
INTERACTIONS BETWEEN PB AND OTHER EXPOSURES

DO INTERACTIONS BETWEEN PB AND OTHER EXPOSURES ENHANCE THE TOXICITY OF EFFECTS?

It is possible that PB may act synergistically with other exposures to lead to adverse or toxic effects that may relate to illnesses in PGW veterans. Some have referred to a putative “toxic cocktail” (Shays, 1997), or to “cumulative health consequences of exposure to multiple risk factors” (Metcalf, 1997) including PB, vaccines, chemical weapons, pesticides, depleted uranium, infection, and smoke from oil fires.

Many ambiguities exist regarding drug-drug and drug-chemical interactions. We know that they are common and often not predicted by the effects of individual drugs given separately; moreover, no FDA regulations require testing of drug combinations for individually approved or licensed agents. While personnel involved in the PGW experienced many drug and chemical exposures, it is not known with certainty whether interactions between these exposures contributed to illnesses in PGW veterans.

However, common mechanisms of effect among certain exposures (such as AChE inhibition by PB, nerve agents, and pesticides), and common side effects of certain exposures (such as enhanced permeability of the blood-brain barrier with chemical mixtures, stress, and aluminum used in vaccine adjuvants) make an effect of drug-chemical interactions possible. These may have contributed to reported illnesses in PGW veterans. Additional research is needed to evaluate the possible impact on reported illnesses of combinations of agents and exposures experienced in the PGW.

This chapter addresses the evidence available regarding the possible role of interactions and illnesses in PGW veterans. It will

- Briefly cite some findings of the Institute of Medicine (IOM) report, *Interactions of Drugs, Biologics, and Chemicals in US Military Forces*

- Review and extend the concept, introduced by the IOM, of matrices of toxicity as a source of investigation for interactions
- Inquire what can be learned from other populations with multiple drug exposures
- Address the evidence regarding interactions between PB and other drug and chemical exposures in the PGW
- Suggest a strategy for investigating PGW drug interactions.

IOM APPROACH TO DRUG/VACCINE INTERACTIONS

The Committee to Study the Interactions of Drugs, Biologics, and Chemicals in U.S. Military Forces, from the Medical Follow-Up Agency of the Institute of Medicine (IOM), drafted a report entitled *Interactions of Drugs, Biologics, and Chemicals in US Military Forces* (Committee to Study the Interactions of Drugs, 1996), to which the interested reader is referred. This 80-page report describes one general approach to investigating interactions, which will not be reprinted in detail here. This report correctly identifies drug interactions as a potential problem when multiple drug or chemical exposures are present, notes the paucity of evidence regarding the scale of the problem with drug interactions, and observes that it is difficult to obtain such data. It identifies difficulties with studying toxic interactions among drugs, due to the rapid growth of possible interactions as more exposures are added. (With n exposures there are on the order of 2^n potential interactions—not counting the need to study different dosage ranges.) And it formulates recommendations for surveillance and testing for interactions in the military.

Relevant points from the IOM report include the following:

- Whereas adverse effects of most single products have been relatively well studied (for instance in data submitted to the FDA for approval of a new drug), for most drugs it is largely unknown whether their combined use may provoke unanticipated reactions.
- The epidemiology of drug interactions is poorly understood, because very little of the literature on drug interactions has resulted from epidemiological investigations. Data derive instead from pharmacokinetic or pharmacodynamic studies, case reports, review articles, and animal and in vitro studies. Consequently, little is known about how often drug interactions actually occur and how often they produce clinically meaningful adverse effects.
- The published scientific literature on the interactions of militarily relevant drugs, biologics, and chemicals does not provide an adequate basis for

assessing the degree of safety; however, no basis was found for extraordinary concern.

- Vaccines have been shown to affect the metabolism of other drugs, possibly by interfering with human liver cytochrome P450 isozymes.
- Newly discovered interactions are not likely to exactly mimic previously described disease and may indeed have unique presentations.
- The process of coding medical information can change that information. Often sentinel events are more difficult to recover from a system once they have been coded.
- Unpredictable interactive toxicities are certain to occur. Thalidomide, benoxaprofen, and other instances are cited. Even less predictable toxicities should be expected when complex mixtures of agents are used together.

