pigs
P.M. Lundy, I.H. Hill, P. Lecavalier, C. Vair, C. Davidson, K.M. Wetherby and B.J. Berger

Contribution of Kinetic Data to the Understanding of Interactions between AchE, OP and Oximes as a Basis for the Development of More Effective Antidotes
F. Worek, H. Thiermann and L. Szinicz

14:30 – 14:45 Coffee Break

A.4.2 Posters

1) **Studies on Cyclooxygenase 2 Induction in the Brain of Soman-Intoxicated Rodents and Consequences of Enzyme Inhibition**
F. Dorandeu, A. Pachot, P. Carpentier, V. Baille, E. Four and G. Lallement

2) **Is Free Radical Production During Soman-Induced Status Epilepticus Relevant to Neuropathology?**
F. Dorandeu, R. Viret, F. Fleury, D. Baubichon, E. Four, C. Masqueliez, R. Gonzalez, C. Amourette and G. Lallement

3) **Brain Polyamine Metabolism During Soman-Induced Status Epilepticus. Modulation with Alpha-DFMO**
F. Dorandeu, D. Baubichon, C. Masqueliez, E. Four, F. Raul and G. Lallement

4) **Liquid VX Skin Exposure in the SKH1 Hairless Mouse: Biological Consequences and Effects of Some Simple Decontaminants**
F. Dorandeu, A. Foquin and G. Lallement

5) **Liquid Sulfur Mustard Percutaneous Intoxication in the SKH1 Hairless Mouse: Biological Consequences and Effects of Some Simple Decontaminants**
F. Dorandeu, A. Foquin, C. Gregoire, B. Lefebvre, B. Brasme, G. Lallement and D. Daveloose

6) **Experimental Design of an Anesthetized Swine Model for Neuroprotection Studies: Issues and Preliminary Results**
F. Dorandeu, A. Foquin, C. Delacour, P. D'Alco, G. Becq, J. Denis, A. Alonso, D. Baubichon, G. Lallement and L. Bourdon

7) **Hydrolysis of Organophosphorus Compounds by Iodosobenzoic Acid-β-cyclodextrin Conjugates**
N. Masurier, F. Estour, M.-T. Froment, B. Brasme, S. Menager, P. Verite, O. Lafont and P. Masson

8) **Optimization of the Expression of Human Butyrylcholinesterase**
F. Nachon, S. Wieseler, P. Masson and O. Lockridge

9) **BB 94, An Inhibitor of Matrix Metalloproteases, Blocks Sulfur Mustard-Induced Epidermal-Dermal Separation**
M.A.E. Mol and R.M. van den Berg

10) **Vaccines and Pyridostigmine Interactions in a Marmoset Model: Preliminary Results**
A.P. Bowditch, S. Chamberlain, G.D. Griffiths, R.J. Hornby, T.M. Mann, P.C. Pearce, D.J. Stevens, E.A.M. Scott and K.E. Williams

11) **Immunological Status of Marmosets Following Co-Administration of Multiple Vaccines and Pyridostigmine Bromide: Preliminary Results**
R.J. Hornby, L.J. Wilkinson and G.D. Griffiths

12) **The Effects of Cell Cycle Regulators on Sulphur Mustard Toxicity in Human Skin Cell Lines**
R. Simpson and C.D. Lindsay

13) **Identification and Characterization of Chemopreventive Mechanisms in Human Skin**
T. Devling, L. McLellan, C.D. Lindsay and J. Hayes

14) **Histopathological Staining Techniques – Application to Skin Studies**
R. Knight, J.C. Platt and J.N. Hughes

15) **Designing Genetically Modified Mice with Low Serum Carboxylesterase Activity**
D.M. Cerasoli, S.M. Chrest, K.J. Skvorak, D.M. Maxwell and D.E. Lenz

16) **Real Life Experiences using the WRAIR Whole Blood Cholinesterase and Pyridostigmine Assays**
R.K. Gordon, J.R. Haigh, S.R. Feaster, B.P. Doctor, G.E. Garcia, D.E. Lenz, M.A. Riel, R.E. Clawson and P.S. Aisen

17) **Update on HD Warfare Agent Decontamination, Detoxification, and Detection using Polyurethane Immobilized Enzymes**
R.K. Gordon, B.P. Doctor, E.D. Clarkson, M.C. Ross, D.M. Maxwell, B.J. Lukey and J.P. Skvorak

Wednesday

Oximes/Nerve Agents Chairs: Doctor (USA) & Mol (NLD)

08:30 – 10:00 **Direct Effects of Physostigmine as a Pretreatment in Guinea Pigs. Side Effects and Efficacy against 2x LD_{50} Soman Induced Incapacitation**
I.H.C.H.M. Philippens, B. Groen and R.A.P. Vanwersch

Biochemical Effects of Low Level Exposure to Soman Vapour
J. Fusek, J. Bajgar, L. Sevelova, G. Krejcova, L. de Jong and H. Benschop

Long Term Effects of the Organophosphate Sheep Dip, Diazinon, On Sleep, the Electrocorticogram and Behaviour in the Common Marmoset (Callithrix Jacchus)
N.G. Muggleton, A.J. Smith, E.A.M. Scott and P.C. Pearce

10:00 – 10:15 Coffee Break

10:15 – 11:00 **Considerations and Findings on the Use of Atropine in Organophosphate Poisoned Patients**
H. Thiermann, F. Worek, L. Szinicz, T. Zilker and P. Eyer

Assessment of a Combination of Physostigmine and Hyoscine as Pretreatment Against the Behavioural Effects of Organophosphates in the Common Marmoset (Callithrix Jacchus)
N.G. Muggleton, A.P. Bowditch, H.S. Crofts, E.A.M. Scott and P.C. Pearce

Radiolabelled Soman Binding to Sera from Rats, Guinea Pigs, Monkeys and Humans
D.M. Cerasoli, C.R. Clark, K.M. Brecht, D.M. Maxwell and D.E. Lenz

11:00 – 12:00 Lunch Break

12:00 – 18:00 Excursion – Bus must leave promptly at 12:00

Thursday

Scavengers Chairs: Lallement (FRA) & Gordon (USA)

09:00 – 10:30 **Pharmacokinetics, Stability, Safety and Toxicity of Purified Human Serum Butyrylcholinesterase in Mice**
W. Sun, M.G. Clark, C. Luo, R. Bansal, B.P. Doctor and A. Saxena

Safety Evaluation of Human Serum Butyrylcholinesterase in Rhesus Monkeys
T.M. Myers, W. Sun, R. Bansal, M.G. Clark, A. Saxena and B.P. Doctor

Application of Adenovirus Expression System for the Production of Recombinant Human Butyrylcholinesterase
N. Chilukuri, K. Parikh, R. Naik, J.M. Watt, P. Tipparaju, B.P. Doctor and A. Saxena

10:30 – 10:45 *Coffee Break*

Scavengers Chairs: Lallement (FRA) & Gordon (USA)

10:45 – 12:00 **Protection Against Soman Poisoning by Human Butyrylcholinesterase in Guinea Pigs and Monkeys**
D.E. Lenz, D.M. Maxwell, I. Koplivitz, C.R. Clark, B.R. Capacio, J.M. Fedorko, C. Luo, A. Saxena, B.P. Doctor and C. Olson

In Vitro Characterization of Recombinant Human Butyrylcholinesterase (Protexia™) as a Potential Bioscavenger
D.E. Lenz, D.M. Cerasoli, E.M. Griffiths, B.P. Doctor, A. Saxena, N.H. Greig, Q.S. Yu, Y. Huang, H. Wilgus and C.N. Karatzas

12:00 – 13:30 *Lunch Break*

13:30 – 14:00 **Wrap-up and Meeting Adjournment**

14:00 – 16:00 **Executive Session**

19:00 – 19:30 *Cocktails*

19:30 *Dinner and Social Evening*

A.5 MEETING OF TG-004 – 23-26 MAY 2005, HRADEC KRÁLOVÉ, CZECH REPUBLIC

Lectures, Posters, Coffee Breaks and Lunch take place at the Faculty of Military Health Sciences, Hradec Králové, Trebesska 1575.

A.5.1 Program

Monday 23 May

08:15 *Bus leaves from Amber Hotel Cernigov*

08:30 – 09:00 **Registration**

09:00 – 09:30 **Opening Remarks**

General Chairs: Lenz (USA) & Fusek (CZE)

09:30 – 10:15 **Medical Management of Chemical Casualties: Virtual Reality Treatment of Nerve Agent Intoxication**
G. Platoff

Whole Blood Robotic Cholinesterase Assay for Organophosphate Exposure
B.P. Doctor

10:15 – 10:30 *Coffee Break*

Vesicants / Medical Countermeasures Chairs: Mol (NLD) & Kehe (DEU)

10:30 – 12:10 **Development of a Medical Countermeasure Against Vesicants. Lead Strategies and Current Status**
W.J. Smith

Effective Medical Countermeasures Against Sulfur Mustard Exposure of Skin and Eye are Closer than Ever Before
M.A.E. Mol

Improved Wound Healing of Cutaneous Sulfur Mustard Injuries in a Weanling Pig Model
J.S. Graham

Round Table Discussion of Vesicant Research vs. NATO Needs
K. Kehe, M.A.E. Mol, W. Smith, et al.

