APPENDIX JJ

INDEPENDENT CONSULTANT REPORT

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Persistent Health Effects of Pesticides and Other Chemicals Used in Desert Storm and Desert Shield:

Prepared for the Senate Subcommittee on Veterans Affairs

by

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Executive summary

This report reviews the known and potential health effects of several groups of chemicals used by U.S. forces during Desert Storm and Desert Shield. It will consist of a review of the literature on the acute and chronic health effects of the main groups of pesticides used during the conflict in the Gulf with discussion of the possible effects of pyridostigmine bromide and nerve gas and will discuss the limited literature on the possible interactions between PB and pesticides. The report addresses the practicality of field level measurements of acetylcholinesterase as a measure of exposure during combat situation and includes possible ways to improve the DOD's current approach. The report briefly addresses the appropriateness of the design of the DOD's Comprehensive Clinical Examination Protocol and the Department of Veterans Affairs Persian Gulf Health Registry and their potential for detecting health problems related to low level chemical exposures. Suggestions are made on how to potentially improve the data quality.

Several different types of pesticides were imported to the Persian Gulf and acquired locally by American forces during Desert Storm and Desert Shield. While use patterns of neither imported nor locally acquired pesticides are documented, the quantities of imported pesticides are documented. Most of the imported pesticides were insecticides or repellents. Pesticides are by nature poisons most of which affect the nervous system. The potential for long term health effects resulting from exposure to many of these chemicals has been demonstrated in numerous studies and case reports with the nervous system being

the principal focus of the majority of these reports.

The oganophosphates, a potent class of pesticides, appear to have been imported in large quantities. These chemicals have been clearly identified in many studies as a cause of both central and peripheral chronic neurological effects in persons who have sustained a heavy exposure (Keifer 1997, Rosenstock 1991, Steenland 1994, Savage 1988, McConnell 1994, Lotti 1986). It is important to note that nearly all cases of chronic neurological effects attributed to organophosphates resulted from overexposure which caused acute severe clinical illness. Most studies of subjects who have sustained less severe exposures or only chronic low level exposure have not observed these chronic neurological outcomes (Ames 1995, Fiedler 1997, Engel 1998).

One organophosphate, chlorpyrifos, which was shipped in large quantities (1580 gallons pure active ingredient, 3841 gallons of formulated product) and has been identified as capable of causing peripheral neuropathy in human beings following heavy exposure (Lotti 1986, Kaplan 1993), has recently come under careful scrutiny in the US because of its extremely broad use by both private citizens and pesticide applicators. The Health Effects Division of the Environmental Protection Agency reviewed the published literature and unpublished case reports and concluded that chlorpyrifos "may be a significant cause of chronic neurobehavioral effects". Unfortunately the report provided no exposure context in which these "chronic effects" might be expected to occur (Blondell 1997). A recent study of morbidity by investigators from the manufacturer of chlorpyrifos identified an elevated risk for five diagnostic categories among its employees exposed to chlorpyrifos: 1. diseases of the ear and mastoid process; 2. acute respiratory infections; 3. other diseases of the respiratory system; 4. general symptoms, signs, and ill defined conditions; and 5. symptoms, signs and ill defined conditions involving the digestive system. (Burns et al. 1998). The illness categories identified by these investigators as showing higher rates in exposed workers reflect a broad assortment of signs and symptoms but of particular interest is the inclusion of the general symptoms category (numbers 4, ICD9 780-799). The medical conditions included in this category are generally those that do not permit strict disease diagnosis by clinicians but interestingly this

symptom category is the same as the third most common diagnosis identified by the Comprehensive Clinical Evaluation Program (CCEP) in evaluating 20,000 Persian Gulf veterans (Joseph et al. 1997). This overlap of diagnosis between workers exposed in an industrial setting and personnel exposed during the Gulf War experience potentially to the same chemical is intriguing. However, it should be pointed out that the situations are not directly comparable. How this chemical was used by personnel in the Gulf is not clearly documented (IOM 1996) where as exposure to the chemical is estimated in the Burns study. Additionally the workers who were reporting these illnesses through the company medical program were presumably actively exposed at the time of their reported illnesses and the CCEP study group was examined and questioned at time when presumably exposure to chlorpyrifos had ceased. Before conclusions that an excess prevalence of this diagnostic category in the CCEP study population is reached an adequate control population would be needed. There was no association drawn in either the EPA report or the morbidity study between chlorpyrifos and peripheral neuropathy, a condition affecting 0.2% of 20,000 veterans examined by the CCEP (Joseph et al 1997).

The other organophosphate pesticides included in the list of imported pesticides include one, dichlorvos, which has been identified in animal models as an inducer of peripheral neuropathy. However this chemical as used in the Gulf was enclosed in pest strips making significant overexposure less likely. No reports were found in the literature that environmental exposure to these pest strips caused significant illness or peripheral neuropathy.

The N-methyl-carbamates were imported in large quantities and while sharing the acute toxicological characteristics of organophosphates, have only rarely been associated with persistent health effects, and then only after chronic heavy exposure (Ecobichon et al 1982). The carbamates are in the same family of chemicals as pyridostigmine, the drug used to prophylax personnel against nerve gas in the gulf. The pyrethroids, another category of pesticides, were brought over in large quantities, but are of relatively low acute toxicity and appear to be relatively safe pesticides (Aldridge 1990, He 1994).

Several other pesticides were brought over in significant quantity. Among theses Aluminum

phosphide, a fumigant, was potentially the most toxic. Brought over as chemical tablets, the aluminum phosphide produces phosphine gas when combined with water. Phosphine is a very toxic gas which can produce severe illness in the setting of sufficient exposure. The illness produced by phosphine exposure would not be easily overlooked (Morgan 1989). Furthermore, based on how aluminum phosphide is generally used it is highly unlikely that low dose exposure to phosphine occurred. There is no evidence in the literature that chronic illness results from low dose exposure to phosphine. The organochlorines pesticides (lindane, pentachlorophenol) the rodenticides, and roach poison (boric acid) are unlikely to have created significant hazard to Persian Gulf personnel in the absence of ingestion.

In the absence of massive overexposure, each of these pesticides by itself, organophosphates, n-methyl-carbamates, pyrethroids, phosphine, organochlorines, rodenticides and roach poison are not likely to have resulted in chronic health effects among even a substantial minority of U.S. troops.

Diethyl-m-toluamide (DEET) was imported in large quantities and presumably used widely as an insect repellent during the conflict. It is also widely used by the U.S. population in general and given its broad use (30+ % of the US population), the chemical has a reasonably good safety record (Veltri 1994). Case reports indicate that this chemical can induce central nervous system effects when absorbed in sufficient quantity but cases usually involve excessive exposure and often involve young children or infants. No reports in the literature describe the long term toxicity of DEET among humans with low level chronic exposure though some permanent residual effects have been noted in at least one case following recovery from what appeared to be an acute intoxication (Knowles 1992). The possibility that even relatively heavy exposure to DEET alone could induce chronic health effects in the Gulf personnel is unlikely.

Pyridostigmine bromide (PB), used by the U.S. forces as a prophylactic agent against the toxicity of nerve gas has demonstrable toxicity for both animal models and humans when given in relatively high dosage. The standard 30 mg three time per day dosage provided to U.S. forces may have caused acute toxicity in particularly susceptible populations such a asthmatics or soldiers with a unique serum

cholinesterase phenotype (Loewenstein-Lichtenstein 1995), or in soldiers who received high per weight dosage because of small body mass (Gouge 1994) but this dosage has been shown to be generally well tolerated by the majority of the population (Blick 1994, Borland 1985, Cook 1992, Glikson 1991).

Studies on animals suggests that under stressful situations the lack of central nervous system penetration which makes PB an attractive prophylactic may not be assured. This central nervous system penetration may lead to acute central nervous system symptoms. Symptom persistence resulting from this increased penetration has not been reported to date in human or animal models, although evidence from one study presented indicated that a central nervous system feedback mechanism may account for changes which may outlast the acute cholinergic effects of the drug (Freidman et al 1996).

No information was found as to whether the bromide in the preparation might have had deleterious effects given bromide's long half-life and the desert conditions of chronic high heat and salt depletion. Despite these caveats, the years of experience in treating patients for myasthenia gravis with PB at doses often much higher than those taken by Gulf War service personnel would suggest that the development of persistent health effects among Gulf War personnel from PB alone is unlikely. The pyridostigmine is rapidly metabolized and the bromide is excreted over several weeks once the drug administration is stopped. The penetration of the blood brain barrier by pyridostigmine under the stress of a combat situation may potentially result in acute effects given sufficient blood levels, but with metabolism of the drug and the reversal of the acute effects, it is unlikely that long term effects would ensue.

The health effects of exposure to nerve gases has been only periodically addressed in the mainstream literature. One excellent study which examined most of the important nerve gases for production of peripheral neuropathy showed that sarin was capable only at super-lethal doses of potentially inducing neuropathy (Gordon et al. 1983). Few cases of known human exposure to nerve gases are available to examine for long term effects, so predictions must be modeled mostly from animal experiments. The Center for Disease Control concluded in 1988 that there appeared to be little risk of adverse health effects from low level long-term exposure to GA, GB, VX, H, HD, HT or lewisite (CDC

1988). In a review of the literature on nerve agents, Gunderson et al. concluded that persistent effects such as psychological and behavioral problems, could result after acute exposure, but that no evidence supported persistent effects from low level exposure to these chemicals (Gunderson et al.1992). A recently published study on survivors of the Japanese subway sarin gas incidents identifies possible delayed effects on balance among surviving female victims. These authors also cite an as yet unpublished manuscript identifying neurobehavioral abnormalities among other victims 6-8 months after the poisoning (Yokoyama et al. 1998). These findings are consistent with problems identified among persons previously poisoned with organophosphate pesticides (Keifer et al. 1997, Steenland et al.1994, Rosenstock et al. 1991, McConnell et al. 1994, Savage et al.1980, Lotti et al. 1986), which are related to the military nerve gases. The literature does not provide evidence to support persistent neurological or other health effects from low-level exposure to nerve gases.