To these the following comments may be added:

- Drug interactions are common.
- Typically, adverse effects of drugs (such as rash or GI symptoms) terminate when the drug is discontinued, but many known exceptions exist. For example, dexfenfluramine was recently removed from the market followed discovery of frequent heart valve abnormalities associated with use of fenfluramine (often in combination with phentermine) as a diet agent. This, and the finding of pulmonary hypertension with fenfluramine, represents recently identified instances of effects that persist beyond drug discontinuation.
- The mechanism of adverse effects is often not understood.

MATRICES OF TOXICITY

The IOM provides a table of sites of action and toxicity for certain agents and suggests that such a table can direct research to sites of common effects between agents. The rationale is that sites of common action or toxicity might more likely be sites at which interactive toxicity may take place.

This approach has value, but also limitations. It is limited conceptually—since interactions may occur from effects on *different* systems that themselves interact. More practically, the approach is limited by the depth of inquiry used to ascertain the actions of the drug in question. Thus, it is constrained both by *available* knowledge (information available in the scientific literature) and per-

haps equally by *accessed* knowledge (the subset of the available literature accessed for use in the matrix).

PB was included in the IOM matrix and provides one example pertinent to the present discussion. Using this matrix, we would not be concerned about interactive toxicities related to, for instance, the gastrointestinal or cardiac system. In the IOM report, the nervous system was the sole cited locus of action or of toxicity for PB. However, PB should have also been noted for toxicity or for site of action in virtually all of the other categories named, and more (Table 9.1). For example, *mucous membranes* are influenced by PB to produce increased secretions, or to reduce mucociliary clearance if ACh downregulation occurs. This latter response could potentially enhance pharyngeal sensitivity to chemical or infectious exposures (see Chapter Eleven, "Multiple Chemical Sensitivity," section on nasopharyngeal factors). The *airways and lungs* are affected by the muscarinic and nicotinic properties of PB, producing bronchorrhea and airway constriction (see Chapter Three's section on muscarinic effects). Indeed, severe exacerbations of asthma in response to PB led some asthmatic PGW personnel to be flown from the theater. *Cardiac* effects not only exist but are expected; generally, relative bradycardia is produced with routine PB administration, with an average five beat per minute reduction in heart rate in persons (see Chapter Three, section on "Side Effects"). Tachycardia may also be produced if the nicotinic effects exceed the muscarinic influences on heart rate. *Hepatic* effects in the form of competition for the hepatic cytochrome P450 system, may be responsible for part of the synergistic toxicity seen with PB and pesticides. *Musculoskeletal* effects are well known; indeed, PB is used therapeutically in myasthenia gravis with the intent of influencing the neuromuscular junction, and motor endplate toxicity with PB is well described (see Chapter Twelve, "Neuromuscular Junction Effects"). Musculotendinous junctions and sarcolemma and sarcoplasm are other sites with cholinergic involvement. ACh receptors exist on *hematologic* (Sastry and Sadavongvivad, 1979) and *immune* cells, so that PB has a potential site of action for these systems (Chapter Fourteen, "Other Considerations"). *Gastrointestinal* effects are common (including cramping, increased intestinal secretions and diarrhea), due to muscarinic effects of PB (see Chapter Three). (The "enteric nervous system has more neurons than the brain" (Patrick, 1997)). *Urologic* effects can occur due to ureteral spasm from muscarinic effects (see Chapter Three). *Endocrine* effects are widely known; indeed, PB is used in testing procedures to induce growth hormone secretion (see Chapter Fifteen). Effects on the *reproductive* system might be anticipated based on evidence that cholinergic signaling is involved in chorionic villi, which express the BChE gene; in the placenta; and in sperm motility, in which ACh, AChE, ChAT (choline acetyltransferase, the enzyme catalyzing production of ACh), and BChE are present (Sastry and Sadavongvivad, 1979; Schwarz, Glick, et al., 1995) (see also Chapter Fifteen).

