

4

POSSIBLE LONG TERM HEALTH CONSEQUENCES OF GULF WAR EXPOSURES: AN INDEPENDENT EVALUATION

INTRODUCTION

This chapter provides an independent examination of the long-term health consequences of Gulf War exposures by nationally recognized scientific experts. Chapter Three reviewed many of the complexities associated with the question of “Why are Gulf War veterans ill?” as well as some of the reasons why this question may never be answered. In an effort to examine what is known regarding the health effects of some of the exposures experienced by troops during the Gulf War, the SIU contracted with the following scientists.

This chapter contains the brief reports prepared by the consultants listed below. (The consultants’ affiliations are provided for identification purposes only.) They are, in the order their reports appear in this chapter:

Fredric Gerr, M.D., Peachtree Environmental Consultants Inc., Decatur, Georgia; and Associate Professor, Department of Environmental and Occupational Health, Rollins School of Public Health of Emory University, Atlanta, Georgia. Dr. Gerr examined the chemicals that were in the Gulf, such as solvents, pesticides, depleted uranium, and others, for their potential health effects particularly upon the brain and nervous system. (Dr. Gerr’s detailed report is at Appendix II.)

Matthew Keifer, M.D., M.P.H., Assistant Professor, Occupational and Environmental Medicine Program, Departments of Medicine and Environmental Health, Harborview Medical Center, University of Seattle, Washington. Dr. Keifer examined the total range of health effects to exposures to pesticides and related chemicals such as pyridostigmine bromide and some chemical nerve agents that are similar to pesticides. (Dr. Keifer’s detailed report is at Appendix JJ.)

James Moss, Ph.D., Gainesville, Florida. Dr. Moss looked at the use of PB as it acts with combinations of other agents such as certain pesticides.

Richard Letz, Ph.D., Peachtree Environmental Consultants Inc., Decatur, Georgia; and Associate Professor, Department of Behavioral Sciences and Health Education, Rollins School of Public Health

of Emory University, Atlanta, Georgia. Dr. Letz evaluated the health effects of stress as an occupational and or environmental exposure in the Gulf.

Michael Lebowitz, Ph.D., Professor of Medicine, Pulmonary and Critical Care Medicine; Professor and Director of Epidemiology, Arizona Prevention Center; Chair, Epidemiology Graduate Interdisciplinary Program, University of Arizona, Tucson. Dr. Lebowitz examined the long-term health effects of sources of indoor and outdoor air pollutants during the Gulf War including oil well fires, sand, space heaters used in unvented tents, and other sources. (Dr. Lebowitz's detailed report is at Appendix KK.)

Kevin Dybvig, Ph.D., Professor, Departments of Comparative Medicine and Microbiology, University of Alabama at Birmingham. Dr. Dybvig evaluated the potential role of infection with *Mycoplasma fermentans* in the health problems of Gulf War veterans.

Shanna Swan, Ph.D., Chief, Reproductive Epidemiology Section, California Department of Health Services. Dr. Swan evaluated reproductive health issues from an epidemiological perspective. (Dr. Swan's detailed report is at Appendix LL.)

Melissa McDiarmid, M.D., M.P.H., Associate Professor of Medicine, Occupational Health Project, University of Maryland; and Director, Depleted Uranium Follow-up Program, Baltimore Veterans' Affairs Medical Center. Dr. McDiarmid examined the chemicals that were in the Gulf, such as solvents, pesticides, and depleted uranium, for their potential to adversely affect reproductive health outcomes. Dr. McDiarmid also examined the chemicals associated with the Gulf War deployment for their potential to increase the risk of cancer among Gulf War veterans. (Dr. McDiarmid's detailed reports are at Appendix MM and NN.)

POSSIBLE POTENTIATION OF PYRIDOSTIGMINE BROMIDE BY PESTICIDES

Prepared by: James Moss, Ph.D., Gainesville, Florida

SUMMARY

The Senate Committee on Veterans' Affairs requested a review and analysis of research on synergism or potentiation of pyridostigmine bromide (PB) toxicity by pesticides. This summary examines reports that indicate PB may become more toxic when an organism is simultaneously exposed to pesticides and other factors. This report suggests that PB has the potential to affect multiple organs and tissues, and that pesticides may synergise or potentiate the effects of PB on various organs and tissues. The author feels that knowledge of which pesticides and other chemicals potentiate PB toxicity will eventually lead to an understanding of the mechanism(s) underlying the observed interactions. When these mechanisms are understood, clearer scientific judgement, and hypothesis based models, can be used so that we may better understand whether PB may contribute to chronic illnesses. Knowledge of which biochemical systems are responsible for pesticide synergism of PB toxicity may allow avoidance of complications of PB use.