12:10 – 13:00 *Lunch Break*

13:00 – 13:30 **Posters (See Section A.3.2.1)**

Vesicants / Toxicokinetics and Molecular Mechanisms Chairs: Smith (USA) & Stetina (CZE)

13:30 – 14:50 **Inhalation Toxicokinetics of Sulfur Mustard in Marmoset**
H.P.M. van Helden

Different Effects of Sulfur Mustard on p53 and p21 Protein in HaCaT Cells and Human Keratinocytes
K. Kehe

The Formation of Inter-Strand DNA Cross-Links in the Pig Skin Treated with Sulphur Mustard in Vivo
R. Stetina

Genomic Analysis of Sulfur Mustard Toxicity
J. Dillman

14:50 – 15:15 **Posters (See Section A.3.2.1)**

15:15 *Bus leaves to Amber Hotel Cernigov*

Tuesday 24 May

08:30 *Bus leaves from Amber Hotel Cernigov*

Nerve Agents / Scavengers Chairs: Masson (FRA) & Bajgar (CZE)

09:00 – 10:00 **Bioscavengers for the Protection of Humans Against OP Toxicity**
B.P. Doctor

Human Butyrylcholinesterase as a Prophylactic Agent for Organophosphorus Poisoning
D.E. Lenz

Effect of Pretreatment with Human Butyrylcholinesterase Scavengers on the Toxicokinetics and Binding of Nerve Agents in Guinea Pigs and Marmosets
M.J. van der Schans

10:00 – 10:30 **Posters (See Section A.3.2.2)**

10:30 – 10:45 *Coffee Break*

Nerve Agents / Prophylaxis Chairs: Doctor (USA) & van der Schans (NLD)

10:45 – 12:00 **Prophylaxis Against Nerve Agent Intoxication: Past, Present and Future**
J. Bajgar

Protection Against Sarin Inhalation Exposure at Low Concentrations
J. Bajgar

Structure/Function Analyses of Rationally Designed Human Serum Paraoxonase (HuPON1) Mutants
D. Cerasoli

An Update on a Study of the Administration of Vaccines and Pyridostigmine Bromide to Marmosets
P. Rice

12:00 – 13:00 *Lunch Break*

13:00 *Bus leaves to Amber Hotel Cernigov*

14:15 – 19:00 *Excursion – Opocno Castle (bus leaves from the Amber Hotel Cernigov)*

Wednesday 25 May

08:30 *Bus leaves from Amber Hotel Cernigov*

Nerve Agents / Oximes Chairs: Szinicz (DEU) & Kassa (CZE)

09:00 – 10:20 **Considerations on Oxime Therapy in Nerve Agent Poisoning**
H. Thiermann

Development of a Dynamic In Vitro Model for the Estimation of Oxime Efficacy in Humans
F. Worek

Species-Related Differences in the Oxime-Induced Reactivation of Acetylcholinesterases Inhibited by Highly Toxic Organophosphates and Organophosphonates
A. Saxena

U.S. Effort to Replace 2PAMCl with an Oxime Having Broader Spectrum Efficacy Against Nerve Agent Intoxication
I. Koplovitz

10:20 – 11:00 **Posters (See Section A.3.2.3)**

11:00 – 11:15 Coffee Break

Nerve Agents / Treatment Chairs: Cassel (SWE) & Thiermann (DEU)

11:15 – 12:30 **The Development of Pharmacological Pretreatment and Antidotal Treatment of Tabun Poisonings**
J. Kassa

Early Treatment of Soman-Intoxicated Rats with Combined Injections of HI-6, Atropine, and Avizafone Plus Adjuncts
P. Aas

How Effective is Treatment of GF Poisoning with a High Atropine Dose? Insights from an Anaesthetized Swine Model
F. Dorandeu

Cutaneous Exposure to GD and VX: Timing of Antidotes
E. Clarkson

12:30 – 13:15 Lunch Break

13:15 – 14:00 **Posters (See Section A.3.2.4)**

14:00 – 14:15 Coffee Break

Varia Chairs: Koplovitz (USA) & Worek (DEU)

14:15 – 16:15 **Ultrapotent Opioids as Non-Lethal Weapons**
L. Hess

Physiological Indices in Freely-Moving Guinea-Pigs: Method Development and Preliminary Results
H. Mumford

Down-Regulation of Genes Associated with Ricin Tolerance in Human RPMI 2650 Cells
L. Wilkinson

Confocal Microscopy of Lung Slice Cultures: Opportunities for Lung Toxicology?
J.E. Tattersall

Translating Laboratory Science into Fieldable Solutions: The UK View
P. Rice

16:15 – 17:15 **Posters (See Section A.3.2.5)**

17:15 *Bus leaves to Amber Hotel Cernigov*

Thursday 26 May

08:30 *Bus leaves from Amber Hotel Cernigov*

Nerve Agents / CNS Chairs: Filbert (USA) & Mikler (CAN)

09:00 – 10:20 **Neuroprotection Against Nerve Agent-Induced, Seizure-Related Brain Damage**
M. Filbert

Neuronal Death Following Soman Intoxication: Necrosis or Apoptosis?
R.K. Kan

Apoptosis is Probably Not a Key Feature of Soman-Induced Brain Damage: A Multiparametric Study in Mice
F. Dorandeu

Effects of Anticonvulsant Drugs on Soman-Induced Seizures in Rats 30 min after Onset
T. Myhrer

10:20 – 10:40 *Coffee Break*

10:40 – 11:40 **A Model for Studying Neuroprotection from Seizure-Induced Brain Injury Caused by Exposure to Organophosphorus (OP) Nerve Agents**
R.A. Bauman

On-Going Studies of Putative Neuroprotectants using Continuous EEG and Physiological, Neurological and Behavioral Assessments after Soman Exposure
D.L. Yourick

The Guinea-Pig Hippocampal Slice as an In Vitro Model for Nerve Agent-Induced Seizure Activity
J.E. Tattersall

11:40 – 12:15 **Posters (See Section A.3.2.6)**

12:15 – 13:00 Lunch Break

13:00 Bus leaves to Amber Hotel Cernigov

13:00 – 15:00 **Executive Session**

15:15 Bus leaves to Amber Hotel Cernigov

19:00 – 22:00 Farewell Party – Hotel GARNI (bus leaves from the Amber Hotel Cernigov)

A.5.2 Posters

Vesicants / Medical Countermeasures

1) **Effect of Skin Decontaminant Desprach on Sulfur Mustard-Induced Toxicity in Rats**
J. Cabal

2) **Cyclodextrines Induced Detoxication of Sulfur Mustard and Soman in Water**
J. Cabal

3) **The Relationship between Resistance to the Cytotoxic Effects of Sulphur Mustard and the Expression of Cell Cycle and DNA Repair Factors**
P. Rice

4) **Cutaneous Full-Thickness Liquid Sulfur Mustard Burn in SKH1 Hairless Mice: Immunological and Hematopoietic Consequences**
F. Dorandeu

5) **pH Dependent Toxicity of Sulphur Mustard In Vitro**
J. Mikler

Nerve Agents / Scavengers

1) **Mutagenesis Strategies for Cholinesterase-Based Catalytic OP Scavengers**
P. Masson

2) **Protective Effect of Equine Butyrylcholinesterase on Sarin Poisoning in Rats**
L. Bartosova

3) **Gene Therapy of Acetylcholinesterase Knock-Out Mice**
A. Hrabovska

Nerve Agents / Oximes

1) **In Vitro Evaluation of Currently Used Acetylcholinesterase Reactivators Against Nerve Agents Intoxications**
K. Kuca

2) **Reactivation of Sarin-Inhibited Acetylcholinesterase in Various Parts of Pig Brain**
K. Kuca

3) **Synthesis of the Novel Series of Bispyridinium Compounds Bearing Xylene Linker and Evaluation of Their Reactivation Activity Against Chlorpyrifos-Inhibited Acetylcholinesterase**
K. Musilek

4) **Antidotal Treatment of Cyclosarin-Poisoned Mice and Rats**
L. Bartosova

5) **Using of Artificial Neural Networks in the Development of New Antidotes Against Nerve Agent Intoxications**
V. Dohnal

6) **Prediction of New Reactivators Against Cyclosarin Poisonings Using Artificial Neural Networks**
V. Dohnal

Nerve Agents / Treatment

1) **The Experimentally GF-Induced Neurotoxicity and its Therapy in Rats**
G. Kunesova

2) **Antidotal Therapy of Tabun-Induced Alteration of Cognitive Function in Rats**
G. Kunesova

3) **Influence of Different Acetylcholinesterase Reactivators on the Process of Human Thrombocyte Aggregation In Vitro**
D. Jun

4) **Differences in Reactivation of VX and Russian VX-Inhibited Acetylcholinesterase by Currently Available Oximes**
D. Jun

5) **Free Radical Scavenging Activity of Antidotes Against CWA Currently Used in the Czech Armed Forces**
V. Koleckar

6) **Further Improvements to Medical Countermeasures Against Nerve Agent Poisoning**
H. Mumford

Varia

1) **Histology and the Brain: A Matter for Thought**
P. Rice

2) **The Inflammatory Response and Innate Immune Activation after Inhalation of Alkylating Chemicals in Rats is Strain-Dependent**
A. Bucht

3) **The Inflammatory Response after Inhalation of Alkylating Chemicals in Mice is Dependent on T Lymphocytes and Natural Killer (NK) Cells**
A. Bucht

4) **Sensitivity of Daphnia Magna Straus to Tabun and its Degradation Products**
S. Vesela

5) **Investigations into the Mechanism of Action of Clostridium Perfingens Type D Epsilon Toxin**
P. Rice

6) **Binding of Organophosphorus Compounds to Sera from Rats, Guinea Pigs, Monkeys and Humans**
D. Cerasoli

7) **Skin Absorption of VX In Vitro**
P. Rice

8) **Electrochemical Measurement of Enzyme Activity**
D. Krejcova

9) **Removal of Barrier Cream from Eyes by Irrigation with Saline**
P. Rice

Nerve Agents / CNS

1) **Effect of Convulsive Dose of Soman on Mouse Brain: A Diffusion-Weighted Magnetic Resonance Imaging (DWI) Study**
G. Testylier

2) **Development of an OP-Induced Seizure Model in Anesthetized Swine**
F. Dorandeu

3) **Evaluation of Anticonvulsants Against Nerve Agent Poisoning in the Guinea Pig**
H. Mumford

Annex B – NERVE AGENT BIOSCAVENGERS: PROGRESS IN DEVELOPMENT OF A NEW MODE OF PROTECTION AGAINST ORGANOPHOSPHORUS EXPOSURE

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The opinions or assertions contained herein are the private views of the authors, and are not to be construed as reflecting the view of the Department of the Army or the Department of Defense.