Table 9.1
Sites of Action of PB; Possible Sites at Which Interaction
May Occur with Other Drugs and Chemicals

Site Of Toxicity	IOM Version	Expanded Version
Nervous system	X	X
Mucous membrane		X
Heart		X
Liver		X
Lung/airway		X
Musculoskeletal		X
GI		X
Reproductive		X
Urinary		X
Hematologic		X
Immune		X

It is possible—perhaps likely—that similar in-depth knowledge of effects of the other identified drugs used in the PGW would lead to identification of effects on many systems rather than the one or two noted in the report. Thus, the strategy of concentrating on interactions in tissues and systems for which there is known toxicity or site of action may be useful in understanding the reason for interaction but less useful in restricting which interactions warrant concern and further study. The prospect of studying all those interactions suspected by commonality of sites of action, using the more complete PB matrix (and more complete matrices for other agents) could be understandably daunting. An alternative approach to evaluating interactive toxicity will be suggested below, although this approach has its own limitations. See Table 9.2 for a summary of known binary interactions.

WHAT CAN WE LEARN FROM OTHER POPULATIONS WITH MULTIPLE DRUG EXPOSURES?

The elderly constitute one population at risk for multiple drug exposures. In this population, as in medically ill populations on multiple medications, it is widely understood that medication effects and interactions represent a common source of morbidity and that drug effects and drug interactions must be considered when new unexplained problems arise. The problem arises in part from the multiplicity of agents and in part from reduced capacity to metabolize administered drugs.

Deployed troops represent another group that may be, in some instances, exposed to multiple medications and other chemicals. Although deployed troops are younger and more likely to have robust liver and kidney function and

Table 9.2

Data Matrix: Interactions Between PB and Selected Exposures

	PB	Nerve Agent	Pesticide	Insect Repellent	Stress	Adrenergic agents	Caffeine	Cipro	Anti-histamines	Vaccines	DU
Demonstrated interaction	NA	+	+	+	+	+	+	-	±	-	-
Closest Model	Primate	Rodent; hen; cockroach	Rodent; hen; cockroach	Rodent	Rodent	Rodent	Rodent				
Evidence Theory	(R)CT ^a ACh action	(R)CT ACh action; common metabolism	(R)CT Enhanced absorption of PB; common metabolism	(R)CT Enhanced penetration of PB into NS	CT Enhanced penetration of PB into CNS	(R)CT Enhanced penetration of PB into NS	Indirect GABA; musculo-skeletal; liver	Indirect ACh	Indirect Liver metabolism; blood-brain barrier	No theory	

^a(R)CT = (Randomized) Control Trial

are therefore more likely to metabolize foreign products expeditiously, here, too, exposures to multiple drugs and chemicals should be considered for their possible role if untoward health effects occur. This is especially true in light of evidence that some of these drug interactions may influence the metabolic systems that normally allow younger persons to tolerate medications with relative facility.

Populations with multiple vaccine exposures will be discussed separately (see *A Review of the Scientific Literature As It Pertains to Gulf War Illnesses*, Vol. 8: *Immunizations* (Golomb, forthcoming)).

SPECIFIC INTERACTIONS OF PB WITH OTHER FACTORS

Interactions with Pesticides and Insect Repellents

Much of the interest in interactions related to PB has concentrated on interactions with pesticides and insect repellents. Several pertinent studies will be reviewed here. Studies have demonstrated additive or synergistic effects of PB with the insect repellent DEET, and with the pesticides permethrin and chlorpyrifos. To date, such studies have been performed in cockroaches, hens, and rats. Further work in this area is ongoing.

Interactant: DEET. DEET (*N,N*-diethyl-*m*-toluamide), an aromatic amide, is an EPA-approved personal insect repellent widely used commercially, though it is known to produce mammalian CNS effects at high exposures by an unknown mechanism. Cases of acute toxicity and death in humans (primarily in children (Osimitz and Murphy 1997)) have been reported following dermal application (De Garbino and Laborde, 1983; Heck, Shipman, et al., 1980; Roland, Jan, et al., 1985; Edwards and Johnson, 1987; Gryboski, Weinstein, et al., 1961; Zadikoff, 1979). Symptoms of DEET toxicity include tremor, restlessness, slurred speech, seizures, impaired cognition, and coma (McConnell, Fidler, et al., 1986). Near lethal doses in rats produce spongiform myelinopathy in cerebellar roof nuclei (Verschoyle, Brown, et al., 1990). The nervous system may not be a selective target for DEET (Schoenig, Hartnagel, et al., 1993; Osimitz and Grothaus 1995; Schoenig, Hartnagel, et al., 1996), and possibly some of the apparent neurotoxicity may be mediated by DEET's postulated ability to allow penetration to the CNS by other agents. (DEET is known for its permeability enhancing effects (Windheuser, Haslam, et al., 1982; Kondo, Mizuno, et al., 1988); however one study found that DEET did not enhance, and in some preparations reduced, absorption of carbaryl and permethrin (Baynes, Halling, et al., 1997).)