Introduction. Pyridostigmine bromide (PB) is a quaternary dimethyl carbamate that has been used to treat myasthenia gravis, a neuromuscular disorder characterized by skeletal muscle weakness (Breyer et al. 1990). Since 1986, PB has been recommended by the United States Army as a prophylactic agent for organophosphate (OP) nerve gas exposure (Dunn and Sidell 1989). Organophosphates bind irreversibly to the enzyme acetylcholinesterase (AChE) in the central (CNS) and peripheral (PNS) nervous systems and thereby prevent hydrolysis (breakdown) of the chemical neurotransmitter acetylcholine (ACh). As a result, ACh accumulates at nerve and muscle receptor sites. At muscles, this can produce excessive stimulation leading ultimately to muscle paralysis and death.

A prophylactic dose of PB (30 mg, every eight hours) binds to AChE, thereby protecting the enzyme from permanent damage by OP chemical warfare agents. Over time the PB is released and AChE activity is restored to a level needed to maintain life, providing that atropine and oxime treatments are also administered at the time of nerve gas exposure (Cook and Kolka 1992). This protocol has been shown to protect primates from the chemical warfare nerve agent Soman (von-Bredow et al. 1991, Wolfe et al. 1992).

Synergism (Potentiation). The possibility that PB could play a role in chronic illnesses increases if conditions potentiate (synergize) PB's toxicity. Such conditions might include simultaneous exposure to other chemicals/toxins such as pesticides. A simultaneous exposure to a toxin and another chemical can produce several different outcomes. These outcomes can range from no increased toxicity, an additive effect or a synergistic effect.

An additive effect is the sum of the independent effects of the chemicals. A dose of "A" may kill 5% of a population and a dose of "B" may kill 5% of a population. The effects would be additive if the same doses of "A" and "B" killed 10% of the population when given together.

Synergism, or potentiation, is an interaction that gives a more than additive effect. In a synergistic interaction, a dose of "A" that killed 5% of a population plus a dose of "B" that killed 5% of a population would kill over 10% and up to 100% of the population, when given together.

When used for nerve gas protection, PB was designed to be taken at doses that would inhibit about 30% AChE activity (Cook and Kolka 1992). Studies have shown that some pesticides increase PB's toxicity from about two-fold to ten-fold (Moss 1996) (Abou-Donia et al. 1996a) (McCain et al. 1997). Even low level potentiation of this specific PB action (AChE inhibition) might inhibit a large proportion of AChE activity, which could be fatal. Any degree of synergism of the effects of PB is therefore relevant.

PB's Effects Outside of Acetylcholinesterase Inhibition (Side Effects). It is possible to have substantial AChE inhibition by some chemicals without a resulting chronic illness. Several hundred humans were exposed to the AChE inhibitor sarin (nerve gas) at doses which caused cholinergic symptoms and substantial AChE inhibition (Sadayoshi et al. 1997), yet the authors reported that chronic delayed effects associated with poisoning by some other OPs were not present.

As mentioned above, PB's main action is acetylcholinesterase (AChE) inhibition. If PB's only action is AChE inhibition, and AChE inhibition is found unlikely to contribute to chronic symptoms, then the likelihood that PB can contribute to chronic illnesses is diminished. However, a different outcome is possible if, in addition to AChE inhibition, PB has some other specific action (side effect). If such a side effect were able to produce chronic outcomes, synergism of the side effect would increase the chronic outcomes. In this review, "side effect" means those effects which are the result of a chemical's action on a molecular target other than the presumed or known primary target for that chemical. For PB, this means effects that are the result of PB actions on a molecular target other than acetylcholinesterase. Possible side effects of PB, may be important if the side effects are potentiated by the actions of pesticides or other factors. Such a potentiation would cause the side effects to increase relative to the known cholinergic effects of PB, and might produce unexpected outcomes.

PB'S Muscarinic Side Effects. ACh causes two major types of response: nicotinic (nicotine sensitive) and muscarinic (muscarine sensitive) (Bowman and Rand 1980). PB produces more of one type of ACh induced response (muscarinic) over the other (nicotinic) (Arce et al. 1991, De-Novellis et al 1994, Muller et al. 1991). This predominantly muscarinic effect would not occur if PB's only action was acetylcholinesterase (AChE) inhibition, because blocking of AChE should elevate ACh at both nicotinic and muscarinic receptors equally. One would not expect to see one or the other effect to predominate. PB is known to directly affect cellular Ach receptors in addition to AChE inhibition (Pascuzzo et al. 1984), and PB binds to ACh muscarinic receptors (Yamamoto et al. 1996). PB therefore has one side effect of activating muscarinic receptors, in addition to its ability to inhibit AChE.