From the information presently available, it does not appear that the DOD has a policy for monitoring cholinesterase or for assessing the physiological effects of the prescribed standard prophylactic dose of pyridostigmine bromide. The broad application of cholinesterase monitoring for all those taking PB doses would probably not be beneficial. Most people taking the drug would probably have a very predictable response to the dosage. The drug generally appears to be safe when taken by individuals of average size (70 kg), with normal uninhibited cholinesterase activity and with no illnesses which would make them particularly susceptible to ill effects from the PB. However, there is a substantial minority of individuals who may be smaller in stature, have illnesses such as asthma or, in rare cases, have congenitally low cholinesterase which makes them sensitive to PB even when taken in the prescribed dose. A mechanism should be in place to identify those who might suffer ill effects and determine how their dosage should be adjusted in order to avoid complications while still providing protection from nerve gases.

While diagnosis of acute symptomatic overexposure to nerve gas or OPs generally does not present a diagnostic dilemma, and urinary and blood tests are available to confirm such acute episodes, low level exposure which results in mild or no illness requires a monitoring strategy. Cholinesterase

monitoring has long been used among pesticide applicators to identify overexposure to organophosphates. It also can potentially be used to identify personnel exposed to organophosphate nerve gas. At present, accurate interpretation requires a pre-exposure baseline on a subject against which to compare subsequent values. This limitation, and problems with the accuracy of commercially available test kits (Wilson 1997), makes cholinesterase testing complicated. Recently, a new approach to identifying overexposure to organophosphate nerve gas has been described. This method reactivates inhibited cholinesterase and reconstitutes the nerve gas molecule which can then be measured (Polhuijs et al. 1997). If this technique shows itself to be sound, it has potential application in determining whether personnel have sustained exposure to nerve gas even several weeks after exposure. This method should be explored for applicability for monitoring exposure to U.S. forces where cholinesterase inhibiting nerve gas exposure is possible.

The potential for chronic health effects resulting from mixtures of chemicals and from mixtures of pyridostigmine bromide and pesticides is a subject of interest and recent investigation, though relatively little has been published to date. Studies on laboratory animals have demonstrated that in sufficient dosage, damage to the nerves of the body can occur with mixtures of some of the chemicals used by service personnel in the Gulf War conflict (Abou donia 1996a & b). An important caveat to these studies is that the dosages used to induce these damages were well above what would have been expected to occur by regular use of these chemicals. Studies of the effects of DEET on the absorption of pyrethroids and carbaryl (an n-methyl-carbamate) do not support the contention that more chemical is absorbed in the presence of DEET (Baynes et al 1997).

The DOD CCEP and the VA PGR programs both appear to be well conceived and well organized based on the information reviewed. Several weaknesses in data quality control were identified and follow-up of ill participants for the purpose of evaluating long -term outcomes among Persian Gulf Veterans was identified by the GAO as a weakness in both programs. Also training of intake personnel does not appear to be contemplated and would be important for quality control of intake info for the VA PGR. Neither

system has an explicit mechanism for incorporating new test tools and data collection methods to explore new hypotheses which may develop within the programs or may be suggested from without. Some flexibility for testing of new hypotheses on subcohorts in each project would be beneficial.

Summary: A fair degree of uncertainty surrounds the exposures that may have occurred to personnel during Desert Shield and Desert Storm. Nevertheless, based on the information available in the literature regarding the pesticides and anti-personnel chemicals to which troops may have been exposed in the GW, chronic health effects would not be expected in any significant number due to low level exposure to these chemicals or to combinations of these chemicals. A small percentage of the population may have had reactions to these chemicals not predicted by animal research or human studies and given exposure sufficient to result in acute toxicity, chronic problems would not be surprising. Information sited in this report does raise questions about the possible non-specific symptoms reported by a substantial percentage of CCEP subjects and how this might relate to pesticide exposures which occurred in GW personnel. This relationship is uncertain but intriguing. The use of PB by the gulf war personnel would probably not cause significant illness in most individuals but might cause problems in some with small stature, asthma or unique biochemistry. The two greatest limitations in identifying illness due to exposures in a theatre of war are the virtual absence of exposure information and the difficulty of evaluating the health status of a self-selected group. In future conflicts, better collection of exposure information and prospective follow-up of a statistically valid sample of the combatant population with an appropriate non-combatant control group would facilitate the identification and characterization of emerging illnesses.

The DOD CCEP and VA PGR programs appear sound but would benefit from greater quality control mechanisms and systematized case follow-up and some flexibility options for incorporating new hypotheses into the data gathering.

Recommendations

A sincere and scientifically valid effort to explore and address health concerns of veterans from military conflicts is an extremely important responsibility that our government has toward its veterans. But communicating in an open, non-defensive manner with the concerned service personnel and the public about the state of knowledge and the progress of knowledge is potentially the greatest challenge facing the Department of Defense and the Veterans Administration with regard to issues of post conflict health of veterans. While the health problems from which Gulf War veterans suffer may never be completely ascribed with certainty to specific exposures that occurred during service in the Gulf, the challenge of identifying, and caring for the health of veteran's and responding to the health concerns of veterans will continue as long as there are veterans. Effective risk communication is essential to maintaining and optimized three way dialog between the veteran-active duty community, the citizenry and the responsible government branches.

Specific Recommendations

This author can not substantially improve on the scientific comprehensiveness of the recommendations made by the Institute of Medicine on improving the surveillance and monitoring capabilities of the DoD regarding health effects of combat service (Institute of Medicine, IOM, 1996). I do believe it is important to add that the IOM report fails to recommend a mechanism whereby the veterans, the U.S. public and active duty personnel might participate in the functioning of an ongoing system of health outcomes monitoring. Potentially the most important predictor of success of this program as judged by these constituencies is the degree to which they can claim ownership of the process. I strongly encourage that a mechanism be established to assure active participation by representatives of the U.S. public, veterans groups and active duty personnel of varied ranks and branches in the design and conduct of any program that is adopted. A mechanism should also be established to regularly communicate with all veterans providing them with ongoing information about new developments and knowledge regarding the

effect of service and health.

Research in basic and applied science

Support for further research on technology for detecting environmental release and personal exposure to war gases should be a particular emphasis of the DOD. Monitors should be developed that are portable, collect and report real time information, and have data storage capabilities and are easily applied by combatants.

Research should be undertaken to develop profiles of individuals who may potentially suffer untoward effects from war gas antidotes (e.g. asthmatics, smaller individuals). Those individuals should have personal drug dosing profiles developed and confirmed by cholinesterase activity levels appropriate to the prophylactic medication taken. Routine cholinesterase testing of all personnel is probably not warranted, but the test should be available on a routine basis for evaluating ill combatants both for overdose of prophylactic medication and for evaluating war gas exposure.

A new technique described by Polhuijs (1997) potentially represents a very significant breakthrough in the detection of cholinesterase inhibited by the nerve gas sarin. Whether this technique is applicable to other nerve gases and pesticides has not been demonstrated to date but suggestions by the author are that the procedure would be applicable to organophosphate pesticides as well. This technique should be explored and amplified if possible for application to exposure assessment of subjects potentially exposed to nerve gases and pesticides.

Organization of this report

The pesticides which are known to have been employed by the American Forces are identified and grouped by chemical category and the general toxicological characteristics of the group are reviewed. The approximate amount of the active ingredient brought over to the Persian Gulf by American forces is estimated based on the a report provided by the DOD on Aug 27, 1997 to Senator A. Specter. The

estimates are included in Table 1. The chemicals are each addressed in groups with their initial acute health effects presented followed by discussion of their potential for prolonged effects. Nerve gases are also reviewed for acute and prolonged toxicity. The potential effects of mixtures of chemicals is discussed. A brief discussion of the Department of Defense Comprehensive Clinical Evaluation Program and the Veterans Administration Persian Gulf Registry is presented and final recommendations are made. A discussion of the appropriateness of cholinesterase monitoring by the DOD and alternative biomonitoring approaches is included.