Several formulations, including 75 percent DEET in ethanol formulation, a 33 percent extended-duration formulation, and 19 percent in stick were prepared

for the U.S. Army. Low usage of insect repellent was reported during the conflict. The cool climate at the time of the war (January and February 1991) resulted in few biting insects, though exposure may have been higher at other times during the deployment. However, these instances would probably not have coincided with PB use. (See also Geschwind and Golomb, forthcoming).

Interactant: Permethrin. Permethrin is a third-generation synthetic (Type 1) pyrethroid insecticide with four stereoisomers (that is, chemicals with the same constituents but different configurations; they may be “cis” or “trans” and “+” or “-”), that often provides insecticidal activity for several weeks following a single application. Permethrin has some esterase-inhibiting activity, as well as other mechanisms of toxicity.¹ Symptoms include hyperactivity, tremor, ataxia, convulsion, and paralysis; peripheral nerve damage has been produced by a near-lethal dose in rats (Abou-Donia, Wilmarth, et al., 1996b) (Rose and Dewar, 1983). Permethrin is metabolized by enzymes termed “hydrolases” and “oxidases”; both esterase and oxidase inhibitors may enhance toxicity in mammals (Pelligrini and Santi, 1972; Casida, Gamman, et al., 1983; Abou-Donia, Wilmarth, et al., 1996b).

Permethrin is EPA approved and commercially available. Aerosol spray cans with 0.5 percent aerosol in a two-gallon compressed air sprayer were available to fewer than 5 percent of deployed units, for impregnation of battle dress uniforms (McCain, 1997). The aerosol spray can method is thought to provide protection for about six washings, or six weeks of use (McCain, 1997). Data in rabbits suggest that permethrin may be resistant to removal with laundering, and may continue to be absorbed into the skin at a constant rate after many washings (Snodgrass, 1992). These data were also noteworthy for one animal that persistently absorbed markedly more than the others, suggesting that dermal absorption differences may constitute yet another of many sources of possible individual differences (see previous Chapter Eight, “Individual Differences in Reactions to PB”).

Interactions among pesticides are not always potentiating. For instance, in one study in rats, the addition of 380 mg/kg and 464 mg/kg of methyl parathion reduced the LD₅₀ of permethrin by 9 percent (NS) and 37 percent ($p < 0.001$) respectively (Ortiz, Yanez, et al., 1995). Permethrin reduced the cholinesterase inhibition of parathion by 50 percent. (These doses are extremely high and bear no relation to exposures that could have occurred in PGW veterans.) (See also Geschwind and Colomb, forthcoming).

¹Other mechanism of permethrin toxicity include delayed closure of sodium channels during a depolarizing pulse, evoking repetitive after-discharges by a single stimulus, inhibition of calcium-magnesium ATPase, and inhibition of calmodulin (Narahashi, 1985) (Abou-Donia, Wilmarth, et al., 1996b).

Interactant: Chlorpyrifos (Abou-Donia, Wilmarth, et al., 1996b). Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloropyridinyl phosphorothioate) is a phosphorothioate insecticide used in the PGW that undergoes first pass metabolism to chlorpyrifos oxon, which in turn inhibits rat brain AChE in vitro (that is, in studies in rat brain preparations not performed in a living animal). Chlorpyrifos toxicity results in muscarinic, nicotinic, and CNS symptoms (see Chapter Three, “Characteristics of PB”), and sensory and CNS toxicity may ensue (Kaplan, Kessler, et al., 1993; Moretto and Lotti, 1998). Moreover, near-lethal doses may produce OPIDN (OP-induced delayed neurotoxicity—also called “OPIDP,” or OP-induced delayed polyneuropathy—see Chapter Fourteen, “Chronic Effects”) in humans (Abou-Donia, Wilmarth, et al., 1996b; Lotti and Moretto, 1986). Doses equivalent to two to three times the LD₅₀, with protection from lethality, produce OPIDN in the hen, suggesting that some humans may be more sensitive relative to the lethal dose than hens. OPIDN consists of delayed protracted ataxia (gait incoordination) and paralysis accompanied by a “Wallerian-type” degeneration of the central and peripheral nervous system. Concurrent exposure to other agents, including an organophosphorus insecticide that does not produce OPIDN, may reduce the threshold dose of chlorpyrifos that produces OPIDN (Abou-Donia, Wilmarth, et al., 1996a). Chlorpyrifos produces an unusually prolonged dose-dependent fall in the activity of brain AChE, lasting for weeks after treatment ends (Chiappa, Padilla et al., 1995), and therefore it might participate in interactions with other agents well after the time of administration.