ABSTRACT

Although treatment for intoxication by organophosphorus (OP) poisons exists, the treatment regimen suffers from undesirable side effects. To overcome these disadvantages the concept of using a bioscavenger has emerged as a new approach to reduce the in vivo toxicity of chemical warfare nerve agents. Bioscavengers fall into two broad categories, stoichiometric, i.e., proteins that bind a poison in some fixed ratio, and catalytic, i.e., proteins that can cause the breakdown of a molecule of a poison, regenerate, and then repeat the process until all of the poison molecules have been destroyed. To be an improvement of over current treatment, a biological scavenger should have no or minimal behavioral or physiological side effects, should provide protection against one or more nerve agents up to 5 LD50 and should reduce or eliminate any behavioral or physiological side effects normally associated with the currently fielded therapy. Studies with equine or human butyrylcholinesterase or fetal bovine serum acetylcholinesterase showed that none these scavengers exhibited behavioral side effects when given alone to rats or monkeys. These three scavengers and carboxylesterase were each capable of providing protection against 2 to 16 LD50s of GD, GB or VX depending on the scavenger and the test species (rat, mouse, rabbit, guinea pig or rhesus monkey). When behavioral testing was performed on animals pre-treated with a bioscavenger, i.e., mouse, rat or rhesus monkey, following administration of up to 5 LD50 of GD or VX, either no, or only very minor, transient deficits were reported. These results were in stark contract to the prolonged, up to 6 to 14 day, behavioral incapacitation experienced by animals pre-treated with pyridostigmine that received the same dose of nerve agent followed by the standard atropine, oxime therapy with or without diazepam. While several challenges still remain to be met before bioscavengers could augment or replace the current therapeutic regimes for nerve agent intoxication, the results to date offer impressive evidence for the value of this approach as the next generation of pharmaceuticals to afford protection against nerve agent poisoning with a virtual absence of behavioral side effects.

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KEYWORDS: Nerve agents, bioscavengers, cholinesterase, organophosphorus poisons, chemical warfare agents, prophylaxis.

B.1 INTRODUCTION

Organophsophorus anticholinesterases (OPs), usually acid anhydride derivatives of phosphoric acid, are among the most toxic substances that have been identified [1]. Originally, OPs were developed for use as insecticides, [2] but their extreme toxicity toward higher vertebrates has led to their adoption as weapons of warfare [3]. The OPs most commonly utilized as chemical weapons (referred to as nerve agents) that NATO forces might expect to encounter are anhydrides of hydrocyanic acid, hydrofluoric acid, (e.g. tabun [GA], sarin [GB], soman [GD] cyclohexylmethyl phosphonofluoridate [cyclosarin, GF]), or of derivatives of thiocholine (e.g., ethyl-S-diisopropylaminoethyl methylphosphonothioate [VX] and Russian V-agent). However, OP pesticides can also be regarded as a potential threat in a terrorist context. Their molecular weights range from 140 to 267 Daltons (Da), and under standard conditions they are all liquids that differ in their degrees of volatility [4]. They have median lethal dose (LD_{50}) values in mammals, including estimates for humans, in the µg/kg dose range for all routes of exposure except dermal, where LD_{50} doses are in the mg/kg range [3]. OPs produce their acute toxic effects by irreversibly inhibiting the enzyme acetylcholinesterase (AChE, E.C. 3.1.1.7) [5, 6]. This inhibition leads to an increase in the concentration of acetylcholine in the cholinergic synapses of both the peripheral and central nervous systems. Because numerous lines of evidence indicate that the sister enzyme butyrylcholinesterase (BuChE, E.C. 3.1.1.8) plays a functional role in the cholinergic system [7], inhibition of BuChE could contribute to the increase in concentration of acetylcholine in the cholinergic synapses, including those in the central nervous system. The physiological consequences of elevated acetylcholine include alterations in the function of the respiratory center [8-11] and over-stimulation at neuromuscular junctions [12-14]. A sufficiently high level of acetylcholine or a sufficiently rapid increase in acetylcholine concentration precipitates a cholinergic crisis, resulting in dimming of vision, headache, shortness of breath, muscle weakness and seizures. In the extreme, organophosphorus intoxication can be a life-threatening event, with death usually resulting from respiratory failure. This is often accompanied by secondary cardiovascular components, including hypotension, cardiac slowing and arrhythmias [6].

This suggests that a prophylactic approach based on the reduction of the concentration of OP toxicant in the blood before it can reach its site of action (synaptic endplates) should be particularly effective; potentially incapacitating or even toxic exposures could be mitigated to mild symptoms such as salivation or shortness of breath and lower level exposures could be rendered inconsequential.

B.2 CURRENT THERAPY FOR NERVE AGENT EXPOSURE

The conventional approach adopted by NATO allies to treatment of OP intoxication involves efforts to counteract the effects of AChE inhibition. Cholinolytic drugs such as atropine are administered at the onset of signs of OP intoxication to antagonize the effects of the elevated acetylcholine levels that result from the inhibition of AChE [15]. Additionally, an oxime nucleophile is given, which reacts with the inhibited "phosphylated' enzyme to displace the phosphyl group and restore normal activity [16]. In the United States, the oxime of choice for treatment of nerve agent poisoning is the chloride salt of 2-PAM, usually referred to as 2-PAM-Cl. In France, the methylsulfate salt of 2-PAM is registered as Contrathion. In the UK, the sulfate salt of 2-PAM, usually referred to as P2S, is the oxime of choice, although bis-pyridinium oximes may be more effective depending on the particular organophosphorus agent [17]. Anticonvulsant drugs such as diazepam are also administered to control OP-induced tremors and convulsions. In conjunction with therapy, individuals at high risk for exposure to nerve agents are pre-treated with a spontaneously reactivating AChE

inhibitor, such as the carbamyl ester pyridostigmine which temporarily masks the active site of a fraction of AChE molecules at recommended dosages, less than 30 % of peripheral AChE, and thus protects the enzyme from irreversible inhibition by the OP agent [18].

While these treatment regimens, with minor variations, have been the standard in NATO countries for many years, they are not ideal and suffer from a number of disadvantages. While current approaches can be effective in preventing lethality, they do not prevent performance deficits, behavioral incapacitation, loss of consciousness or permanent brain damage, all of which can result from acute OP toxicity [19].

Several nerve agents, including cyclosarin, sarin, and in particular soman, present an additional therapeutic challenge in that after they inhibit AChE, they undergo a second reaction in which the phosphonyl group attached to the inhibited enzyme is dealkylated. This process, known as aging, results in a phosphonylated AChE that is refractory to either spontaneous or oxime-mediated reactivation [20]. The rate of aging depends on the chemical structure of the alkoxy chain being dealkylated; the bulkiest branched chains promote very rapid aging, e.g., the half time of aging of soman-phosphonylated human AChE is 2 min at 37°C, whereas that of the sarin-phosphonylated enzyme is about 4 hours [21]. The ineffectiveness of therapeutically administered oxime as a treatment for some nerve agents explains the continued research efforts aimed at alternative approaches to protection [22]. One approach in particular has focused on preventing the critical enzyme AChE from becoming inhibited in the first place. Although the currently used pre-treatment/therapy regimen is able to protect soldiers against the otherwise lethal effects of nerve agents, it does not adequately protect against the incapacitation that results from high levels of nerve agent exposure. Furthermore, it appears that greater than marginal improvement of these pharmacological approaches will be difficult because stronger drugs or higher doses are likely to produce unacceptable performance decrements by themselves [22, 23].

B.3 NERVE AGENT BIOSCAVENGERS: AN ALTERNATIVE TO CONVENTIONAL APPROACHES

While successful, current treatments for acute nerve agent poisoning always result in the victim suffering a toxic insult that subsequently must be therapeutically managed. To avoid the onset of a toxic insult, recent efforts have focused on identifying proteins that can act as biological scavengers of organophosphorus compounds and can remain stable in circulation for long periods of time. The concept of using a protein that can react with a nerve agent, either stoichiometrically or catalytically, to protect against the toxic effects of those compounds, either acute or low level, is not new. As early as 1956 it was shown that injection of exogenous paraoxonase (EC. 3.1.8.1) could protect rats against several times the LD_{50} of paraoxon [24]. This approach avoids the side effects associated with current antidotes [22, 25-32] and the requirement for their rapid administration by prophylactically inactivating (through sequestration or hydrolysis) anticholinesterase agents before they can react with the target AChE. The time frame for this inactivation to occur before endogenous AChE is affected is quite narrow (estimated to be approximately two minutes in humans [33]), so especially for situations involving acute exposure, the scavenger function must be very rapid, irreversible and specific. Ideally, the scavenger would enjoy a long residence time in the bloodstream, would be biologically innocuous in the absence of nerve agent and would not present an antigenic challenge to the immune system. For these reasons, a formal program to identify candidate bioscavengers was begun in 1993 and has focused on enzymes of mammalian (usually human) origin.

Candidate bioscavenger proteins, in general, function either by stoichiometrically binding and sequestering the anticholinesterase or by catalytically cleaving the OP substrate into biologically inert products. In the former category are naturally occurring human proteins that bind and/or react with nerve agents, including

enzymes such as cholinesterases (ChEs) and carboxylesterases (CaEs), as well as antibodies specific for nerve agent haptens. Each of these stoichiometric scavengers has the capacity to bind one or two molecules of nerve agent per molecule of protein scavenger. While this approach has been proven to be effective in laboratory animals, it has the disadvantage that the extent of protection is directly proportional to the concentration of unexposed, active scavenger in the bloodstream at the time of nerve agent exposure. Since the molecular weight of a protein scavenger is in the range of 80,000 Da and the molecular weight of the nerve agents is about 160 Da, the concentration mass ratio of scavenger to nerve agent is 500:1. Thus, a high concentration of scavenger protein in circulation is necessary to protect against exposure to multiples of an LD_{50} dose of nerve agent, although lower concentrations would be sufficient to prevent inactivation of synaptic AChE after a low-dose exposure. It might be possible to mitigate the need for large amounts of scavenger by also administering, either prophylactically or immediately post-exposure, a currently fielded oxime. Oxime treatment would allow the continual reactivation of the bioscavenger *in vivo*, in effect converting the stoichiometric scavenger into a pseudo-catalytic one (cf. Enzymes, *vide infra*).

Candidate enzymes with *bona fide* catalytic activity against nerve agents include the human organophosphorus acid anhydride hydrolases (OPAHs) [34], such as paraoxonase (Hu-Pon). Additionally, the ability to generate catalytic antibodies in response to appropriate transition state analogs [35-40] suggests that nerve agent-specific antibodies that catalyze hydrolysis of their ligands could be effective bioscavengers. Finally, the ability to engineer site-specific amino acid mutations into naturally occurring scavenger enzymes can allow investigators to alter the binding and/or catalytic activities of these enzymes. In general, the use of scavengers with catalytic activity would be advantageous because small amounts of enzyme, meaning lower concentrations in circulation, would be sufficient to detoxify large amounts of nerve agent (as in an acute exposure).