Table 1. Pesticide categories, names and amount of active ingredient brought by U.S. forces to the Persian Gulf

Pesticide Type	Pesticide name	active ingredient brought to the PG
Organophosphate	Chlorpyrifos	4690.19 gallons
Organophosphate	Diazinon	108.65 gallons
Organophosphate	Malathion	4665.07 gallons
Organophosphate	Dichlorvos	25200 pest strips
Organochlorine	Lindane	111199.00 units
Organochlorine	Pentachlorophenol	20 units
Pyrethroid	D-trans-allethrin	3.84 gallons
Pyrethroid	allethrin	9 gallons
Pyrethroid	Resmethrin	5.12 gallons
Pyrethroid	D-phenothrin	9 gallons
Pyrethroid	Pyrethrin	675 gallons 19.69 units
Pyrethroid	Permethrin	36813.94 gallons
Repellent	N,N'-Diethyl-M-Toluamide	7657.1 gallons
Repellent	Benzocaine	gallons
Repellent	Sulfur	10.14 gallons
N-methyl-carbamate	Propoxur	16.91 pounds
N-methyl-carbamate	Carbaryl	254 pounds
N-methyl-carbamate	Bediocarb	11.4 pounds
N-methyl-carbamate	Methomyl	916 pounds
Anticoagulant rodenticide	Dipacinone	10 blocks
Anticoagulant rodenticide	Warfarin	25490 pounds
Anticoagulant rodenticide	Bromadiolone	8954 cans
Anticoagulant rodenticide	Pindone	31 pounds
Anticoagulant rodenticide	2-sovalery-1,3-indandione	31 pounds
Roach killer/bait	Boric acid	168 pounds
Roach killer/bait	Amidinohydrazone	960 bait stations
Fumigant	Aluminum phosphide	10956 tablets

Organophosphates (OP)

Chemicals: chlorpyrifos, diazinon, dichlorvos, malathion

Acute toxicity of organophosphates

Potentially the most important group of pesticides identified by the DOD as having been used during Desert Storm and Desert Shield with respect to human health effects are the organophosphates. The organophosphates gain this prominence due the frequency with which they are responsible for human pesticide poisonings and their ubiquity as insecticides. The organophosphates are a broad group of

chemicals with a common mechanism of acute toxicity which they share with the n-methyl-carbamates, another important group of insecticides, and many of the nerve gases. This mechanism of toxicity is the inhibition of acetylcholinesterase. Acetylcholinesterase, abbreviated AChE, is an essential enzyme in the function of the nervous system. The enzyme serves to metabolize acetylcholine, the chemical messenger which crosses the small space, called the "synaptic space" between many nerve cells and other nerve cells, nerve cells and glands, and nerve cells and muscle cells. Figure 1 below presents an enlarged view of the contact point between a nerve ending and a muscle cell. All nerves which depend on this chemical messenger are called cholinergic. When an impulse reaches the "nerve ending", the "packets of acetylcholine" fuse with the membrane of the nerve ending. This causes release of the contained acetylcholine into the "synaptic space". Acetylcholine diffuses across the space and binds to specialized receptor molecules on the "endplate folds". The presence of acetylcholine on these receptors causes a change in the conformation of the receptors and ions are permitted to flow through small pores within the molecules into the interior of the cell. If enough acetylcholine crosses the synapse and enough flow of ions is permitted the endplate depolarizes. This means that the charge of the cells interior reverses suddenly. When this happens the depolarization spreads to the entire cell. If depolarization occurs in a nerve cell, the impulse is carried on to the next nerve or other tissue. If it happens in a gland, such as the salivary glands, the gland secretes. If it happens in a muscle, the muscle contracts. The enzyme acetylcholinesterase is strategically placed in the endplate folds so as to metabolize acetylcholine to acetic acid and choline and inactivate it as a chemical messenger. This allows the endplate and the cell to return to its resting state and to stop functioning.

Figure 1. Nerve Ending Interfacing with Effector Tissue

When a human is exposed to sufficient organophosphate pesticide, phosphorylation of the active site of the acetylcholinesterase enzyme occurs. When sufficient acetylcholinesterase is inhibited acetylcholine builds up at the effector tissue synapse. The result is over-stimulation of the effector organ or tissue. This over-stimulation results in the problems characteristic of acetylcholinesterase inhibition. These problems include over-secretion of glands resulting in excessive salivation, and thick and abundant secretions from the mucous glands in the lungs; contraction of voluntary muscle (the muscles that we control voluntarily such as arms legs and including diaphragm). The latter results in weakness, fatigue and paralysis, and difficulty breathing. This over-stimulation effects the central nervous system resulting in lightheadedness, confusion and if severe enough, convulsions and coma with arrest of breathing. The over stimulation of the peripheral nervous system can result in a slow heart rate with conduction abnormalities of the heart, and over stimulation of the involuntary muscle of the gut results in cramping and sometimes diarrhea. The effects on the gut and the brain combine to induce nausea and sometimes vomiting (Namba 1971). In most cases recovery of the acetylcholinesterase enzyme activity occurs as the inhibited enzyme is replaced by new enzyme. This occurs at an estimated 1% per day. Acute symptoms generally abate relatively soon after the poisoning even before all the acetylcholinesterase is replaced. If death does not occur, recovery from all effects is the rule. Below are presented some persistent health effects which have been identified in some individuals following an acute intoxication.

Prolonged effects of organophosphates

In dealing with the health problems following exposure to organophosphates one must make the distinction between the type of problem, persistent or transient and the nature of the exposure, acute intoxication or chronic non-acutely intoxicating. There do exist four distinct moderately well characterized

medical conditions which may follow an acute OP overexposure. The first is the acute intoxication stage representing cholinergic crisis while the toxin is still present in the body and interfering with the function of the nervous system as described above. Following recovery from the acute phase several other syndromes or complexes of symptoms have been identified. These include post intoxication paralysis, referred to as the "intermediate syndrome"; given the appropriate chemical the organophosphate induced delayed polyneuropathy (OPIDP); and sometimes the less well characterized post-OP intoxication myopathy. These three complexes of sequelae are more the exception than the rule in recovering patients but among a sizable percentage other persistent symptoms may occur which include somatic, psychiatric, central and or peripheral nervous system complaints that do not fit well into the three syndromes described above (Whorton 1983, Tabershaw 1966). Several studies have focused on these symptoms and attempted to characterized the neurophysiological and neuropsychological abnormalities which appear to be associated with them. Slightly fewer studies have examined problems following ongoing or the cessation of chronic non-intoxicating exposures to organophosphates and the results of these studies are more mixed. Several case reports describe asthma, which may be prolonged or pancreatitis, which is generally short lived in connection with OP exposure.

Organophosphate Induced Delayed Polyneuropathy (OPIDP)

Probably the best characterized persistent condition following poisoning by OPs is organophosphate induced delayed peripheral neuropathy (Hierons 1978). This is a condition in which the peripheral nerves die back from distal to proximal direction. While sensory complaints are common at the onset of the condition, the motor nerves are primarily affected and the condition can result in permanent weakness of the distal muscles if severe. Recovery may be possible in less severe cases. The long tracks of the spinal cord may also be involved resulting in a combined spastic and peripheral neuropathic condition. The first signs of the condition begin 10-14 days after the acute OP intoxication with pain and parasthesias in the lower extremities. A marker for the potential neurotoxic effect of OPs has been

identified and is referred to as neuropathy target esterase. Those pesticides identified as causing this condition are capable of irreversibly inhibiting NTE (Lotti 1986). One important note is that the OPIDP has only been identified in association with severe OP pesticide intoxication and only a small group of OPs have been associated with the development of the full blown syndrome. This appears to be due to a threshold level of inhibition of the NTE necessary for the pathological changes in the nerve cells to proceed and the specific ability of certain OPs to age the NTE (Lotti 1986, Johnson 1980). Among the pesticides used during Dessert shield and Desert storm, chlorpyrifos (Lotti 1986) and malathion (Argiles 1990) have been identified as capable of inducing this syndrome in heavily overexposed individuals and dichlorvos (DDVP) has been shown in experimental animals to be capable of inducing the same problem (Johnson 1981). A review of the literature revealed no reports to date of diazinon producing peripheral neuropathy either in animal models or in humans.

While OPIDP from organophosphate pesticides has only been described in severe intoxications with specific organophosphates, there have been mixed results from studies of the peripheral nervous system of workers with chronic exposure to organophosphates or with acute overexposure to organophosphates previously thought not to be neurotoxic. McConnell (1992) and colleagues showed marked differences in peripheral vibratory sensation among individuals who had suffered acute overexposure to pesticides not previously identified as causing OPIDP. In a controlled cross-sectional study of 36 workers previously hospitalized for poisonings with organophosphates, vibration perception threshold (indicating reduced vibration perception) was elevated as compared to controls who had not suffered an acute medically treated intoxication. The differences were most marked between subjects who had suffered exposure to a known peripheral neurotoxic organophosphate, methamidafos, but were also apparent among workers who had been poisoned with pesticides previously unknown as neurotoxins. In another study of previously poisoned workers, Steenland showed that having suffered an acute organophosphate pesticide poisoning was a significant predictor of increasing vibration threshold and slowing of peripheral nerve conduction (1994). Two studies which sought signs of neuropathic effects

from chronic exposure to pesticides without acute intoxication have not found differences between exposed and unexposed but several other studies have found differences. Ames studied 40 workers who had suffered sufficient overexposure to organophosphates to demonstrate either a 30% drop on acetylcholinesterase or a 40 % drop in plasma cholinesterase (another form of cholinesterase found in the blood). He compared these workers after recovery to 90 non-poisoned controls and did not find any differences in peripheral nervous system function (Ames 1995). Engel et al. compared 67 Hispanic farm workers with chronic exposure to principally organophosphate pesticides from agricultural foliar residues to 68 unexposed controls. No significant differences were detectable in nerve conduction of sensory or motor nerves nor in repetitive stimulation electromyography (Engel 1998). Two other studies did show some effects on the peripheral nervous system which appeared to correlate with exposure to organophosphate pesticide use. In a study of postural sway, measured to reflect proprioception among pesticide applicators, Sack (1993) found that an increase in postural sway was associated with work as a pesticide applicator. The implications of this finding were said by the authors to not be clear as they also reported that peripheral nervous system function as measured by conduction velocities did not show an association with exposure category. In a study of vibration threshold of pesticide applicators in New York, Stokes et. al. showed increased vibration threshold among pesticide applicators when compared to randomly selected controls (Stokes 1995). These changes were associated with having handled pesticides on a long term basis.