PB's Calcium Side Effects. LoPachin and Lehning (1997) stated that "Studies conducted over the past two decades indicate that calcium accumulation in injured axons has significant neuropathic implications and is a potentially unifying mechanistic event." PB induced muscle damage is probably caused by calcium leakage into cells through calcium channels, because a calcium channel blocker was able to reduce PB induced muscle damage (Meshul 1989).

PB'S Neurotoxic Esterase Side Effects. Another potential side effect target of PB is on an enzyme called neurotoxic esterase (NTE). NTE inhibition is believed to be associated with organophosphate induced delayed neuropathy (OPIDN). Some OP acetylcholinesterase inhibitors (in addition to their AChE inhibition), also inhibit NTE, and such exposure can lead to OPIDN (delayed neuropathy) in experimental animals (Lotti et al. 1993).

Many OPs inhibit both AChE and NTE (Ehrich et al. 1995). The type of toxic effect can range from purely AChE inhibition (rapid death from respiratory failure), to mostly delayed neuropathy (caused by NTE inhibition) (Lotti et al. 1993). Mixed effects can be exhibited by a single compound. Selective synergism of the NTE effect would result in selection for OPIDN symptoms over cholinergic symptoms. An example of this type of chemical manipulation was the production of OPIDN in cats by chlorpyrifos which normally causes only cholinergic symptoms (Fikes et al. 1992).

PB is a carbamate, and an AChE inhibitor. Some carbamates (in addition to AChE) inhibit NTE and therefore have the potential to cause delayed neuropathy if given chronically, or at high doses. A carbamate (PMBC) has been shown to cause delayed neuropathy in hens with repeated doses (Lotti et al. 1993). A series of other carbamates have been synthesized that also inhibit NTE (Randall et al. 1997). Carbaryl, a carbamate pesticide, has been reported to cause delayed neuropathy in a human (Dickoff et al. 1987). PB therefore has the potential to inhibit NTE and synergism of that side effect is a possible route to PB induced delayed neuropathy.

Target Organs. PB has predominately muscarinic side effects and many organs and tissues are affected by muscarinic, cholinergic chemicals such as PB (Bowman and Rand 1980). Many organs and tissues are therefore potential targets of synergised, muscarinic, side effects of PB. Examples are

the human central nervous system (CNS) which has PB sensitive, muscarinic receptors (Valcavi et al, 1991, Mazza et al. 1994, O'Keane et al. 1992). PB does not easily cross the blood-brain barrier (BBB) under "normal" conditions, however, the BBB may be more permeable under some conditions such as stress (Friedman et al. 1996). The BBB is not completely impermeable to PB, under any circumstances. PB causes CNS mediated behavioral changes in rats (Wolthuis and Vanwersch 1984), rhesus monkeys (Blick et al. 1994) and humans (Borland et al. 1985). Chronic dosing of PB resulting in a constant exposure of the BBB could result in significant amounts of PB in the CNS.

Other examples of organs and tissues that have muscarinic receptors which are potential targets of PB effects are peripheral neural tissue such as the guinea pig myenteric plexus (Mike 1994) and the rat superior cervical sympathetic ganglion (Ramcharan and Matthews 1996). There are also muscarinic receptors in the hearts of humans (Bowman and Rand 1980) and in blood vessels in the human brain (Tsukahara et al. 1989a), human skin (Stephenson and Kolka 1990), rat mesenteric vascular bed (Pinaridi et al. 1992), the rabbit thoracic aorta (Tsukahara et al. 1989b) and the rat liver (Pfaffendorf and Van-Zwieten 1993). Other organs or tissues that are sensitive to muscarinic effects are the retina (Hutchins 1994) the eye's ciliary body (Farahbakhsh and Cilluffo 1994), salivary gland (Iwabuchi and Masuhara 1992), pancreas (Kato et al. 1992), tracheal smooth muscle (Thomas and Ehlert 1996), adrenal cells (Aguilar et al. 1992), gut smooth muscles (Reddy et al. 1995), the spleen (Sandberg, 1994), kidney cells (Mohuczy and Garg 1992), the bladder (Kumamoto et al. 1990), gallbladder smooth muscle (von-Schrenck et al. 1993) and lung (Mak et al. 1992). Immune system cells (thymocytes and lymphocytes) are also sensitive to muscarinic chemicals (Kubera et al. 1992).