PB Interaction Studies

Cockroach Study. (PB, lambda-cyhalothrin, permethrin) (Moss, 1996). Synergism of DEET with lambda-cyhalothrin, permethrin, and PB. Studies in adult male German cockroaches demonstrated that DEET toxicity was increased by lambda-cyhalothrin and permethrin as well as by PB. Specifically, the LD₅₀ for DEET, in µg/g, was reduced from 2,711 to 404 with addition of PB at a dose of 2,049 µg/g, an effect that was highly statistically significant. DEET, in turn, at a dose of 7.003 µg/g, contributed synergistically to the toxicity of pyridostigmine, changing the LD₅₀ from 7.003 to 1,868, an effect that was statistically significant. Synergism was also produced by DEET for malathion and carbaryl, but not bendiocarb or chlorpyrifos.

Hen Studies (Abou-Donia, Wilmarth, et al., 1996c). Hens are used as a model of human anticholinesterase effects because hens, like people (and unlike many mammals) are susceptible to anticholinesterase effects, including OPIDN.

Doses of PB (5 mg/kg in water—about 12 times the PB dose, unadjusted for route), DEET (500 mg/kg, “neat”—that is, undiluted), and chlorpyrifos (10

mg/kg in oil) were given parenterally five days a week for two months to hens. Individually these doses produced no deaths (although they produced clinical illness). In combination they led to mortality (or euthanization due to severe illness) in approximately 20 percent with PB and chlorpyrifos, 35 percent for PB and DEET, 65 percent for all three; and 75 percent for DEET plus chlorpyrifos. Significant reductions in body weight (up to 20 percent) were seen for chlorpyrifos alone and for all combinations.

A measure of severity of clinical signs, as well as locomotor dysfunction (abnormal walking), histopathological changes in the cervical and thoracic spinal cord, and the mean of ranked scores of these tests were all increased in hens given drug combinations compared to hens given any drug individually. These abnormalities were greatest in those hens given all three agents. Histopathological alterations were increased with all combinations but were greatest with PB plus chlorpyrifos.

Plasma BChE (see Chapter Eight, "Individual Differences in Reactions to PB") was significantly reduced with each drug individually, and with all combinations. (This reduction was maximal with PB and combinations including PB; and values were as low as 10 percent of control with PB and DEET; however, these employ levels that produce significant immediate toxicity.) Brain AChE was reduced with chlorpyrifos (to about 70 percent of control) and to a greater extent with all combinations, including chlorpyrifos (to approximately 30 percent of control with DEET, 55 percent of control with PB, and 25 percent of control with all three). Neuropathy target esterase was significantly reduced with all combinations containing chlorpyrifos (to about 75 percent of control in all cases) but not with chlorpyrifos alone.

Similar findings were produced in another study in hens, by the same research group, involving PB, DEET, and permethrin (Abou-Donia, Wilmarth, et al., 1996c). Doses that resulted in minimal toxicity when given individually produced more significant toxicity when given in combination. Measures used included clinical evaluation (of walking, flying, tremor, leg movement, ability to enter home cage, weight loss, and death), histopathological assessment of spinal cord and sciatic nerve, BChE and AChE determination, and a neurotoxicity score using the above factors. No mortality was seen in the control hens or with those given a single drug (PB, permethrin, or DEET), but combinations produced about 20 percent (PB+P), 40 percent (D+P, or D+PB), or 80 percent mortality (PB+D+P). Days to onset of clinical signs were affected with all single drugs except permethrin alone, and with all drug combinations. (All three drugs in combination led to clinical signs within several days; this compares to no signs by 60 days—the end of the experiment—in control and in permethrin animals.) Locomotor dysfunction (problems walking) resulted from all drug combinations but not from single drugs, and the same results