By nearly all criteria, the use of biological scavengers, either stoichiometric or catalytic, as a prophylactic approach to providing protection against an exposure to either a low-level or a lethal dose of a nerve agent offers numerous advantages over conventional treatments. In fact, the half time for reaction of a nerve agent with a biological scavenger can be calculated using some very conservative assumptions. Based on toxicity estimates in humans, the expected concentration of a nerve agent in the blood at an LD_{50} dose would be about 8×10^{-7} M [41]. The bimolecular rate constant for reaction of soman with AChE is ~9×10^{7} M^{-1} min^{-1} [42, 43]. If a scavenger were present in blood at a concentration of 1 mg/mL (1×10^{-5} M), then the rate constant for reaction of scavenger with toxicant would be pseudo first order and the $t_{1/2}$ for the reduction of toxicant would be ~$3\text{-}7 \times 10^{-4}$ min. Under those conditions, which assume perfect mixing and that all of the scavenger and all of the toxicant remain in the bloodstream, the concentration of toxicant would be reduced to $1/1000^{th}$ of its initial concentration within 10 half-lives ($2\text{-}4 \times 10^{-3}$ min). Where actual measurements have been made of the rate of reduction of concentration of soman in animals (guinea pigs), it was found that, in the absence of an exogenous scavenger, the concentration of a 2 LD_{50} dose of soman in circulation was reduced by 1000-fold in about 1.5 minutes.[44] These results support our contention that, if a bioscavenger were present in circulation at the time of exposure, the reduction in toxicant concentration to a physiologically insignificant level (with no measurable inhibition of AChE) would be very rapid, and would certainly occur in less than one circulation time at most concentrations of OP that might be encountered in a battlefield setting. The need to administer, repetitively, a host of pharmacologically active drugs with a short duration of action at a precise time following exposure is all but eliminated if a scavenger is used. The potential for having to use mission oriented protective posture (MOPP) gear is greatly reduced. Finally, with the appropriate scavenger(s), such an approach could afford protection against all of the current threat agents, including those that induce rapid aging of AChE and are refractory to treatment by the current atropine and oxime treatment regime.

B.4 STOICHIOMETRIC SCAVENGERS AND THE PROTECTION THEY OFFER

B.4.1 Antibodies

More than 25 years ago efforts were undertaken to protect animals by actively immunizing them with analogs of paraoxon or soman attached to appropriate protein carrier molecules, to elicit an antibody response against these two highly toxic organophosphorus compounds [44, 46]. As summarized in Table B-1, rabbits that developed antibodies against paraoxon were protected against 2 to 3 times the LD_{50} of paraoxon [47]. The extent of protection was found to be directly related to the concentration of the paraoxon specific antibodies in circulation. Significantly, the protected animals were essentially asymptomatic and did not require the administration of any additional therapeutic drugs. Rabbits immunized with an analog of soman were not protected against the administration of a lethal dose of that compound. Subsequently it was determined that the polyclonal antibodies induced in these animals were not of sufficiently high affinity to successfully compete with AChE for the binding of soman [46].

Table B-1: Protection from Organophosphorus Intoxication by Antibody Bioscavengers

Bioscavenger	Test Species	Nerve Agent	Protection (LD_{50})[a]	Serum $T_{1/2}$[b]	Reference
Polyclonal Antibodies [c]	Rabbit	Paraoxon	2 – 3	Days to Weeks	[47]
Polyclonal Antibodies	Rabbit	GD	–	Days to Weeks	[46]
Monoclonal Antibody [c]	Mouse	GD	- / extended mean survival time	(6 – 8 days)	[46], [103]

[a] Values represent multiples of median lethal doses (LD_{50}s) of nerve agent survived after antibody administration.

[b] Half-life of antibodies in blood circulation.

[c] Polyclonal antibodies: the endogenous serum titer after priming with nerve agent analogs. Monoclonal antibody: produced *in vitro* by a hybridoma, then passively administered to naïve mice.

Based on these limited but promising results, efforts were made to generate high affinity monoclonal antibodies that could be used to afford passive protection from nerve agents. Hunter et al. [48] reported the production of the first anti-soman monoclonal antibodies, which were subsequently shown to be of sufficiently high affinity to compete with AChE for soman binding *in vitro* [46]. When mice were passively immunized with these antibodies they failed to show any protection against the *in vivo* toxicity of soman, although the time to death was almost doubled in the animals pre-treated with antibody [46]. Further *in vitro* characterization of the monoclonal antibodies showed that their anti-soman binding constants were only in the micro-molar range, but that they were highly soman-specific, in that they did not bind the structurally related nerve agent sarin [49]. Subsequent calculations suggest that to afford protection on a stoichiometric level against soman or sarin, a monoclonal antibody must have a binding constant in the 50 nano-molar range [41]. Work by Erhard et al. [50-52], Glikson et al. [53] and Miller and Lenz [54] on monoclonal antibodies against soman produced similar results with respect to affinity.

In addition to efforts to produce antibodies that might be capable of affording protection against chemical warfare agent exposure, there have been additional efforts, primarily, but not exclusively, in NATO countries to develop antibodies against chemical warfare agents for use as diagnostic tools. Antibodies specific for a variety of chemical warfare agents to include both polyclonal [55] and monoclonal [56, 57] antibodies against

VX, polyclonal antibodies against sarin [58-60] and both polyclonal [61] and monoclonal antibodies [62] against sulfur mustard and sulfur mustard DNA adducts [63, 64] have been developed. To date only the antibodies against sulfur mustard adducts [63, 64], have been utilized as diagnostic reagents.

B.4.2 Enzymes

A number of different enzymes that react with OPs but do not catalyze their hydrolysis have been tested for their ability to provide protection against nerve agent poisoning. Wolfe et al. first reported the use of exogenously administered AChE as a bioscavenger (Table B-2) [65]. In that study, fetal bovine serum acetylcholinesterase (FBS-AChE) was administered to mice 20 hours before a multiple LD_{50} challenge of VX was administered. Complete protection was afforded against a 2 x LD_{50} dose of VX (100% survival of exposed animals), while moderate protection (80% survival of exposed animals) was observed after a challenge of 3 times the LD_{50}. No protection was observed against higher multiple LD_{50} challenges of VX. When animals pre-treated with FBS-AChE were exposed to soman, little protection was afforded. However, FBS-AChE pre-treatment in conjunction with post-exposure atropine and 2-PAM treatment protected mice from 2 x LD_{50} of soman. The authors reported that animals displayed no detectable side effects in response to administration of FBS-AChE.

Table B-2: Protection from Organophosphorus Intoxication by the Bioscavenger FBS-AChE

Bioscavenger	Test Species	Nerve Agent	Protection (LD_{50})[a]	Serum $T_{1/2}$[b]	Reference
FBS-AChE	Rhesus Monkey	GD	2 – 5	30 – 40 Hrs	[66], [69]
FBS-AChE	Mouse	GD	2 (w/ Atropine + 2-PAM)	40 – 50 Hrs	[65]
FBS-AChE	Mouse	GD	2 (after CBDP treatment)	~24 Hrs	[82]
FBS-AChE	Mouse	GD	2 – 8	24 – 26 Hrs	[67], [71], [83]
FBS-AChE	Mouse	MEPQ	4	~24 Hrs	[71], [82]
FBS-AChE	Mouse	VX	2 – 3, 6	~24 – 50 Hrs	[65], [71], [82]

[a] Values represent multiples of median lethal doses (LD_{50}s) of nerve agent survived after FBS-AChE administration.

[b] Half-life of administered FBS-AChE in blood circulation.

Maxwell and co-workers carried out a similar set of experiments using rhesus monkeys pre-treated with the scavenger FBS-AChE [66]. When monkeys pre-treated with FBS-AChE were challenged with either 1.5 or 2.5 x LD_{50} of soman, there was protection (Table B-2) with no decrements in performance on the serial probe recognition (SPR, discussed in Behavioral Effects, below) task as compared with animals treated with FBS-AChE alone. The animals were also monitored for the generation of an antibody response against the administered FBS-AChE, but none was detected. The authors caution, however, that whenever a foreign protein is administered to an animal, the potential for an antibody-mediated immune response must be assessed on a case-by-case basis. Maxwell and co-workers also compared the relative protection against soman afforded to mice by three different treatments: pyridostigmine pre-treatment with atropine therapy post-exposure, post-exposure oxime (HI-6) and atropine therapy, or FBS-AChE pre-treatment alone [67]. The authors concluded that

the FBS-AChE pre-treatment offered superior protection against both soman toxicity (survival after 8 to10 x LD_{50} doses) and behavioral incapacitation. The results of these and other studies using FBS-AChE are summarized in Table B-2.

Broomfield and co-workers reported that equine butyrylcholinesterase (eq-BuChE) afforded complete protection against a 2 x LD_{50} challenge dose of soman in rhesus monkeys (Table 3) with no supporting therapy and against 3 to 4 x LD_{50} doses when atropine was administered (post-exposure) [68]. Protection against a single LD_{50} dose of sarin was also demonstrated. There were no fatalities in any of these cases (Table 3). Furthermore, when animals were assessed for behavioral deficits, again using an SPR task, they all returned to baseline performance within nine hours after soman exposure (*vide infra*) [23].

In a related study, Wolfe et al. assessed the ability of pre-treatment with either FBS-AChE or eq-BuChE to protect rhesus monkeys against multiple LD_{50} doses of soman (Tables B-2 and B-3) [69]. Survival and the ability to perform the primate equilibrium platform (PEP) behavioral task were the variables assessed. The animals that received FBS-AChE as a pre-treatment were protected against a cumulative exposure of 5 x LD_{50} of soman and showed no decrement in the PEP task. Two of the four monkeys that received purified eq-BuChE did show some transient decrement in PEP task performance when the cumulative dose of soman exceeded 4 LD_{50}s. All of the experimental animals were observed for an additional six weeks, and none displayed any residual or delayed performance decrements suggesting no residual adverse effects. These results were reviewed and expanded upon by Doctor et al., in studies where mice pre-treated with FBS-AChE were also administered the oxime HI-6 immediately post-exposure to sarin [70]. In theory, the oxime will continuously regenerate the inhibited scavenger enzyme *in vivo*; this approach is predicted to increase the amount of sarin that could be scavenged by a given amount of AChE, making this stoichiometric scavenger pseudo-catalytic. The therapeutic addition of HI-6 after pre-treatment with FBS-AChE was found to enhance the efficacy of the scavenger enzyme against sarin *in vivo*, increasing the ratio of neutralized organophosphorus compound per FBS-AChE molecule from 1:1 (in the presence of AChE alone) to roughly 65:1.