Recently, combinations of organophosphates and other chemicals given in a specified order to experimental animals appear to have consequences not seen if either of the single chemicals are given alone at the same concentrations. A combination of phenylmethanesulfonyl fluoride (PMSF) and neurotoxic organophosphates (Moretto 1992 , Randall 1997) has been identified as facilitating the development of peripheral neuropathy at lower doses of the organophosphate than previously thought necessary. The disorder appears to be the same as OPIDP and the mechanism is thought to be the same as that for OPIDP with inhibition of NTE being the essential first step. The addition of a second chemical

which also specifically interacts with NTE (though not irreversibly inhibiting it) is thought to be a second essential step (Randall 1997). The importance of this finding is twofold. Firstly evidence now exists for the facilitation of peripheral neuropathy at lower doses of neurotoxic organophosphate pesticide than previously thought necessary. Secondly and importantly, in this model, a specific relationship does exist between the two chemicals responsible and the nerve cell enzyme essential in the development of OPID. Both chemicals inhibit NTE, albeit differently. This is important from the perspective of specificity of effects. It is likely that a specific sequential combination of inhibitors is necessary for this unique instance of the development of OPIDN.

Intermediate syndrome: Short duration paralysis following acute intoxication

Several investigators have reported a paralytic syndrome following severe acute intoxication with organophosphate pesticides which has been referred to as the "intermediate syndrome" (Senanayake 1987 Karademir 1990). The syndrome is characterized by diffuse weakness, greatest in the trunk muscles. If sufficiently severe, respiratory paralysis may ensue and mechanical ventilation may be needed to sustain life. The syndrome onset is generally within a few days of the onset of acute symptoms of intoxication and may last several days to weeks. In a prospective study of newly diagnosed organophosphate intoxications 8 of 19 went on to develop intermediate syndrome. The results of this study effectively reverse what had been the accepted belief that "intermediate syndrome" is a rare sequelae to OP intoxication. Recovery appears to be complete if other complications of the intoxication do not ensue. The syndrome has only been described in association with a few organophosphate pesticides and appears to be unrelated to the pesticides ability to produce OPIDN (de Bleeker 1992,1993). Like the OPIDN the syndrome has not been found to occur with low level exposure to OPs.

Organophosphate induced myopathy

This is a well characterized syndrome in experimental animals but relatively little has been written about its occurrence in humans following acute intoxication (de Bleeker 1994, de Bleeker 1992, Gupta 1992). The syndrome appears to result from overstimulation of the muscle by the excess of acetylcholine resulting from the inhibition by organophosphates. Substantial work on this subject has been done in relation to pyridostigmine bromide and is presented below. Damage to voluntary muscles resulting from overexposure to organophosphates could potentially explain the prolonged weakness described by some investigators as persisting beyond the acute intoxication (Whorton 1983).

Prolonged central nervous system effects of organophosphates:

Several studies which have examined patients after recovery from acute overexposure to organophosphates have documented the existence of persistent differences in neuropsychological performance and subjective symptoms (Tabershaw 1966, Whorton 1983, Savage 1982, Rosenstock 1991, Steenland 1994). The earliest observations on this phenomena come from physicians attending to patients recovering from acute organophosphate intoxications which were common with the introduction of these chemicals to agriculture. Reports by several authors who attended acute intoxications ponder the persistence of symptoms after recovery from acute intoxications (Holmes and Gaon 1956, Hirshberg and Lehrman 1984).

Based on the impetus of these early observations several studies have examined individuals with organophosphate pesticide exposure for central nervous system changes both following recovery from acute overexposure and during ongoing chronic exposure. In a survey of over 100 subjects previously poisoned with organophosphate pesticides a substantial percentage complained of persistent symptoms they felt originated with their poisoning (Tabershaw 1966). In another large study conducted in the U.S. comparing subjects with histories of acute organophosphate pesticide poisonings to controls, approximately 25% of the previously poisoned subjects were found to be functioning in the "impaired" range based on a broad neurobehavioral test battery. This finding was evident despite the fact that testing

was done on average, about a decade after the acute intoxication. Both cognitive tests, and measures of fine motor coordination, were significantly impaired relative to control subjects (Savage 1982). In a study of 36 Nicaraguan agricultural workers it was shown again that even single episodes of acute medically treated organophosphate intoxication may lead to chronic neuropsychological dysfunction (Rosenstock 1991). The study applied a battery of neurobehavioral tests on average two years after an acute intoxication. Significant cognitive impairments in the poisoned group were present when compared to the performance of an age and socioeconomically matched control group. Verbal and visual attention, visuomotor speed, visual memory, sequencing, and problem solving were notably different between the groups. A study of individuals who had been reported to the California pesticide poisoning registry, persistent central nervous system effects appeared to be present after recovery from acute organophosphate poisoning (Steenland 1994). Tests of both visual attention and mood were significantly worse in the previously poisoned group.

In a review of the nervous system effects of pesticides, Keifer and Mihuran note that personality changes are also commonly reported following exposure to organophosphates with symptoms such as anxiety, restlessness, inner "tension", and apprehension. (Keifer 1997, Ecobichon 1982, Levin 1974, Levin 1976).

Prolonged effects of long-term low level exposure to organophosphates

Animal experiments suggest that chronic exposure to organophosphate pesticides may lead to changes in central nervous system function, particularly impaired spatial learning (Wolthius 1984, Uphurch 1987, McDonald 1988). Less evidence is available from well conducted human studies to support this contention. The earliest studies of chronic exposure to organophosphate exposure used either clinically based standardized test batteries or electroencephalographic analysis. Korsak (1977) examined workers with chronic exposure to both organophosphate and orgnanochlorine pesticides and found worse performance on two of several neurobehavioral tests. Metcalf found abnormalities in EEGs, visual memory and an increase in "soft" neurological signs among organophosphate exposed workers but the

absence of clear methods, exposure criteria and comparisons between exposed and controls makes the study difficult to interpret (Metcalf 1969). A study of pesticide applicators conducted while exposure was ongoing demonstrated no significant neurobehavioral effects except for increased anxiety in the exposed group (Levin 1976). Three recent studies come to differing conclusions regarding neuropsychological effects of chronic exposure to organophosphates. An investigation of organophosphate exposed sheep-dippers in Wales examined subjects for chronic neuropsychological effects and found significant differences in neuropsychological performance on tests of sustained attention, and speed of information processing after several months away from exposure (Stephens 1995). This suggests that chronic exposure to organophosphates may result in persistent alterations in some neurobehavioral functions. In contrast, a study of pesticide applicators in Washington State showed no significant persistent effect on neurobehavioral function after exposure had ceased for a season (Daniell 1992), and a study of life long orchardists compared to age, and education matched controls found the only significant difference to be a reduction in dominant hand reaction time among the exposed members of the study (Fiedler 1997). These studies suggest that the effects found by Stephens may be due to a characteristic of either the exposure or the subjects not shared with Fiedler's and Daniell's study.

Recently the Health Effects Division of the Environmental Protection Agency reviewed the published literature and unpublished case reports on chlorpyrifos a very widely used organophosphate pesticide and one that was used extensively by the U.S. forces in the Desert Storm/Desert Shield and concluded that chlorpyrifos "may be a significant cause of chronic neurobehavioral effects". This report examined numerous case reports and pesticide poisoning registries looking at the health effects of chlorpyrifos. The report identified several acute pesticide poisonings as a consequence of chlorpyrifos overexposure but also identified "chronic effects" as occurring in a number of individuals as a result of exposure. Unfortunately the report provided no exposure context in which these "chronic effects" might be expected to occur (Blondell 1997). A recent study of morbidity among employees exposed to chlorpyrifos by investigators from the manufacturer of the chemical identified an elevated risk for five diagnostic

categories: 1. diseases of the ear and mastoid process; 2. acute respiratory infections; 3. other diseases of the respiratory system; 4. general symptoms, signs, and ill defined conditions; and 5. symptoms, signs and ill defined conditions involving the digestive system. (Burns et al. 1998). The illness categories identified by these investigators as showing higher rates in exposed workers reflect a broad assortment of signs and symptoms but of particular interest is the inclusion of the general symptoms category (numbers 4, ICD9 780-799). The medical conditions included in this category are generally those that do not permit strict disease diagnosis by clinicians but interestingly this symptom category is the same as the third most common diagnosis identified by the Comprehensive Clinical Evaluation Program (CCEP) in evaluating 20,000 Persian Gulf veterans (Joseph et al. 1997). This overlap of diagnosis between workers exposed in an industrial setting and personnel exposed during the Gulf War experience potentially to the same chemical is intriguing. However, it should be pointed out that the situations are not directly comparable. How this chemical was used by personnel in the Gulf is not clearly documented (IOM 1996) where as exposure to the chemical is estimated in the Burns study. Additionally the workers who were reporting these illnesses through the company medical program were presumably actively exposed at the time of their reported illnesses and the CCEP study group was examined and questioned at time when presumably exposure to chlorpyrifos had ceased. Before conclusions that an excess prevalence of this diagnostic category in the CCEP study population is reached an adequate control population would be needed. There was no association drawn in either the EPA report or the morbidity study between chlorpyrifos and peripheral neuropathy, a condition affecting 0.2% of 20,000 veterans examined by the CCEP (Joseph et al 1997).