Potential Pesticide Synergists of PB Toxicity. This table is a partial list of pesticides ordered through the federal supply system for operations Desert Shield and Desert Storm (U.S. Senate 1995b). The insecticides with question marks (?) have not yet been evaluated for the ability to potentiate the toxicity of PB.

Pesticide	Insecticide Class	Synergizes PB?
permethrin	pyrethroid	yes
chlorpyrifos	organophosphate	yes
lindane	organochlorine	yes
DEET	repellant	yes
propoxur	carbamate	?
carbaryl	carbamate	?
diazinon	organophosphate	?

dichlorvos	organophosphate	?
methomyl (Fly bait)	carbamate	?
malathion	organophosphate	?
pyrethrins	pyrethroid-like	?

Of these insect control chemicals, DEET, permethrin and lindane are designed to be used in a manner that was likely to involve close personal human contact. Interest in the synergism of PB by DEET and permethrin arose as a result of disclosures to the U.S. Senate Veterans' Affairs Committee (U.S. Senate 1995a) that DEET and permethrin caused increased PB toxicity in cockroaches. Abou-Donia et al. (1996b) recently reported that the organophosphate insecticide chlorpyrifos, PB, and DEET interact synergistically.

The pesticides discussed below potentiate PB toxicity in various animals. Little is known about the specific mechanisms of these synergistic mechanisms. It will be difficult to predict whether these interactions would cause chronic health consequences until the specific mechanisms of synergistic interactions are understood.

Permethrin. Permethrin is a pyrethroid insecticide. Pyrethroids are generally thought to kill by modifying sodium channel function in nerve fibers. This leads to excessive leakage of sodium ions in nerve fibers which leads to excessive depolarization and excitation of the neurons (Matusmura 1985). Pyrethroid insecticides can also directly inhibit an enzyme that removes (pumps) calcium from inside cells of the rat brain (Alrajhi1990). Combined effects of PB (increased calcium leakage into the cells) plus permethrin (blocked calcium removal by pumps) could lead to a co-synergistic increase by these chemicals on cellular calcium. The outcome would be potentiation, by permethrin, of PB induced damage. Calcium loading, and subsequent damage, would be possible in tissues that had muscarinic (PB) receptors and permethrin sensitive calcium pumps.

PB toxicity is potentiated by permethrin in cockroaches (Moss 1996), chickens (Abou-Donia et al. 1996a), and rats (McCain et al. 1997). It is not clear whether this potentiation was caused by permethrin's actions on sodium channels, calcium pumps, or another action of permethrin. Abou-Donia et al. (1996a) suggested that, in chickens, PB prevented the breakdown of permethrin, that the permethrin action was responsible for the toxicity, and that PB was simply increasing the permethrin concentration (and therefore its effect). However, the damage and clinical signs reported in this study (Abou-Donia et al. 1996a) were similar to the results of organophosphate induced delayed neuropathy (OPIDN) and not pyrethroid poisoning. In addition to this, Buchholz et al. (1997) found that when rats were simultaneously dosed with PB and permethrin, PB caused the central nervous system tissue levels of permethrin to be lowered by 30%.

Either pyrethroid mechanism (sodium or calcium disruption) can lead to an ion imbalance within nerve cells which can lead to over-excitation and eventual direct damage to the nerves (LoPachin and Lehning (1997)). This over-excitation also leads to an inappropriate release of neurochemicals from nerves that leads to secondary physiological effects (Bowman and Rand 1980). Any of these permethrin effects have the potential to synergise the primary action of PB, or PB's known and potential side effects. The long term consequences of a simultaneous exposure to PB and permethrin cannot be predicted without knowledge of which biochemical effects are responsible for the synergism of PB toxicity.

Chlorpyrifos. Chlorpyrifos is an organophosphate (OP) insecticide which inhibits acetylcholinesterase. It can also cause organophosphate-induced delayed neuropathy (OPIDN) (Fikes et al. 1992). Because OPIDN may be related in some way to the disruption of calcium levels in cells (Abou-Donia 1993), the possibility also exists that some interaction between PB and chlorpyrifos is from the effects of both compounds on calcium maintenance in nerve cells.