Table B-3: Protection from Organophosphorus Intoxication by the Bioscavenger eq-BuChE

Bioscavenger	Test Species	Nerve Agent	Protection (LD_{50})[a]	Serum $T_{1/2}$[b]	Reference
eq-BuChE	Rhesus Monkey	GB	1	620 Hrs	[68]
eq-BuChE	Rhesus Monkey	GD	2 (4 w/ atropine)	620 Hrs	[69]
eq-BuChE	Rhesus Monkey	GD	5	30 – 40 Hrs	[69]

[a] Values represent multiples of median lethal doses (LD_{50}s) of nerve agent survived after eq-BuChE administration.

[b] Half-life of administered eq-BuChE in blood circulation.

Maxwell et al. identified carboxylesterase as another enzyme with the potential to be a good anti-organophosphorus scavenger molecule (summarized in Table B-4) [71]. While AChE and BuChE were found to be more efficient scavengers for soman in mice than CaE (i.e., they have higher bimolecular rate constants), the latter enzyme was capable of affording equal protection on a molar basis. Carboxylesterases (CaE; EC 3.1.1.1) catalyze the hydrolysis of a wide variety of aliphatic and aromatic esters and amides [72, 73]. Catalysis occurs by a two-step process in which the substrate acylates the active site serine of CaE, which subsequently deacylates by the addition of water [74]. CaE can be distinguished from AChE and BuChE by the fact that AChE and BuChE react with positively charged carboxylesters, such as acetylcholine and butyrylcholine, and are readily inhibited by carbamates, while CaE does not react with positively charged

substrates and is inhibited by carbamates only at high concentrations [74]. These differences in substrate specificity also extend to the reaction of CaE with OP compounds. Positively charged OP compounds, such as VX, react poorly with CaE while neutral OP compounds, such as soman, sarin and paraoxon, react rapidly. Dephosphorylation of the active site phosphorylated serine of CaE is a slow process compared to deacylation [75], and therefore CaE has usually been considered to be a stoichiometric detoxification mechanism for OP compounds.

Table B-4: Protection from Organophosphorus Intoxication by Endogenous Plasma CaE

Bioscavenger	Test Species	Nerve Agent	Protection (LD_{50})[a]	Reference
CaE [b]	Mouse	GD	16	[77]
CaE	Guinea Pig	GD	3.5	[77]
CaE	Rabbit	GD	3	[77]
CaE	Rat	GD	8 – 9	[77], [109]
CaE	Rat	GD	8	[111]
CaE	Rat	GB	4 – 5	[111]
CaE	Rat	VX	1	[111]
CaE	Rat	Paraoxon	2	[111]

[a] Values represent multiples of median lethal doses (LD_{50}s) of nerve agent survived due to the presence of CaE. Because CaE is an endogenous plasma protein in these species, the protection it offers was measured by comparing LD_{50} values in untreated and CBDP-treated animals; 2 mg/kg CBDP completely abolishes endogenous plasma CaE activity [111].

[b] For each species, the activity of the host's endogenous CaE was tested.

CaE is 60-kDa enzyme that is found in many mammalian tissues – lung, liver, kidney, brain, intestine, muscle and gonads – usually as a microsomal enzyme. In some species CaE is also found in high concentration in plasma; plasma CaE is synthesized in the liver and secreted into the circulation via the Golgi apparatus of hepatocytes [76]. Secretion of CaE appears to be controlled by the presence or absence of a retention signal at the carboxy terminal of the enzyme (Figure B-1). CaE that is retained in the liver has a highly conserved carboxy terminal tetrapeptide sequence (HXEL in single letter amino acid code, where X represents any amino acid), while the secretory form of CaE has a disrupted version of this retention signal in which the terminal leucine residue is replaced by either histidine-lysine or histidine-threonine [76]. Mammalian species that have high levels of secretory CaE in their plasma require much larger doses of OP compounds to produce toxicity than do species with low levels of plasma CaE [77]. For example, the LD_{50} dose for soman in rats is 10-fold larger than the LD_{50} in non-human primates, which correlates with the differences in the plasma concentrations of CaE found in these species (Figure B-2). Although human CaE has been cloned and expressed [76], there is no commercial source of highly purified CaE for use in vivo testing of protective efficacy. Therefore, the primary evidence demonstrating the effectiveness of CaE as a stoichiometric scavenger against OPs, especially sarin and soman, has been by comparison of OP LD_{50}s in animals with high endogenous plasma levels of CaE to OP LD_{50} levels in animals of the same species whose plasma CaE has been chemically inhibited [77]. For example, inhibition of plasma CaE prior to the LD_{50} determination of soman in rats reduces its LD_{50} by approximately eight-fold (Table B-4) strongly suggesting that circulating CaE is an effective bioscavenger against OP compounds.

Enzyme	COOH-Terminal Residues	Reference
Intracellular CaEs		
Rabbit Es-1	..TE**H**I**EL**	[115]
Rabbit Es-2	..QK**HTEL**	[116]
Hamster AT51p	..GK**HSEL**	[117]
Human CaE-1	..TE**H**I**EL**	[118]
Human CaE-2	..ER**HTEL**	[119]
Pig CaE	..IK**HAEL**	[120]
Rat Es-10(pI6.1)	..WK**HVEL**	[121]
Rat Es-B	..PH**H**N**EL**	[122]
Mouse Es-X	..RE**HVEL**	[123]
Mouse Es-22	..TE**HTEL**	[124]
Consensus	..**HXEL**	
Secreted CaEs		
Mouse Es-1	..TE**HTE*HK***	[125]
Rat Es-1	..TE**HTE*HT***	[126-128]

Figure B-1: Biochemical Basis for CaE Cellular Trafficking.

The carboxy-terminal amino acid residues of carboxylesterase enzymes from disparate species are aligned to show the conserved "HXEL" motif found among intracellular enzymes (shown in bold letters), and the disrupted versions of this retention motif found in the mouse and rat secreted carboxylesterase isoenzymes (alterations to the motif shown in italics). The capacity of the carboxy-terminal "HXEL" motif to act as an endoplasmic reticulum retention signal has been directly demonstrated.[129]

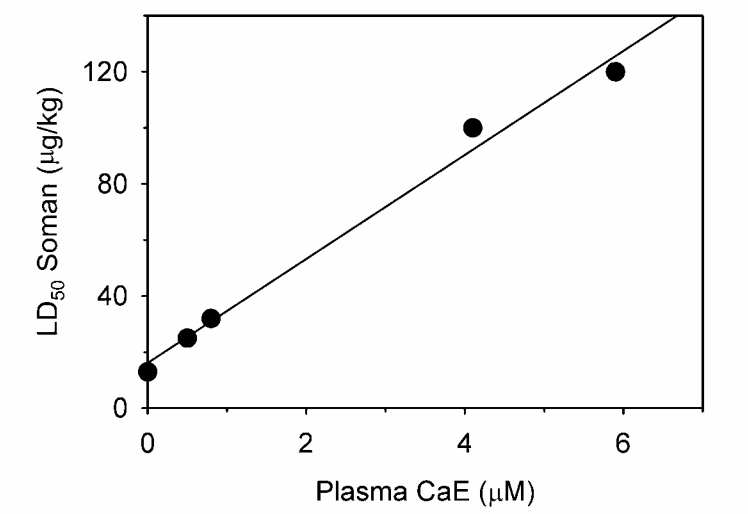

Figure B-2: Effect of Plasma CaE Concentration on Soman LD_{50} (administered subcutaneously) in Different Species. Data points (from lower left to upper right of graph) for species were monkey, rabbit, guinea pig, rat and mouse. Data taken from Maxwell et al. [71].

Recent investigations of the reactivation of OP-inhibited CaE have suggested that it may be possible to increase its potential as an OP scavenger by exploiting its turnover of OP compounds. Maxwell et al. observed

that OP-inhibited CaE does not undergo the aging process that prevents oxime reactivation of OP-inhibited cholinesterases [78], while Jokanovic et al. found that OP-inhibited CaE from plasma underwent spontaneous reactivation with a half time of 1 to 2 hours [79]. Comparisons of the amino acid sequences of CaE, AChE and BuChE are informative with regard to the critical amino acid residues required for occurrence of aging versus spontaneous reactivation. Of the seven conserved amino acid residues that have been identified by theoretical studies and confirmed by site-directed mutagenesis to be important for aging of OP-inhibited AChE and BuChE, only two are conserved in CaE [80]. Conversely, a highly conserved histidine found in CaE from six mammalian species and two insect species, but not in mammalian cholinesterases, correlates with the higher level of spontaneous reactivation of OP-inhibited CaE in comparison to OP-inhibited cholinesterase [78]. Interestingly, introduction of a histidine into BuChE at a position nearly identical to the position of the conserved histidine of CaE, i.e., in the oxyanion hole, produces spontaneous reactivation of OP-inhibited BuChE [81].

A more detailed discussion of the relative merits of FBS-AChE, eq-BuChE and plasma CaE as scavengers, which describes the extent of protection they offer against a variety of nerve agents, both *in vitro* and *in vivo* in mice, was presented by Doctor et al. [82]. The authors note that some of the *in vivo* differences in sensitivity and protection seen may be due to variations in the circulatory pharmacodynamics of the different organophosphorus compounds, such that those inhibitors that distribute more slowly from circulation are more readily scavenged. This concept supports the feasibility of using scavengers to protect against low-level exposures of nerve agent. Raveh et al. have provided additional examples that agree with those conclusions [83-85]. The extent of protection afforded by FBS-AChE against soman in marmosets and rhesus monkeys with respect to survival was determined and found to be the same in both species. Significantly, the stoichiometry of the protective dose of FBS-AChE scavenger to organophosphorus compound was experimentally determined to be one to one on a molar basis in both species of monkey, suggesting that a similar ratio will be maintained in other species, including man. Finally, none of the animals pre-treated with scavenger displayed any adverse symptoms following an LD_{100} challenge dose of soman.