Other persistent health effects of organophosphates

Exposure to organophosphate pesticides has been linked to the development of asthma in a minority of reports (Bryant 1985) . Also there have been reports of pancreatitis in association with organophosphate exposure (Marsh 1988). Pesticides in generally, and specifically the organophosphates

have been associated with the development of several cancers (Zahm 1997) and pesticides in general have been linked in studies to decreased measures of immunity (Reppetto 1992) . Though no reports yet have linked pesticides to frank immunosuppresion resulting in associated diseases.

The literature suggests that prolonged health effects may result from exposure to organophosphate pesticides. These prolonged effects generally follow upon an acute intoxication with these chemicals and whether low level exposure even for a prolonged duration can cause health problems is less clear. Given the exposure that was likely to have been sustained by PG personnel, the development of persistent health problems resulting from OPs alone in the absence of acute intoxication is unlikely.

N-methyl-Carbamate pesticides

Propoxur, Bendiocarb, Carbaryl, Methomyl

Acute toxicity of N-methyl-carbamates

N-methyl carbamate insecticides, often referred to simply as carbamates, like organophosphate pesticides and nerve gas, inhibit cholinesterase. They differ from the organophosphates principally in that they carbamoylate rather than phosphorylate the active enzyme cholinesterase. Carbamoylation with the active site of the enzyme is more easily reversed than phosphorylation and as a result carbamate inhibition is less stable (Ecobichon 1982). These characteristics provide that intoxications with carbamates are generally of shorter duration and generally have been less problematic with respect to persistent health effects. The mechanism of illness due to acute overexposure to carbamates is virtually identical to that of organophosphate pesticides and will not be repeated here. The symptoms of acute intoxication are also virtually identical. The reader should refer back to the information provided above under organophosphates for details on the mechanism and acute toxic syndrome.

Prolonged effects of N-methyl-carbamates

The carbamates have achieved much less notoriety for persistent health effects than the OPs.

Ecobichon reports on a case of an older gentleman who was drenched in carbaryl while applying. He became acutely ill but even after recovery had persistent memory problems and visual problems that lasted over a year (Ecobichon 1982). In another clinical case report, recurring neurological and gastroenterological symptoms occurred in a 75-year-old man who suffered chronic exposure to dust containing 10% carbaryl in his home. Principal complaints lasting over a ten month period included confusion, weakness, memory loss, muscle cramps, fasciculations and anorexia. Following discontinuation of exposure the subject developed a progressive stocking-glove neuropathy (Branch 1986). Neuropathy ascribed to carbamates was also described in a Japanese woman. This 55 year-old female ingested 200 ml of m-tolylmethyl carbamate. During the acute toxicity phase the patient required ventilatory support. 6 days subsequent to ingestion, symptoms of numbness in her lower extremities began (Umehara 1991). Another case of peripheral neuropathy was described in which a man ingested carbaryl and following the acute cholinergic crisis developed peripheral neuropathy (Dickoff 1987).

The medical literature provides relatively little support for the ascription of persistent illnesses to non-intoxicating chronic N-methyl-carbamate exposure. One report was found of a cross-sectional study of self-reported respiratory symptoms and farming which drew an association between N-methyl-carbamates and asthma. Little else in the literature supports or refutes this studies conclusions (Senthilselvan 1992). It appears that N-methyl-carbamate exposure alone is unlikely to have caused significant persistent illness in PG personnel.

Pyrethroids and Permethrin

Resmethrin, D-trans-allethrin, D-phenothrin, , allethrin, pyrethrin, permethrin

Acute toxicity of pyrethroids and permethrin

Pyrethroids comprise a widely used group of insecticides with low mammalian and human toxicity. The pyrethroids are a synthetic modifications of the natural insecticidal pyrethrins, which are derived from chrysanthemums. Pyrethroids are more environmentally stable, are less allergenic, and have

higher and broader insect toxicity than pyrethrum. The chemicals when given in toxic doses induce a strong excitatory action on the nervous system and increase the reactivity of the nervous system to external stimuli. The chemicals appear to do this by interfering with sodium channel action and, much like organochlorines, cause overexcitation of excitable tissues and, ultimately, repetitive discharging of nervous tissue and convulsions.

Two distinct nervous system syndromes have been identified in association with pyrethroid overexposure in animals. These are the coreoathetosis salivation-syndrome (CS-syndrome) characteristic of the alpha cyano pyrethroids such as deltamethrin and the tremor syndrome (T-syndrome) seen in the non cyano pyrethroids such as resmethrin and permethrin. There also appears to be an intermediate syndrome called now the TS syndrome which includes characteristics of both of the above. Workers with dermal exposure to pyrethroids or pyrethrins often report symptoms of tingling, burning, and numbness of extremities and mucous membranes. High-level exposure may induce prolonged course muscular fasciculations, opisthotony, and seizures. To date few human deaths have been attributed to pyrethroid intoxications and no significant permanent organ damage appears to be a usual consequence of pyrethroid exposure in the absence of death. Human recovery from overexposure has generally been reported to be complete. One acute health effect which may occur even when exposure is very limited is an allergic response (Morgan 1989). This appears to be much less of a problem with the synthetic pyrethroids, but if a person is allergic to a chemical a severe reaction may be life threatening. Following an acute allergic reaction complete recovery is the rule but the sensitivity may persist for a lifetime.

Prolonged effects of pyrethrin and pyrethroids.

Animal studies have demonstrated that high level exposure to pyrethroids is capable of inducing peripheral neuropathic changes. The dosage levels necessary to induce these changes were near lethal and no detectable changes were noted at lower dosages (Aldrige 1990, Vijvergerg 1990). The levels of exposure to these chemicals in usual use or even heavy use is unlikely to be sufficient to induce peripheral

neuropathic changes in humans. While human exposure to the pyrethroids have been studied less well than organophosphates, as a group they appear to be relatively safe. Excepting occasional allergic conditions and skin disorders, there have been no reports in the literature which suggest that health problems persist after an acute or chronic exposure to pyrethroids. Several studies have followed cohorts of workers with chronic exposure to these chemicals but no evidence from these reports suggest that health effects persist after exposure ceases (He 1994, Chen 1991, Zhang 1991).

Organochlorine Pesticides

Lindane, Pentachlorophenol

Acute toxicity of organochlorine pesticides

The organochlorine pesticides induce toxicity by interfering with normal ion flow across membranes in tissues such as nerve cells. Their capacity to cause nervous tissue excitability is responsible for the seizures which are commonly associated with overdosage to these chemicals. Among the organochlorine chemicals listed as imported for use by PG forces is Lindane. Lindane has a long history of use as a miticide. It has been used for control of scabies and lice in adults and children for many years. While its acute toxicity is similar to most organochlorines, it has a relatively good safety record considering its long history of use and particularly its use in human skin applications. Pentachlorophenol while sharing the basic chemical components of organochlorine pesticides, manifests its toxicity in a far different way. This chemical interferes with the oxidative phosphorylation chain resulting in the futile utilization of metabolic energy. The results of overexposure to are excessive metabolic activity, hyperthermia and in severe cases death. Seizures can occur but the main toxicity of this chemical is hypermetabolism (Morgan 1989).

Prolonged effects of Organochlorine Pesticides

The organochlorine pesticidal chemicals imported for use by PG personnel are not known for

significant long term toxicity. There have been some case reports of a possible association between both pentachlorophenol and lindane and blood dyscrasias but these have been relatively few in number. Pentachlorophenol has been associated with chloracne in some reports and this outcome has been attributed to the presence of dioxin impurities. Overall these two pesticides have a relatively benign history and have not been strongly connected with long term health effects (Hayes 1991). Lindane was imported as a dust and probably used for lice and mite control. It is not clear how pentachlorophenol was used but only a small quantity appears to have been imported. Neither of these chemicals would have likely presented a significant acute health hazard to PG personnel and neither are likely to have caused any significant long term health effects.

Aluminum Phosphide

Acute toxicity of Aluminum Phosphide

Aluminum phosphide, a fumigant, was also imported in substantial quantities (20,020 tablets). These chemical tablets produce phosphine gas when combined with water. Phosphine is a very toxic gas which can produce severe illness in the setting of sufficient exposure. The illness produced by phosphine exposure would not be easily overlooked (Morgan 1989). Furthermore, based on how aluminum phosphide is generally used it is highly unlikely that low dose exposure to phosphine occurred among PG personnel.

Prolonged effects of Aluminum Phosphide

There is no evidence in the literature that chronic illness results from low dose exposure to phosphine gas.

Rodenticides

Diphacinon, bromadiolone, pindone

Acute toxicity of rodenticides

The rodenticides imported into the PG were anticoagulant in nature. These chemicals have virtually no acute toxicity except by inducing bleeding over several days after ingestion. These chemical interfere with blood coagulation and are only effective if ingested (Morgan 1989). It is extremely unlikely that rodenticides could induce illness in any way in PG personnel.

Prolonged effect of rodenticides

None known for human beings.

Boric Acid

Acute toxicity of Boric Acid

Boric acid is a general cellular toxin if ingested. It has very low dermal toxicity (Morgan 1989). Skin exposure would be very unlikely to cause significant illness and oral exposure would be unlikely except by intention. It is extremely unlikely that boric acid would induce illness in any way in PG personnel.

Prolonged toxicity of boric acid

None known for human beings.