were found with tremor (Abou-Donia, Wilmarth, et al., 1996b, 1996c). Neuro-pathic changes were nonexistent in PB+P, mild in D+P, mild to moderate in PB+D, and mild to severe in PB+D+P.² Plasma BChE activity was markedly reduced to 83 percent of that of the control group in D+P ($p < 0.05$), to 26 percent with PB+D+P, to 20 percent with PB+P, and to 8 percent with PB+D (all $p < 0.01$). No changes were seen in brain AChE with any treatment. A “Mean Rank” score, based on ranking in the above categories, was significantly greater for two than for one agent, and for three than any combination of two agents in all cases except one: PB+P results did not differ significantly from P alone. It must be emphasized that these studies used extremely high doses of P, PB, and D.

Rats (McCain, 1997). Studies have sought to evaluate potential toxic interactions of PB, DEET, and permethrin given by gastric lavage (that is, by tube through the mouth to the stomach) to male rats. Rats are more closely related to humans than are hens or cockroaches, though they are not susceptible to certain adverse effects of cholinesterase inhibitors that have been identified in humans and hens (such as OPIDN). An oral route for PB more closely mimics the PB route of administration to veterans in the PGW. DEET and permethrin were also administered orally, in contradistinction to the probable route of delivery in PGW veterans, who would most likely have been exposed through skin contact or perhaps by inhalation. However, the oral route allows more control over dosing than the dermal and inhalational routes.

This study determined the LD₁₆ (dose that was lethal in 16 percent of rats) for all three agents individually, then determined what happened to rats when two of the three were given at the respective LD₁₆, and the third was varied in dose. A clear synergistic effect (more than additive toxicity) was demonstrated. While only about 10 percent of animals died when given permethrin and DEET each at its respective LD₁₆ (compared to 32 percent predicted, or 16 percent for each drug), when the LD₁₆ of PB was added (45.76 mg/kg or 107 times the PGW dose) mortality rose to approximately 90 percent—far more than the 26 percent expected on top of the dual drug effect or the 48 percent expected if the three drugs behaved independently. (Actually, the predicted deaths should be slightly less than 26 percent and 48 percent assuming independence of effect, because some of the animals that would have been killed with the first drug, or a pair of drugs, might be the same that would have been killed with the second; therefore, the actual predicted rates would have been 24 percent and 41 percent. Consequently, the synergism is more striking.) Similar studies were con-

²These results reinforce the idea that individual variation in the development of illness is the rule, even in genetically homogeneous animals with highly similar exposures. Such variation would be expected a fortiori in genetically heterogeneous humans with highly diverse exposures (see Chapter Eight).

ducted holding PB and DEET at their LD₁₆s and adding different doses of permethrin; or holding PB and permethrin at their LD₁₆s and adding different doses of DEET. In each case, toxicity—gauged by lethality—exceeded that expected by independence.

Mice Studies. One study in ICR mice, to date published only in abstract form, found that combinations of PB (2.3 mg/kg, or 5.2 times the PGW dose, intraperitoneally) and DEET (200, 300, 400, 550 mg/kg intraperitoneally contralaterally) resulted in a significant increase in lethality compared to either agent alone (Chaney, Moss, et al., 1997). Moreover, caffeine (5 mg/kg) given 15 minutes prior to one or both led to increased PB but not PB/DEET lethality in mice. Selected adrenergically active agents enhanced lethality of PB alone (α blockers, including phentolamine (mixed α 1/ α 2) 1 mg/kg; prazosin (α 1) 2 mg/kg; yohimbine (α 2) 1 mg/kg). Several enhanced PB and PB/DEET lethality (α - and β -agonist epinephrine 5 mg/kg; β 1 and β 2 agonist isoproterenol 3 mg/kg; and a β 2 agonist salmeterol 0.4 mg/kg). Several other agents did not significantly increase either PB- or PB/DEET-induced lethality (α 2 agonist clonidine, 1 mg/kg; β 1 antagonist acebutolol 1, 5 mg/kg; β 1 β 2 antagonists propranolol 1.5, 3 mg/kg and nadolol 1, 5 mg/kg; and β 2 agonist terbutaline 1 mg/kg) (Chaney, Moss, et al., 1997; Hardman, Limbird, et al., 1996).