Ultimately, the goal of research on scavenger molecules is to generate a means to protect humans from the toxic effects of nerve agents. In an effort to minimize any physiological, immunological or psychological side effects of scavenger use in humans, research efforts are now focused on the use of human BuChE (HuBuChE), human CaE and/or, as a model system, FBS-AChE (which does not induce an immune response in rhesus monkeys [66]). In a series of studies, Ashani and his co-workers examined the scavenger properties of FBS-AChE and particularly HuBuChE in mice, rats and rhesus monkeys with respect to several different nerve agents as well as other organophosphorus compounds (Table B-5) [83-85]. They found that following administration of exogenous cholinesterase, there was a linear correlation between the concentration of cholinesterase in the blood and the level of protection against organophosphorus poisoning. Furthermore, the extent of protection granted to mice was sufficient to counteract multiple LD_{50} doses of soman. When the protective effect of pre-treatment with HuBuChE was compared in mice and rats, it was found that in both species the same linear correlation existed between blood concentration of HuBuChE and protection against soman, sarin or VX (Table B-5). They further noted that to be effective, a scavenger had to be present before exposure to the organophosphorus compound, because (as discussed above) the nerve agent had to be scavenged within one blood circulation time period [84]. In the final paper in this series the authors report similar protection results against a 3.3 LD_{50} dose of soman or a 2.1 LD_{50} dose of VX in rhesus monkeys [85]. They also report considerable protection against soman-induced behavioral deficits in a spatial discrimination task.

Table B-5: Protection from Organophosphorus Intoxication by HuBuChE Bioscavengers

Bioscavenger	Test Species	Nerve Agent	Protection (LD_{50})[a]	Serum $T_{1/2}$[b]	Reference
HuBuChE	Rhesus Monkey	GD	2	~30 Hrs	[85]
HuBuChE	Rhesus Monkey	VX	1.5	~30 Hrs	[85]
HuBuChE	Rat	GD	2 – 3	46 Hrs	[84]
HuBuChE	Rat	VX	2	46 Hrs	[84]
HuBuChE	Mouse	GD	2.1	21 Hrs	[85]
HuBuChE	Mouse	GB	1.6	21 Hrs	[85]
HuBuChE	Mouse	GA	1.8	21 Hrs	[85]
HuBuChE	Mouse	VX	4.9	21 Hrs	[85]

[a] Values represent multiples of median lethal doses (LD_{50}s) of nerve agent survived after BuChE administration.

[b] Half-life of administered BuChE in blood circulation.

B.5 CATALYTIC BIOSCAVENGERS

While stoichiometric scavengers are able to afford good protection as long as they reside at high levels in the blood stream, they suffer the disadvantage that they are all molecules of high molecular weight (*vide supra*); a comparatively large quantity is required to neutralize a small amount of nerve agent. A catalytic scavenger, even having the same high molecular weight, could be administered in smaller quantities and could produce the same or greater degree of protection. It would also have the advantage of not being consumed in the process of detoxifying the nerve agent, so it would be available to protect against multiple exposures of either high or low dose. Some of these potential bioscavenger proteins along with parameters of their catalytic activities are summarized in Tables B-6 – B-8. As discussed above, in conjunction with an oxime such as HI-6, cholinesterases that have not undergone aging can be continually reactivated to function pseudo-catalytically, eliminating substantially more moles of organophosphorus compounds than would be predicted based on binding alone. Furthermore, some enzymes, such as the OPAH from *Pseudomonas diminuta* [86], the prolidase from A*lteromonas haloplanktis* [87], or the human Pon (Hu-Pon) [34, 88-91] have intrinsic catalytic anti-organophosphorus activity. The *Pseudomonas diminuta* enzyme has been shown to afford protection against soman lethality in mice and to protect against behavioral side effects (Table B-6) [92]. However, since this bacterially derived enzyme has no known mammalian homologues, it will likely be a potent initiator of immune responses and is therefore unlikely to be appropriate for use as a prophylactic scavenger in humans. However, bacterial enzymes could be used for skin protection as active components of topical skin protectants (TSPs) or covalently bound to the cornified layer of epidermis [93]. Detoxification of OPs can also be achieved through enzymatic oxidation of their alkyl chains. In particular, breakdown of VX by horseradish peroxidase [94] or by *Caldariomyces fumago* chloroperoxidase [95] could be used in polyfunctional active TSP and for skin decontamination. Additionally, the *Pseudomonas diminuta* OPAH could be used as a one-time pre-treatment either in addition to or in place of conventional therapy, since in the short term this enzyme is highly effective against GD, GB and VX, and alone induces no known behavioral effects. The Hu-Pon enzyme has been identified as having a similar potential for affording protection (Table B-6), but without the complication of inducing an immune response; the human immune system would recognize it as “self” so it would not be an immunogen. There are no available data on the efficacy of Hu-Pon

against OP toxicity in a mammalian model system [34, 88-92]. A preliminary study showed that in animals not pre-treated with CBDP to inhibit plasma CE [77] a dose of 1 mg/mouse of Hu-Pon administered in the vein tail 5 min prior to subcutaneous injection of soman does not provide protection against ≥ 1xLD_{50} of this OP; however, a mild protective effect in terms of seizure rate and incapacitation was observed for lower doses of soman (Lallement et al., unpublished). Since the effects of plasma CaE were not reduced [77, 80], these results suggest that protection against multiple LD_{50}s of nerve agents could be achieved using either higher doses of wild-type Hu Pon (5 – 10 mg/mouse) or using mutants of the enzyme having an enhanced catalytic activity toward OPs. In addition the effects would be expected to be more pronounced in a species with little or no plasma CaE.

Table B-6: Kinetic Properties of Naturally Occurring Catalytic Bioscavengers

Bioscavenger	**Source Species**	**Substrate Specificity**	**Km (μM)**	**Vmax ($nmol \bullet min^{-1} \bullet mg^{-1}$)**	**Reference**
Phosphotriesterase	*P. Diminuta*	GD	36 / 500	15 / 7.3	[92], [112]
Phosphotriesterase	*P. Diminuta*	GD	700	N.D.[a]	[112]
Phosphotriesterase	*P. Diminuta*	Paraoxon	50	3200	[112]
Phosphotriesterase	*P. Diminuta*	DFP	100	64	[112]
			Bimolecular Rate Constant (k_{cat}/Km [$M^{-1} \bullet min^{-1}$]) Q191 / R191 [b]		
Pon	Human	GD	2.8×10^6 / 2.1×10^6		[34]
Pon	Human	GB	9.1×10^5 / 6.8×10^4		[34]
Pon	Human	DFP	3.7×10^4 / N.D.		[34]
Pon	Human	Paraoxon	6.8×10^5 / 2.4×10^6		[34]

[a] Not Determined.

[b] Two naturally occurring allelic variants of Pon (Q191 and R191) have been identified. The activity of each form is shown.

While the enzymes discussed above possess the desired catalytic activity, none of them is fast enough for use as a nerve agent pre-treatment. Since the organophosphorus anticholinesterases have been in the environment for only a little over 50 years, it is not likely that any of the enzymes we identify as OPAHs have as their primary function the destruction of OPs. In fact, an OPAH from an *Alteromonas* species has been identified as a prolidase, a dipeptidase that cleaves at a penultimate proline from the carboxyl end of a peptide [87]. Recently, Pon was shown to be a homocysteine thiolactone hydrolase and can protect against protein N-Homocysteinylation [96].

A functional catalytic scavenger must have both (*)[1] a lower Km (a measure of the strength of binding of a substrate to the enzyme) and a higher turnover number than have been found to date among these naturally

[1] (*) Actually, the ratio k_{cat}/K_m must be as high as possible because the concentration of OP in blood, [OP], is far below K_m and, therefore, the rate of hydrolysis (v) of OP is first-order (cf. ref. 34): $v = (k_{cat}/K_m)$ [OP].[E].

occurring catalytic enzymes, since agent must be cleared from the bloodstream within the one to two minutes before it reaches critical targets [68]. Therefore, it was decided to attempt to create such an enzyme by specific mutation of existing human enzymes. Obvious candidates for such attempts include members of the cholinesterase family (including carboxylesterase) and the paraoxonases, which already possess the desired activity but at insufficient levels. The rationale for the design of mutations in the cholinesterase family was based on the fact that for these enzymes, the organophosphorus inhibitors are in reality hemisubstrates; their initial reaction with enzyme is similar to that of normal substrates. However, the subsequent reaction, equivalent to deacylation of the active site serine, is blocked because of the geometry of the active site. The amino acid group responsible for deacylation is not in an appropriate position to effect dephosphorylation [97, 98].

The perceived solution to this problem was to insert a second catalytic center into the active site specifically to carry out the dephosphylation step of the reaction [98]. Applying this rationale, the human wild-type BuChE has been mutated in the oxyanion hole (Figure B-3 and Table B-7) to express a mutated enzyme, G117H, with the ability to catalyze the hydrolysis of sarin, DFP, paraoxon, VX and other non-aging nerve agents. [98, 99] Aging and reactivation are parallel first-order reactions in phosphylated enzymes. In the reactivation reaction the phosphoryl group is removed from the active site serine residue (S198), restoring activity, whereas in the aging reaction one of the alkyl groups is removed from the phosphoryl group, rendering the group non-reactivatable. To effect the hydrolysis of rapidly aging nerve agents such as soman it is necessary to inhibit the aging reaction so that reactivation is faster. This was accomplished by replacing the carboxyl group (Glutamic acid, E197) adjacent to the active site serine with an amide (Glutamine, Figure B-4) [99]. Unfortunately, these mutants have catalytic activities that are too slow for practical use (Table B-7), and thus the search for a faster enzyme continues. For example, human CaE and Pon are currently being subjected to mutation in efforts to generate additional, faster catalytic anti-nerve agent enzymes. It is important to note that in the case of Pon, the desired catalytic activity is present at low levels in the native enzyme; since OPs are “accidental” substrates for Pon (see above [34, 89]) it is likely that improvement in activity can be realized through protein engineering. Because Pon is a naturally occurring plasma enzyme produced in the liver, an alternative would be to enhance the endogenous enzyme biosynthesis by inducing increased activity of its gene promoter. Recent results on increased expression levels of Pon by HuH7 hepatoma cells upon action of fibrates are promising (C. Gouédard, C, Barouki and Y. Morel, unpublished).