Pyridostigmine Bromide

Acute toxicity of pyridostigmine bromide

Pyridostigmine bromide (PB) is a quarternary carbamate compound that is used medically for the treatment of myasthenia gravis and is used prophylactically to reduce the mortality associated with exposure to cholinesterase inhibiting nerve gas. This drug, like the pesticidal N-methyl-carbamates, organophosphates and the nerve gases, actually inhibits the enzyme acetylcholinesterase. But unlike the nerve gases and organophosphates, PB does so temporarily. When a human is exposed nerve gas, it quickly reacts with and permanently inhibits available cholinesterase. When PB is present, it also binds to the active site of the enzyme and temporarily occupies the reactive site on the cholinesterase enzyme denying

access to the nerve gas. The PB is then metabolized by the body in the following hours and the protected cholinesterase reactivates, thus saving the life of the protected subject (Hussain 1988, Maxell 1988). PB has been evaluated in numerous animal models for its ability to protect against nerve gas toxicity. It appears to be particularly effective against some nerve gasses and its protective effect is most definite when used in conjunction with antidotes including atropine and valium or 2-pam (Koplvitz 1992). Its use in a cocktail of several drugs has also been explored in animal models and it performs less well than physiostigmine in protecting the central nervous system from nerve gas toxicity (Harris 1984). This is explained by the fact that pyridostigmine does not penetrate into the central nervous system as readily as physiostigmine.

In tests on animals pyridostigmine only achieved inhibition of CNS acetylcholinesterase when nearly half lethal dosage of the drug was used. Even then only 17% of control levels of acetylcholinesterase were inhibited (Xia 1981). As a result, pyridostigmine alone may even enhance the central nervous system toxicity of some of the nerve gases as shown by Shiloff where pre-treatment with pyridostigmine to >65% inhibition of AChE decreased the time to onset of seizure in rats exposed to soman. Less inhibitory doses did not have as significant an effect as the near 65% inhibitory dose and did not differ significantly from controls (Shiloff 1986).

The acute toxicity of PB has been explored in numerous publications and its effects are largely those that would be predicted by its capability to inhibit cholinesterase. Like the organophosphates and the carbamates the inhibition of cholinesterase leads to the accumulation of acetylcholine causing overstimulation of the cholinergic nervous system. This leads to the classic presentations of salivation, lacrimation, urination, diaphoresis, gastroenteric cramping, and emesis. As with other cholinesterase inhibitors, the respiratory system and the muscular systems are also affected. The dosage necessary to induce toxicity was explored in one study using dogs. Pyridostigmine toxicity was shown to be evident at levels above 2 mg/kg in beagle dogs. Prominent problems in treated dogs were gastrointestinal distress, emesis, diarrhea, redenning of the stools and death caused by intestinal intussesception (Kluwe 1990).

Normally the dosage given for prophylaxis against nerve gas poisoning is 30 mg, three times per day. This amounts to about 1.3 mg/ kilogram(kg) per day for the average person but only 0.43 mg per kg per doseage interval. In beagles dosages of pyridostigmine of what would have been equivalent in humans to 35 mg three times per day hours produced inhibition of cholinesterase to 50% of control activity in the beagles indicating relative equivalence with human ChE inhibtion by pyridostigmine.

Recent reports have identified individuals in the population who might be more susceptible to the toxicity of PB than others due to preexisting chronic illness or genetic differences in their cholinesterase. Even the standard 30 mg three time per day dosage provided to U.S. forces may have caused acute toxicity in these particularly susceptible populations such a asthmatics or soldiers with a unique serum cholinesterase phenotype (Loewenstein-Lichtenstein 1995). Soldier who received high per weight dosage because of small body mass (Gouge 1994) might also be more susceptible to the toxic effects of PB. Nevertheless, the 30 mg three times per day dosage has been shown to be generally well tolerated by the majority of the population (Blick 1994, Borland 1985, Cook 1992, Glikson 1991).

Studies on animals suggests that under stressful situations the lack of central nervous system penetration which makes PB an attractive prophylactic may not be assured. This central nervous system penetration may lead to acute central nervous system symptoms. Symptom persistence resulting from this increased penetration has not been reported to date in human or animal models, although evidence from one study presented indicated that a central nervous system feedback mechanism may account for changes which may outlast the acute cholinergic effects of the drug (Freidman et al 1996).

No information was found as to whether the bromide in the preparation might have had deleterious effects given bromide's long half-life and the desert conditions of chronic high heat and salt depletion. Despite these caveats, the years of experience in treating patients for myasthenia gravis with PB at doses often much higher than those taken by Gulf War service personnel would suggest that the development of persistent health effects among Gulf War personnel from PB alone is unlikely. The pyridostigmine is rapidly metabolized and the bromide is excreted over several weeks once the drug

administration is stopped. The penetration of the blood brain barrier by pyridostigmine under the stress of a combat situation may potentially result in acute effects given sufficient blood levels, but with metabolism of the drug and the reversal of the acute effects, it is unlikely that long term effects would ensue.

Another possible source of toxicity which has been associated with pyridostigmine use is that due to the bromide ion with which it is conjugated. Bromide is treated like chloride by the body and with a half life of 12 weeks it may accumulate to a sufficient degree over a long period of time to cause changes in central nervous system function such as depression, irritability, confusion muscle weakness and inappropriate behavior leading to psychotic behavior. A acne like rash is also common in cases of bromine toxicity (Goldfrank 1986). Bromism is historically a well known condition but unusual today because of the rarity of the use of the ion in modern medications. One report was found in the literature of bromism induced by overuse of pyridostigmine bromide in a post surgical patient. The patient showed central nervous system symptoms characteristic of bromine intoxication and following reduction of the patients dosage recovery appears to have been complete (Rothenberg 1990). Bromism is unlikely to occur in recruits given the doses recommended for prophylaxis for nerve gas though no literature was found that reviewed the potential for accumulation of bromide ion given the desert environment the long half life of bromide ion and the salt depletion that might occur under those conditions.

The safety of pyridostigmine at the levels used during the Persian Gulf activities is supported by several publications which have focussed on the acute short term effects of the drug. Only recently have studies begun to examine whether long term effects may be present after discontinuation of therapy. The acute behavioral toxicity of PB was determined using rhesus monkeys and a continuous performance test. PB was dosed at a 0.12, 0.24,0.48,0.96 mg/kg. Only the highest dose consistently produced performance decrements but the authors estimated the dosage that would likely produce decrement in 50% of subjects at 0.66mg/kg in the rhesus monkeys. This dose interpolated to an AChE inhibition of 77%. It should be noted that the authors point out that peripheral signs of PB toxicity were present in animals which showed no performance decrement. No evaluation of persistence of problems was performed in this study (Blick

1994). Pyridostigmine in animal models appeared to reduce heat tolerance when dosage was given to rats by parenteral route (Francesconi 1984) but this effect was less pronounced when given by oral route (Francesconi 1986). A study of four men given PB 30 mg q 8hrs for 3 days showed minimal changes in all neuropsychological parameters. Daily variations were more prominent than drug effects. Two visual parameters were improved by the drug where as a slight decrease in the visuomotor coordination was observed. (Borland 1985). One study looked for side effects among seven male soldiers in a desert environment given 30 mg PB three times per day. There were no symptomatic differences between placebo and PB therapy in this double blind study. Slight differences in grip strength, pupillary size, peak rectal temperature and diastolic blood pressure were noted. (Cook 1992). Pyridostigmine given to volunteer subjects at 30mg T.I.D. showed no demonstrable effect on muscular function, nerve conduction or muscle endurance. Cholinesterase activity showed that this dose caused an average inhibition of 23% below control levels (Glikson M 1991).

Because the cholinergic system is important in control of the heart and lungs, specific focus has been given to its effects on these organs both in animals and man. A study or the respiratory and cardiovascular effects of various dosages of PB on dogs showed that at dosages as high as 2 mg/kg, airway resistance began to show definite increases suggesting brochoconstriction and or secretion excess. An increase in airway resistance of 10 fold was noted at 5mg/kg. Cardiac output was not impaired but heart rate did decrease. At lower levels of the drug (0.5mg/kg) no effects were noted. The study did not test whether long term effects of the low dose or even higher dose regimens might exist after cessation of therapy. (Cadwel 1989). In a study of respiratory function of human volunteers, asthmatic and non asthmatic subjects given PB at 30 mg/kg three times per day showed no significant changes in airflow but statistically significant changes were noted in normals at 60 mg. ChE activity was reduced by an average of 24 % of control activity with the 30 mg dosage (Zviram 1991).

Exacerbation of asthma was found to occur in 7 of 10 asthmatic subjects given the prescribed 30 mg/kg during active duty in the desert conflict. While non-asthmatics did not appear be affected, symptoms

of respiratory distress in asthmatics were clearly more evident particularly in soldiers who weighed less and thus received higher per kilogram doses (Gouge 1994). Calculation of the dosages given to soldiers receiving 30 mg three times per day indicates that a dosage of 0.56 mg/kg q 8 hrs was given to the most severely affected soldier (30mg/54 kg the weight of the smallest individual). This is a substantial dose per kilogram and may be responsible for the toxicity experienced in this individual..

Several other studies have looked at symptoms and physical evidence of effects of PB in active duty soldiers. A survey of Israeli soldiers taking PB at 30 mg three times per day demonstrated that symptoms characteristic of cholinesterase inhibitor appeared in only about 1/5th of participants. Of the symptom reporters, 61% reported a moderate symptom and 37% reported at least one severe symptom. The survey was conducted during active duty and no good control was provided. In the absence of a non-combat threatened control group the survey results are difficult to interpret as to whether do to PB or combat stress or a combination (Sharabi 1991).