Suggested mechanisms. Several possible mechanisms for an interaction have been proposed (McCain, 1997; Abou-Donia, Wilmarth, et al., 1996b, 1996c).

First, it has been suggested that DEET (and perhaps permethrin) may act as a permeability-enhancing agent for PB and pesticides; or more generally that concurrent exposure to chemicals may increase their absorption. PB is poorly absorbed by the gut and has a steep dose-response curve, so that enhanced absorption leading to increased bioavailability of PB could lead to increased toxicity or lethality (McCain, 1997). It has further been suggested that the epidermis may serve as a depot site for DEET, resulting in its slow release into the circulation (Blomquist and Thorsell, 1977; Spencer, Hill, et al., 1979; Snodgrass, Nelson, et al., 1982), so that exposures need not be concurrent for synergistic interaction to occur. (Similarly, DEET could enhance the dermal absorption of such pesticides as chlorpyrifos, which are known to produce OPIDN). DEET has been used to increase permeability of other agents dermally (Windheuser, Haslam, et al., 1982; Hussain and Ritschel, 1988).

Second, enzymes including plasma esterases, hydrolases, and amidases are involved in metabolism of these compounds, enhancing their removal from the circulation by scavenging or breakdown into water-soluble metabolites (Abou-Donia, 1995). PB may bind to and inhibit nonspecific esterases (such as BChE) preventing detoxification of other chemicals, such as permethrin (an ester). More generally, the compounds may compete for metabolizing enzymes, lead-

ing to decreased breakdown and increased delivery of toxic compounds to nervous tissues (Abou-Donia, Wilmarth, et al., 1996b, 1996c; McCain, 1997).

Third, pyrethroids, like carbamates, are also metabolized by cytochrome P-450 in the liver (Kostka, Palut, et al., 1997). Once again, there may be competition for degradation leading to decreased breakdown and increased circulating levels of these agents (McCain, 1997). A mechanism in which there is an increased effective dose of the agents caused by competition for metabolizing enzymes is consistent with the result that inhibition of brain AChE and NTE was greatly amplified after combined exposures of PB and/or DEET with chlorpyrifos, possibly causing increased chlorpyrifos oxon in the brain tissue (Abou-Donia, Wilmarth, et al., 1996c).

Fourth, PB in concert with other agents may increase permeability of the blood-brain barrier, leading to enhanced access of toxins into the brain (see Chapter Seven, "Blood-Brain Barrier Passage"), including access of normally excluded PB to the CNS. Increased permeability of the blood-brain barrier may occur through vascular damage or trauma (perhaps including chemical trauma or alterations of membrane lipids by lipid-soluble pesticides and nerve agents), or it may relate to inhibition of BChE and AChE in the capillary wall and in astrocytes (supporting cells in the nervous system). Increased permeability of the blood-brain barrier has been reported with physostigmine (a centrally penetrating analog of PB) and with ACh (Greig and Holland, 1949), suggesting that AChE inhibition, or increased ACh activity, may itself enhance permeability of the blood-brain barrier, leading to increased access by toxins. Further evidence for a mechanism involving the blood-brain barrier is suggested by findings that "stress" (at least of certain types), has been reported to increase blood-brain barrier permeability in animal studies (see Chapter Seven); in other studies, adrenergic agents enhance PB- and PB/DEET-induced lethality (in mice) (Chaney, Moss, et al., 1997). These findings suggest that a stress-induced adrenergic surge, or heightened adrenergic tone, may play a role in the breach of the blood-brain barrier caused by stress.

Limitations. First, these studies are limited by the use of nonhuman animal models. Insects are phylogenetically distant from humans; hens are not mammals, though they share with humans a susceptibility to at least one long-term consequence of OP toxicity that most evaluated mammals do not, namely OPIDN; and mice and rats, although mammals, are neither close mammalian relatives nor ones that experience OPIDN. Animals differ in susceptibility to adverse outcomes from chemicals and chemical combinations compared to humans. Nonetheless, preservation of the finding of synergism between PB and other chemicals across such diverse species strongly suggests that such synergism, if not its particulars, is conserved and may occur in humans.