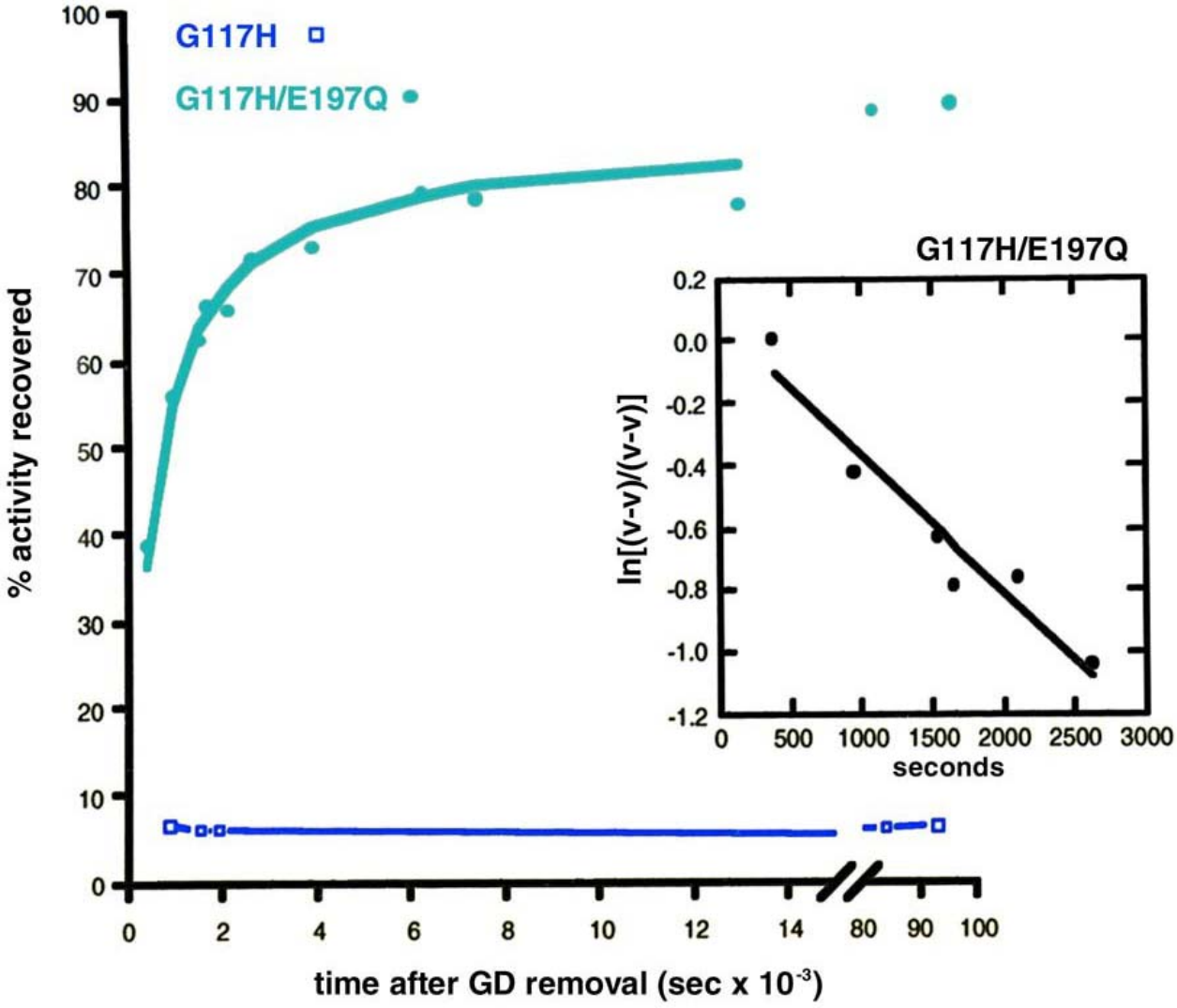

Figure B-3: Comparative Reactivation Kinetics of Soman-Inhibited Human Butyrylcholinesterase Single Mutant G117H (ϒ) and Double Mutant G117H/E197Q (●).

Note that the recovery rate of the double mutant is very fast (with reaction rates of 77,000 and 128,000 per minute for the P_sC_s and P_sC_R isomers of soman, respectively), while the single mutant does not recover measurably. The insert shows that reactivation of the double mutant with soman can be treated as a first order reaction for at least 2.5 x 10^3 sec.

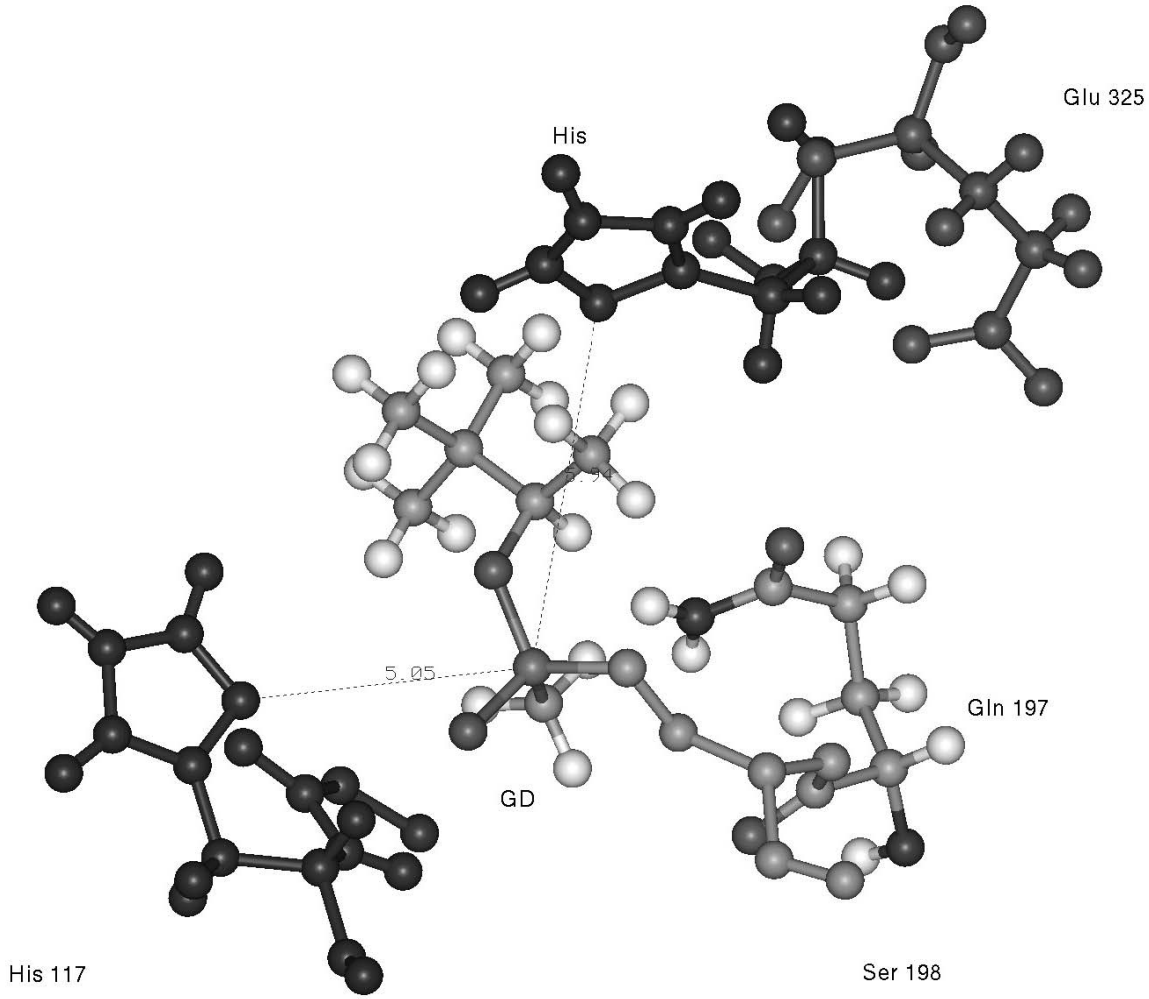

Figure B-4: A Ball and Stick Computer Model of the Active Site of the Double Mutant of Butyrylcholinesterase G117H/E197Q.

In addition to the His 117 and Gln 197, the active site triad amino acid residues of His 438, Ser 198 and Glu 325 are also depicted with soman at the active site. The distances between the phosphorus atom of soman and His 117 is 5.05 Å and distance between the phosphorus atom of soman and the active site His 438 is 5.94 Å.

Table B-7: Kinetic Properties of Catalytic Mutated BuChE Bioscavengers

Bioscavenger	Substrate Specificity	Spontaneous Reactivation Rate Constant (x 10^3 min^{-1})[a]	Reference
Wild type BuChE	GB	< 0.05	[100]
Wild type BuChE	VX	< 0.05	[100]
Wild type BuChE	GD	< 0.05	[100]
G117H BuChE [b]	GB	5	[100]
G117H BuChE	VX	7	[100]
G117H BuChE	GD	< 0.05	[100]
G117H E197Q BuChE [c]	GB	62	[100]
G117H E197Q HuBuChE	VX	78	[100]
G117H E197Q HuBuChE	GD	6	[100]
G117H E197Q HuBuChE	GD	6	[100]
G117H E197Q HuBuChE	GD	77	[100]
G117H E197Q HuBuChE	GD	128	[100]

[a] The rate-limiting step in the hydrolysis of organophosphate nerve agents by mutated HuBuChEs is the enzyme reactivation step [100].

[b] A version of BuChE in which the glycine at amino acid residue 117 has been replaced by histidine.

[c] A double mutant of BuChE containing both histidine (rather than glycine) at amino acid residue 117 and glutamine in place of glutamic acid at residue 197.

[d] The reactivity with each of the four stereoisomers of GD was determined independently.

Finally, through the careful design and synthesis of transition state analogs of the hydrolysis of soman, it has been possible to immunize mice and recover hybridomas whose antibodies display slow catalytic activity (Table B-8) towards soman [35, 36, 101]. Such catalytic antibodies could be “humanized” to reduce their immunologic antigenicity [102], thereby prolonging their serum half-life into the range of days to weeks, as reported for other mammalian species [103]. While most of these catalytic enzymes and antibodies have not yet been tested in mammalian systems, they are indicative of the types of drugs that may soon be available for use in animals, including humans. Since mutated BuChE, CaE and Pon are based on human proteins, and catalytic antibodies can be rendered predominantly human in structure, the expectation is that these proteins would have no immunological or behavioral side effects.