Animal studies suggest that PB may induce weakness in skeletal muscles when given in sufficient dosage that is not explained by cholinesterase inhibition alone (Anderson 1988). This effect may be due to reduction of acetylcholine release at the synapse, but a well known phenomena of diffuse myopathy can be induced by high levels of cholinesterase inhibition as well. This myopathy was first demonstrated by injecting experimental animals with potent cholinesterase inhibitors such as paraoxon, the active metabolite of parathion. The resulting muscle necrosis appears to be dependent upon degree and duration of AChE inhibition. Temporary inhibitors such as pyridostigmine can induce similar damage when infused for sufficient duration but do not cause such damage when the duration of ChE inhibition is short. (Wecker L 1978). Pyridostigmine appears to be capable of inducing this myopathic picture in rats (Gebbers 1986, Meshul 1985, Schuschereba 1990). In these experimental models, the development of myopathy appears to requires substantially higher dosages than would normally be given for nerve gas prophylaxis.

The doses used for nerve gas prophylaxis are expected to induce about 30% cholinesterase inhibition in the subjects taking 30 mg 3 times per day. Due to its charged form pyridostigmine is

normally prevented by the blood-brain barrier from entering the central nervous system to any large extent. This is one of the distinct advantages of pyridostigmine over other temporary cholinesterase inhibitors. The lack of its entrance into the nervous system permits the central nervous system (CNS) cholinesterase from becoming inhibited and interfering with normal brain function. The unfortunate downside to this is that the brain cholinesterase is also not protected from the effects of the cholinesterase inhibiting nerve gases which do penetrate the blood brain barrier. Several studies have examined the central nervous system effects of pyridostigmine. Four men given PB 30 mg q 8hrs for 3 days showed minimal changes in all neuropsychological parameters. Daily variations were more prominent than drug effects. When data were grouped, two visual parameters were improved by the drug where as a slight decrease in the visuomotor coordination was observed. (Borland 1985). One rat study concluded that exposure to PB at less than 10% the LD50 could influence learning of tasks that require higher CNS structures and motor activity (Wolthius 1984). In one study of myasthenia gravis patients (often treated with PB), visual evoked potential amplitudes (as measured by p100) were shown to improve significantly in subjects following treatment with PB (Fotiou 1994) demonstrating that CNS penetration was sufficient to have demonstrable CNS effects. The latter studies are either devoid of dosages or the conversion to a dose comparable to human dosage is difficult.

Pyridostigmine bromide (PB), used by the U.S. forces as a prophylactic agent against the toxicity of nerve gas has demonstrable toxicity for both animal models and humans when given in relatively high dosage. The standard 30 mg three time per day dosage provided to U.S. forces may have caused acute toxicity in particularly susceptible populations such a asthmatics or soldiers with a unique serum cholinesterase phenotype (Loewenstein-Lichtenstein 1995), or in soldiers who received high per weight dosage because of small body mass (Gouge 1994) but this dosage has been shown to be generally well tolerated by the majority of the population (Blick 1994, Borland 1985, Cook 1992, Glikson 1991).

Prolonged effects of pyridostigmine bromide

Very little literature exists which has examined the possibility that effects on the nervous system persist after discontinuation of exposure to PB. One study looked at the effects of the startle response in rats treated 7 days with 2 mg/kg/day PB. These rats showed continued sensitivity to startle stimuli as far out as 14 days after discontinuation of treatment (Natelson B 1996 abstract.). This article has not been found in full format and as far as can be ascertained to the present is only available in abstract. An important article addresses the difference in brain penetration by PB that might exist under stressful situations. In a mouse model, measurable PB levels appear in the CNS to a greater degree under stressful situations than in the absence of stress (Friedman 1996). Increased penetration itself causing the temporary inhibition of ChE would explain increased symptoms during administration of the drug, it would not offer any contribution to persistent symptoms. The authors of the above paper go on to report that demonstrable RNA transcription in the CNS of the stressed mouse may indicate potential feedback responses which would presumably outlast the acute effects of the ChE inhibition from the drug. (Friedman 1996).

Another recent publication may offer insight into the acute symptomatology of some soldiers who took PB during the conflict. Differences in susceptibility to the side effects of PB may at times be due to the relatively rare phenotype of atypical BChE (0.04% of the population, 0.6% in some populations). This papers posits and supports with limited data that individuals with this phenotype may be more subject to the cholinergic effects of PB as they have less active BChE to "scavenge" the free BChE which is not bound to AChE (Loewenstein-Lichtenstein 1995). This differential susceptibility to PB might explain acute symptoms in some soldiers and would potentially make these individuals more susceptible to long-term CNS effects of PB is any exist

Little evidence in the present literature suggests that persistent health effects would be expected following recovery from chronic or acute overexposure to pyridostigmine bromide. The issues raised by the increased CNS penetration of PB seen under stressed conditions and the unique sensitivities that some individuals may have to the drug due either to pre-existing asthma, small body mass or differences in cholinesterase phenotype are intriguing and the question of bromide toxicity from chronic dosing in a

desert environment is not addressed in the literature. Nevertheless, persistent effects from pyridostigmine alone would not be likely after the discontinuation of the exposure.

Diethyl-m-toluamide (DEET)

Acute toxicity of DEET

Diethyl-m-toluamide (DEET) was imported in large quantities and presumably used widely as an insect repellent during the conflict (DOD on Aug 27, 1997 to Senator A. Specter). It is also widely used by the U.S. population in general and given its broad use (30+ % of the US population), the chemical has a reasonably good safety record (Veltri 1994). Case reports indicate that this chemical can induce central nervous system effects when absorbed in sufficient quantity but cases usually involve excessive exposure and often involve young children or infants.

Prolonged effects of DEET

No reports in the literature describe the long term toxicity of DEET among humans with low level chronic exposure though some permanent residual effects have been noted in at least one case following recovery from what appeared to be an acute intoxication (Knowles 1992). The possibility that even relatively heavy exposure to DEET alone could induce chronic health effects in the Gulf personnel is unlikely.

Nerve Gases

Acute effects of Nerve Gas

Nerve gas behaves in the body in a manner very similar to organophosphate pesticides. The molecule directly binds to the enzyme cholinesterase and inhibits its ability to metabolize acetylcholine. This leads to the accumulation of this neurotransmitter at target tissues resulting in the overstimulation of glands, muscles and other nerve to nerve cholinergic synapses. The primary cause of death from nerve gas

is paralysis of the respiratory system from the combination of decreased central drive, increased airway resistance from secretions and bronchospasm, and decreased muscle strength from respiratory muscle paralysis.

Prolonged effects of Nerve Gas

The health effects of exposure to nerve gases has been only periodically addressed in the mainstream literature. In 1982 the National Research Council examined the effects of low level anticholinesterase exposure on 1,400 military volunteers and could draw no firm conclusion as to whether long term effects were caused by exposures (NRC 1982). No other major studies on humans is available examining long term health effects of low level nerve gas exposure. One excellent study which examined most of the important nerve gases for production of peripheral neuropathy in rodents showed that sarin was capable only at super-lethal doses of potentially inducing neuropathy (Gordon et al. 1983).

Few cases of known accidental or intentional human exposure to nerve gases are available to examine for long term effects, so predictions must be modeled mostly from animal experiments. A study reported by the National Research Commission of exposure of 1400 military volunteers to low level exposure to anticholinesterases including nerve gas could not rule out chronic health effects from these exposures (NAS, 1982). But the Center for Disease Control concluded in 1988 that there appeared to be little risk of adverse health effects from low level long-term exposure to GA, GB, VX, H, HD, HT or lewisite (CDC 1988). In a review of the literature on nerve agents, Gunderson et al. concluded that persistent effects such as psychological and behavioral problems, could result after acute exposure, but that no evidence supported persistent effects from low level exposure to these chemicals (Gunderson et al.1992). A recently published study on survivors of the Japanese subway sarin gas incidents identifies possible delayed effects on balance among surviving female victims. These authors also cite an as yet unpublished manuscript identifying neurobehavioral abnormalities among other victims 6-8 months after the poisoning (Yokoyama et al. 1998). These findings are consistent with problems identified among

persons previously poisoned with organophosphate pesticides (Keifer et al. 1997, Steenland et al.1994, Rosenstock et al. 1991, McConnell et al. 1994, Savage et al. 1982, Lotti et al. 1986), which are related to the military nerve gases. The literature does not provide evidence to support persistent neurological or other health effects from low-level exposure to nerve gases. So at present there is little support in the literature for the conclusion that in the absence of acute intoxication with these chemicals neurobehavioral symptoms would develop.

The Possible effects of Mixtures of Chemicals in the Gulf

The effects of mixtures of chemicals on human health is an understudied area. Due to the potential number of combinations of chemicals which could be studied, the number of experiments necessary to evaluate even a fraction of the possible combinations is staggering. Several of the chemicals to which U.S. Persian Gulf personnel were exposed share mechanisms of toxicity. The Organophosphate pesticides, the carbamate pesticides and the carbamate pyridostigmine bromide all cause toxicity by inhibiting the cholinesterase enzyme. Given sufficient expose to any one or combinations of these chemicals acute toxicity would result with relatively characteristic findings. As discussed above, there have been persistent health effects noted following severe acute intoxications with cholinesterase inhibitors, but these have not been convincingly confirmed to occur following long term low level exposure in humans.