PB and chlorpyrifos potentiate the toxicity of each other in chickens. A suggested reason for this was that both compounds block a detoxifying esterase enzyme that breaks down both chemicals. The neuropathy was attributed to the action of chlorpyrifos which was synergized because its breakdown was prevented by PB (Abou-Donia et al. (1996b)). The authors suggested that these combined chemicals may be responsible for some manifestations of chronic illnesses in Persian Gulf War veterans. It was also suggested that the neuropathy seen was not from the effects of neurotoxic esterase (NTE) inhibition, but the symptoms reported were consistent with the effects of neurotoxic esterase (NTE) inhibition (Lotti et al. 1993, Johnson 1990).

Other Pesticides. Other pesticides may have been locally obtained. Those from the OP, carbamate and pyrethroid classes of pesticides have the potential to synergize PB toxicity because of similar modes of action. No information was found that ruled out or confirmed synergism of PB toxicity by those pesticides.

DDT is available outside of the U.S. and may have been present in the Persian Gulf. DDT does not strictly fit into the above classes, however, the mode of action of DDT is close to that of the pyrethroids in insects and vertebrates (Matusmura 1985). PB potentiates the toxicity of DDT in cockroaches and DDT may potentiate PB toxicity (Moss, unpublished data). It is therefore possible that DDT would also be a PB synergist in mammals.

Lindane. Lindane is a common organochlorine de-lousing agent. Lindane toxicity is potentiated fourteen fold in cockroaches by a sub-lethal dose of PB (Moss, unpublished data). No published research was found that dealt with synergism between PB and lindane on vertebrates. Lindane blocks inhibitory actions in the nervous system which results in over-excitation (Matusmura 1985). One of the side effects of lindane is the inhibition of a calcium ATPase, a pump that removes calcium from cells (Basavarajappa and Salimath 1990). Combined effects of PB (increased calcium leakage)

plus lindane (blocked calcium removal by pumps) would probably lead to a co-synergistic increase by these chemicals on cellular calcium. The outcome would be potentiation, by lindane, of PB induced damage. Calcium loading, and subsequent damage, would be possible in tissues that had muscarinic (PB) receptors and lindane sensitive calcium pumps. Synergistic interactions between PB and lindane in vertebrates should be investigated.

DEET (N,N-Diethyl-m-toluamide). The insect repellent DEET was developed by the U.S. Department of Agriculture in the 1950's (McCabe et al. 1954). The mechanism(s) of the repellent and toxic action(s) of DEET are still unknown. Some reports indicate that excessive doses of DEET may be toxic to humans (Clem et al. 1993, Lipscomb et al. 1992, Schaefer and Peters 1992) and non-human vertebrates (Mount et al. 1991, Schoenig et al. 1993, Verschoyle et al. 1992).

DEET and PB synergize each other's toxicity in cockroaches (Moss 1996), rats (McCain et al. 1997), chickens (Abou-Donia et al. 1996a), and mice (Chaney et al. 1997a). In chickens, the synergism of DEET has been attributed to blocking of degrading enzymes (esterases) by PB so that more DEET could cross the blood-brain barrier (BBB) (Abou-Donia et al. 1996a).

We cannot understand the sub-lethal, possible long term consequences of this chemical mixture of PB and DEET without knowing DEET's mode of action. One cannot tell from current experiments which of the two (DEET or PB), is the primary toxicant, the synergist, or if both contribute to synergism and toxicity.

Moss (1996) hypothesized that DEET might have actions similar to the insect neurochemical octopamine, or the human neurochemical adrenaline. Based on that speculation, Chaney et al. (1997a,b) tested the ability of both DEET, adrenaline, and adrenergic drugs to potentiate the toxicity of PB. Chaney et al. (1997a) found that both DEET and beta-adrenergic drugs (including the native neurochemical adrenaline) synergised the toxicity of PB in mice. The synergistic interactions between PB and DEET, and PB and adrenergic drugs, were probably caused by the muscarinic side effects of PB because atropine (a muscarinic receptor blocker) eliminated the synergistic interactions (Chaney et al. 1997a). DEET's synergism of PB toxicity may be the result of adrenergic effects of DEET.

The possibility that PB will synergise the effects of adrenergic stimulation should also be investigated. In preliminary experiments (J. Moss and J. Schiffenbauer, unpublished data) it was found that PB and salbutamol (a beta-adrenergic PB synergist in mice [Chaney et al. 1997a,b]) interacted synergistically in mouse T-lymphocytes. Combined, PB and salbutamol inhibited mouse T-cell proliferation while the same drugs alone had no effect. Adrenergic drugs were originally investigated because DEET mode of action research raised the possibility that DEET had adrenergic effects. The effects on lymphocytes might range from subtle short-term effects which could be stimulation or suppression, depending on the particular type and stage of development of the cells or the effect could be outright mortality of the cells.

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