Table B-8: Kinetic Properties of Mouse Derived Catalytic Antibody Bioscavengers

Bioscavenger	Substrate Specificity	Km (μM)	Vmax ($nmol \bullet min^{-1} \bullet mg^{-1}$)	Reference
Antibody IIA12-ID10	GD, others?	330	25	[36]
Antibody DB-108Q	GD, others?	110	16	[37]
Antibody DB-108P	GD, others?	100	53	[37]

B.6 BEHAVIORAL EFFECTS

Since overt signs, symptoms or physiological responses may not accompany many low-level exposures, behavioral toxicological measures may be chosen to detect any toxic changes wrought. Under such conditions, it is important to ensure that biological scavengers, either elevated levels of naturally occurring proteins or mutagenized forms thereof, do not elicit behavioral effects of their own after administration. Other considerations are potential behavioral effects that might result after pre-treatment with a biological scavenger followed by exposure to a nerve agent, as well as a comparison of the extent of behavioral side effects that ensue from pre-treatment with scavenger followed by nerve agent exposure versus exposure to nerve agent followed by conventional therapy. The discussion here will be limited only to the side effects, if any, resulting from administration of scavengers alone. The other topics, including the ability of scavengers to ameliorate behavioral side effects following nerve agent exposure and the advantages of scavengers versus conventional therapy are discussed in detail elsewhere [104].

B.7 BEHAVIORAL EFFECTS OF SCAVENGERS ALONE

Most studies that have examined the behavioral effects of biological scavengers have done so by comparing a behavior before scavenger administration, after scavenger administration, and then after exposure to nerve agent [see 104]. There are, however, several studies that have examined the behavioral effects of the biological scavengers themselves in the absence of cholinesterase inhibitors. In a study by Genovese and Doctor, rats were trained to perform three behavioral paradigms: a passive avoidance task, a motor activity and a scheduled-controlled behavior (Table B-9) [105]. The performance of animals before and after administration of purified eq-BuChE at a dose that would be expected to provide protection against an exposure of several LD_{50}s of an organophosphorus compound was assessed. They determined the pharmacokinetic profile of eq-BuChE in rats and then examined the behavior of the animals in the passive avoidance task when the levels of administered eq-BuChE were maximal. Subsequently, the animals were tested after enzyme levels had started to diminish, to enhance the opportunity of detecting any behavioral effects. During the activity tests, individually housed animals were allowed to habituate. Enzyme was given such that maximum levels would be present in circulation about one hour before the beginning of dark cycle. Motor activity was then monitored for ten days. As a final test, the effects of excess enzyme were examined in rats trained to perform a VI56 s schedule of food reinforcement. Previously, cholinergic compounds had been shown to disrupt performance of this task. Animals were observed for ten days to ensure that any prolonged or delayed effects would be noted. In all cases for all test paradigms, the authors report that eq-BuChE did not disrupt performance of any of the learned tasks, did not upset the circadian cycle of light/dark activity and had no effect on motor activity. They noted that these outcomes were in contrast to those observed when the standard cholinolytic, atropine, was administered. Finally, they evaluated the protective effects of the levels of enzyme given to the rats in the behavioral studies against

MEPQ, a peripherally active organophosphorus compound. While the level of protection observed was lower than the theoretical prediction, the authors suggested that the simultaneous administration of scavenger and MEPQ might have reduced the efficacy of the administered eq-BuChE.

Table B-9: Extent of Behavioral Deficits Following Bioscavenger Administration or Conventional Therapy

Bioscavenger	Species	Behavioral Test(s)	Impairment[a]	Recovery Time[b]	Reference
Atropine	Rat	Passive Avoidance, VI56 s Schedule	Total	> 1 Week	[105]
eq-BuChE	Rat	Passive Avoidance, Motor Activity, VI56 s Schedule	None	Immediate	[105]
BuChE	Rat	Morris Water Maze	None	Immediate	[107]
Pyridostigmine	Rhesus Monkey	Primate Equilibrium Platform (PEP)	Substantial	N.D.[c]	[113]
eq-BuChE	Rhesus Monkey	Serial Probe Recognition (SPR)	None	Immediate	[106]
eq-BuChE	Rhesus Monkey	Observation, SPR	Subtle SPR Defect	~6 Days	[114]
eq-BuChE	Rhesus Monkey	Observation, SPR	None	Immediate	[106]
BuChE	Rhesus Monkey	Spatial Discrimination	Minor (1/4 had errors)	> 1 day	[85]

[a] Behavioral impairment relative to untreated animals.

[b] Time elapsed before performance returns to pre-treatment levels.

[c] Not determined.

In a separate study also using eq-BuChE, rhesus monkeys were trained to perform a SPR task [106]. Using a six-object list, the monkeys were tested for same-different discrimination and delayed same-different discrimination. Once the animals became proficient at the task (80% correct for three successive sessions on three consecutive days) they received eq-BuChE in a dose similar to that reported by Broomfield et al. as sufficient to afford protection against 2 or 3 multiples of an LD_{50} soman challenge (*vide supra*) [63]. The authors reported that in their study, repeated administration of commercially prepared eq-BuChE had no effect on the behavior of the monkeys as measured by the SPR studies (Table B-9). Given the lack of behavioral effects and the relatively long *in vivo* half-life of the eq-BuChE, they concluded that this biological scavenger was potentially more effective than current chemotherapeutic treatments for organophosphorus intoxication. Other studies in rodents or monkeys using human BuChE also showed virtually no behavioral effects following administration of this enzyme [85, 106-107]. In particular, it has recently been reported that administration of high doses (90 mg/kg, i.e., a dose 30-fold higher than that necessary for protection of 2 LD_{50} of soman in humans) of BuChE to mice induced no toxic behavioral effects [108].

B.8 SUMMARY

Organophsophorus nerve agents represent a very real threat not only to warfighters in the field but also to the public at large [109]. Nerve agents have already been used by terrorist groups against a civilian population and, due to their low cost and relative ease of synthesis, are likely to be used again in the future [110]. In addition, many commonly used pesticides and chemical manufacturing by-products can act as anticholinesterases, and may be a low-dose exposure threat to workers in a variety of professions. Also, we cannot rule out the possibility of the use of anticholinesterase pesticides against civilians in a terrorist context. Current therapeutic regimes for acute nerve agent exposure are generally effective at preventing fatalities if administered in an appropriate time frame. For acute multi-LD_{50} levels of exposure, pyridostigmine pre-treatment coupled with post-exposure administration of an oxime, atropine, and an anti-convulsant does not prevent the substantial behavioral incapacitation or, in some cases, permanent brain damage that can result from organophosphorus poisoning although it does enhance survival. It is therefore important from both military and domestic security perspectives to develop novel defenses against nerve agents, including the use of bioscavenger molecules that avoid many of the difficulties associated with current treatments. While the use of nerve agents on the battlefield may be somewhat predictable, their use in a terrorist situation will be, in all probability, an unanticipatable event. The ability to afford long-term protection for first-responders exposed to toxic, or incapacitating concentrations of OPs is a notable potential advantage of biological scavengers.

The use of bioscavengers as a defense against OP intoxication has many advantages and few apparent disadvantages. As discussed in detail above, bioscavengers can afford protection against not only mortality, but also most or all of the adverse physiological and behavioral effects of nerve agent exposure. They can be administered prophylactically, precluding the need for immediate post-exposure treatment. In addition, the use of bioscavengers has several psychological benefits that are likely to result in a higher degree of user acceptability than exists for conventional therapy. No post-exposure auto-injectors are necessary, and protection is afforded with little chance of short- or long-term side effects. Of particular significance is the fact that current candidate bioscavenger proteins are, for the most part, enzymes of human origin. From a scientific standpoint, these proteins are good candidates because they are less likely to be recognized by cells of the immune system, and will enjoy prolonged residence times in circulation. From a user point of view, individuals are in essence being protected against nerve agents using a substance that their bodies already produce, rather than being injected with drugs and enzyme inhibitors that alone can produce potent side effects; such a distinction may enhance the comfort and compliance of end users.

There are several challenges that must be met in the future before bioscavengers can augment or replace the current therapeutic regimes for nerve agent intoxication. First, scavenger proteins, either alone or in combination, with a range of specificities that encompasses all known nerve agents must be defined. In one of the NATO countries (United States), BuChE is being produced in sufficient quantity to carry out safety and efficacy studies in guinea pigs and non-human primates. It is expected that the data from those studies will be used to prepare an Investigational New Drug filing with the US Food and Drug Administration to begin human safety testing. It is recognized that the immunogenicity and serum half-life of the scavenger(s) must be determined in humans, and efforts may be required to minimize the former and maximize the latter. Finally, appropriate dosages of scavenger(s) must be determined that will, based on animal models, protect against concentrations of nerve agents likely to be encountered under a wide range of scenarios. While the majority of the research to date has focused on stoichiometric scavengers, the use of either naturally occurring or genetically engineered enzymes with catalytic activity holds the greatest theoretical promise for the development of a broad specificity prophylactic scavenger. Future efforts are likely to focus on generating, characterizing and utilizing such enzymes in rodent and non-human primate models.

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Annex C – COMPLETE SCIENTIFIC PROGRAMS FOR THE TG-004 MEETINGS HELD FROM 1999 – 2005

Click on the links below to view the complete scientific programs:

1999 – Meeting held in Brussels (BEL) 22-26 March

2000 – Meeting held in The Hague (NLD) 1-5 October

2002 – Meeting held in Oslo (NOR) 4-7 November

2003 – Meeting held in Medicine Hat, Alberta (CAN) 29 September – 3 October

2005 – Meeting held in Hradec Králové (CZE) 23-26 May

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14. Abstract

The NATO HFM-041 / RTG Prophylaxis and Therapy Against Chemical Agents was active between 1999 and 2005. It was the primary forum for exchange of scientific information between NATO member countries on medical defence against chemical warfare threats. Five international scientific meetings were held to discuss progress in the development of new medical therapeutics. The topics discussed included the need for a new broader spectrum oxime and the development of a new anticonvulsant to replace diazepam. Biological scavengers as an approach to chemical medical defence became a topic of increasing scientific activity. That effort complimented the overall interest in shifting from medical treatment to prophylactic antidotes. Concerns regarding the use of sulfur mustard led to the identification of new classes of therapeutic compounds. Discussions focused on developing closer cooperation between the NATO member nations to foster interoperability in the development and utilization of new drugs for medical chemical defence. During this period, pyridostigmine bromide gained approval by the USFDA as a pre-treatment for soman poisoning and human butyrylcholinesterase was granted investigational new drug (IND) status by the FDA as a pre-treatment for nerve agent poisoning. Several joint international scientific projects were undertaken and completed.

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