The potential for chronic health effects resulting from mixtures of chemicals and from mixtures of pyridostigmine bromide and pesticides has been a subject of recent investigations. In the few studies located which have examined the question, exposure of animals to mixtures of chemicals which individually are not know to produce nerve damage did induce damage. The chemicals tested in two of the studies specifically examined chemicals which were used by service personnel in the Gulf conflict (Abou donia 1996a & b). These chemical mixtures included OPs and DEET as well as PB and pyrethroids. Earlier studies have shown that peripheral neuropathy can be caused by combining in a specific sequence

non-neuropathic doses of organophosphate chemicals with other non-neuropathy inducing chemicals in animals. The order of the administration of the chemicals appeared to be important in these studies, but the combination of OPs and non-neuropathy inducing chemicals causing neuropathy raises the question of whether some fortuitous combination of such chemicals could induce neuropathy in humans (Morretto et al 1992). An important caveat to these studies is that the dosages used to induce these damages were well above what would have been expected to occur by regular use of these chemicals by Persian Gulf U.S. forces and would probably produce identifiable acute toxicity in human subjects.

One important question regarding the combination of DEET and carbamate insecticides and DEET and pyrethroid insecticides was addressed by Baynes et al (1997). These researchers examined whether DEET might facilitate the penetration of pesticides through the skin. Because of the extensive use of DEET directly on the skin of U.S. Persian Gulf service personnel and their simultaneous exposure to insecticides of these categories the question was raised as to whether skin penetration of toxic chemicals might have been facilitated by DEET. Their work with animals does not support that such facilitation is likely to have taken place. (Baynes et al 1997).

Whether prolonged health problems would result from the combinations of chemicals that PG veterans were likely to encounter during their time in the Persian Gulf cannot be completely answered by modeling in animals alone. There is clearly the possibility that rather high levels of mixtures of chemicals can induce neuropathic injury in animals but whether the same is true in humans at lower, non-intoxicating levels is not clear. The combination of DEET and chlorpyifos or DEET and pyrethroids would not be uncommon in many situations where insect repellent is used in the presence of insecticides. The addition of PB to the mix is probably rather unique. Further research on mixtures of such chemicals is clearly needed but realistic exposure levels need to be applied to confirm that at expected dosages of these mixtures disease does or does not occur.

Cholinesterase Monitoring for Exposure to Nerve Gas, Organophosphate pesticides and PB

Cholinesterase monitoring has long been used to identify overexposure to organophosphates among pesticide applicators (Wilson et al. 1997). It also can potentially be used to identify personnel exposed to organophosphate nerve gas because of the similar mechanisms of toxicity. Presently, to detect low levels of exposure, accurate interpretation requires a pre-exposure baseline on a subject against which to compare subsequent values. This limitation, and problems with the accuracy of commercially available test kits, makes cholinesterase testing more complicated than simply measuring a value in blood as can be done with testing for some substances such as lead.

From the information presently available, it does not appear that the DOD has a policy for monitoring cholinesterase or for assessing the physiological effects of the prescribed standard prophylactic dose of pyridostigmine bromide or for routinely evaluating for exposure to nerve gas. The broad application of cholinesterase monitoring for all those taking PB doses or to all combatants for evaluation of nerve gas overexposure would probably not be beneficial. Most people taking the PB as a drug would probably have a very predictable response to the dosage. The drug PB generally appears to be safe when taken by individuals of average size (70 kg), with normal uninhibited cholinesterase activity and with no illnesses which would make them particularly susceptible to ill effects from the PB. However, there is a substantial minority of individuals who may be smaller in stature, have illnesses such as asthma or, in rare cases, have congenitally low cholinesterase which makes them sensitive to PB even when taken in the prescribed dose. A mechanism should be in place to identify those who might suffer ill effects and determine how their dosage should be adjusted in order to avoid complications while still providing protection from nerve gases. Regarding the broad application of ChE monitoring for identifying exposure to nerve gases; in the absence of symptoms the potential for identifying a person with exposure to nerve gas is probably small. The variability inherent in the test procedure makes the likelihood of false positive results an important consideration. The added issue of sorting out low level nerve gas inhibtion from pyridostigmine effect in the same individual reduces substantially the practicality of this test for this purpose.

Other options for exposure biomonitoring

Recently, a new approach to identifying overexposure to organophosphate nerve gas has been described. This method reactivates inhibited cholinesterase and reconstitutes the nerve gas molecule which can then be measured by various methods. The technique has been used on samples from the sarin gas attacks in Tokyo and cholinesterase activity could be recovered and varying levels of sarin could be found in reactivated sample. If this technique shows itself to be sound, it has potential application in determining whether personnel have sustained exposure to nerve gas or organophosphates even several weeks after exposure (Polhuijs et al. 1997).

Review of DOD Comprehensive Clinical Evaluation Program and VA Persian Gulf Registry

The CCEP was reviewed in a report issued by the Institute of Medicine in 1997. The review carefully documented the methods by which the DOD evaluates PG personnel. At the time of the report the CCEP had completed evaluations on more that 24,400 individuals. The IOM concluded that the CCEP is appropriately structured for screening for significant illness in the PG personnel and that the system would likely be capable of identifying significant illness were it present in the evaluated population. The IOM was generally complimentary of the program but provided some recommendations that included: improved documentation of screening histories and exams, improved history taking and standardization across sites, and ensuring that a referral neurologist and psychiatrist be available for primary physicians (IOM 1997).

In a report published in March 1997, results as of April 1996 were reviewed on 20,000 participants in the CCEP (Joseph et al. 1997). Descriptions of demographics and primary and secondary diagnosis were provided. Based on this report and the review by the IOM, the CCEP appears to be an efficient system for capturing a broad group of conditions. It is likely to pick up most diseases that might result from exposures in the PG. Its multi-phase approach appears to efficiently prioritize diagnostic issues for appropriate evaluation by specialists. The recommendations made by IOM regarding documentation and standardization will, if incorporated, provide added value to the present system both for use in

evaluating the problems of PG veterans but will also serve for evaluation of similar health issues among veterans of future conflicts. Lacking in the IOM report was recommendations regarding quality assurance (QA) mechanisms to be incorporated into the CCEP.

A review of the Persian Gulf Registry Protocol (V.A. 1995) suggests no explicit mechanism for checking the quality of data collected by primary care practitioners who collect enrollment information from new enrollees. While the material reviewed for this report was limited, it was not clear from the protocol manual that any mechanism was contemplated for giving hands on training to primary care practitioners in the completion of the forms required for the VA Persian Gulf Registry. There was also no mention of mechanisms for primary data quality evaluation to be applied once data began to flow. A GAO report (GAO 1997) also finds lack of QA for care after initial assessment and a lack of systematic follow-up of veterans referred on or diagnosed on the initial evaluation. While no more recent information other than the GAO report were available to this author at the time writing of this report, this criticism should be acted upon and mechanisms for QA should be developed and incorporated into both the VA and DOD systems.

Both CCEP and the VAGRP data collection systems provide opportunities to test new hypotheses about causation of GW illness. In the descriptions of the systems it is not apparent how new data collection tools would be incorporated so as to test new hypotheses as the develop. As causation is clearly not established for the GW illness, a mechanism should be in place to permit new tools to be interjected into the data collecting systems to permit new hypotheses testing.

Recommendations

A sincere and scientifically valid effort to explore and address health concerns of veterans from military conflicts is an extremely important responsibility that our government has toward its veterans. But communicating in an open, non-defensive manner with the concerned service personnel and the public

about the state of knowledge and the progress of knowledge is potentially the greatest challenge facing the Department of Defense and the Veterans Administration with regard to issues of post conflict health of veterans. While the health problems from which Gulf War veterans suffer may never be completely ascribed with certainty to specific exposures that occurred during service in the Gulf, the challenge of identifying, and caring for the health of veteran's and responding to the health concerns of veterans will continue as long as there are veterans. Effective risk communication is essential to maintaining and optimized three way dialog between the veteran-active duty community, the citizenry and the responsible government branches.

Specific Recommendations

This author can not substantially improve on the scientific comprehensiveness of the recommendations made by the Institute of Medicine on improving the surveillance and monitoring capabilities of the DOD regarding health effects of combat service (Institute of Medicine, IOM, 1996). I do believe it is important to add that the IOM report fails to recommend a mechanism whereby the veterans, the U.S. public and active duty personnel might participate in the functioning of an ongoing system of health outcomes monitoring. Potentially the most important predictor of success of this program as judged by these constituencies is the degree to which they can claim ownership of the process. I strongly encourage that a mechanism be established to assure active participation by representatives of the U.S. public, veterans groups and active duty personnel of varied ranks and branches in the design and conduct of any program that is adopted. A mechanism should also be established to regularly communicate with all veterans providing them with ongoing information about new developments and knowledge regarding the effect of service and health. This appears now to exist at least to some extent through the used of the Internet, though certainly not all veterans are users of the internet at present.

Research in basic and applied science

Support for further research on technology for detecting environmental release and personal exposure to war gases should be a particular emphasis of the DOD. Monitors should be developed that are portable, collect and report real time information, and have data storage capabilities and are easily applied by combatants.

Research should be undertaken to develop profiles of individuals who may potentially suffer untoward effects from war gas antidotes (e.g. asthmatics, smaller individuals). Those individuals should have personal drug dosing profiles developed and confirmed by cholinesterase activity levels appropriate to the prophylactic medication taken. Routine cholinesterase testing of all personnel is probably not warranted, but the test should be available on a routine basis for evaluating ill combatants both for overdose of prophylactic medication and for evaluating war gas exposure.

A new technique has been described which potentially represents a very significant breakthrough in the detection of cholinesterase inhibited by the nerve gas sarin. This technique could potentially be used to identify exposure to nerve gas and may have application in detecting exposure to organophosphate pesticides. This technique should be explored and amplified if possible for application to exposure assessment of subjects potentially exposed to nerve gases and pesticides.

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