Second, these studies assume concurrent exposure. As previously noted, insect biting was viewed as infrequent in January and February, the times of the air and ground wars (PGW, 1997) when PB use is believed to have predominantly occurred. (However, some veterans report having taken PB for prolonged periods (Zeller, 1997; Hamden, 1997).) Insect biting did not increase until March, and pesticide use may have occurred predominantly starting in March, after most PB use is believed to have ceased. (Nonetheless, it is not possible to exclude significant use of pesticides in the theater before that time.) The effects of exposures staggered in time would likely differ, though it has been suggested that DEET, for one, may be stored in the epidermis for slow release into the circulation (Blomquist and Thorsell, 1977; Snodgrass, Nelson, et al., 1982; Spencer, Hill, et al., 1979), and permethrin, if uniforms are impregnated, may remain in the material and continue to be absorbed slowly through the dermis at a constant rate despite many launderings (Snodgrass, 1992).

Third, these studies employ different routes of exposure than those experienced by PGW veterans and employ doses (per kg of body weight) markedly in excess of doses that might conceivably have been experienced by PGW veterans. For instance, for the rat study, corresponding per-kg oral doses in a 70 kg person would be: 107 30-mg PB tablets; 23 6-oz cans of 0.5 percent permethrin; and six two-ounce tubes of 33 percent DEET. In the hen studies, the corresponding doses are 467 PB tablets, 1,667 permethrin cans, and 76 DEET tubes (Young, undated). However, while these doses vastly exceed those that might have been experienced by PGW veterans, the outcome measures—e.g., lethality—are also greatly in excess of the more subtle effects on sleep, energy, cognition, and joint pain experienced by many veterans. Thus, while these studies by no means confirm a connection between PB-chemical interactions and illnesses in PGW veterans, they are mutually consistent and clearly do not exclude such a connection. The persistence of additive or synergistic toxicity across species suggests that interaction studies need to be done in more physiologically plausible dose ranges—but also perhaps with more-sensitive outcome measures. In addition, none of these studies discontinued the exposures and performed long-term follow-up to determine whether persistent neurological problems were evident.

PB and Nerve Agents

Although PB is used in the military for *protection* against the effects of the nerve agent soman, in some instances no effect (Kerenyi, Murphy, et al., 1990) or potentiation of a toxic effect (Shiloff and Clement, 1986) for soman has been reported. (The studies finding no effects took place in rats. Moreover, PB doses in studies demonstrating a toxic effect are much higher than those used in the PGW.) PB confers protection against soman if given in advance of soman expo-

sure; however, additive toxicity, rather than protection, may occur if PB is administered following nerve agent exposure. (This is because PB will bind to, and inhibit, additional AChE, without “blocking” nerve agent from binding, since the nerve agent has already had the opportunity to bind AChE and undergo aging.) PB may lower the PR for some nerve agents (for lethality), but protection against lethality using standard postexposure protocols remains quite good despite addition of PB, at least in nonprimate mammals (see Chapter Three, section on “Military Uses”). Further work is needed on sublethal exposures and on primates. DoD reports that up to 100,000 troops may have been exposed to low levels of sarin in association with the Khamisiyah ammunitions depot demolition which occurred particularly on March 10, 1991 (U.S. troops are believed to have destroyed chemical weapons at Khamisiyah on March 4, 10, and 12 1991, but primarily March 10) (PGW, 1997; OSAGWI, 1997). The exposures, based on modeling of data guided by simulations, were to very low doses—below those needed to produce “first effects” (such as runny nose or tearing) but above the exposure limit considered safe for long-term exposure for the public. Most administered PB is believed to have been given in association with the air and ground wars, which began respectively on January 17, 1991, and February 24, 1991, with cease-fire declared February 28, 1991 (PGW, 1997). Thus, it would be expected that most personnel would have terminated use of PB prior to any exposure to sarin associated with the Khamisiyah incident.

PB and Combat Anesthesia

It has been noted that the presence of a chemical threat (with a consequent decision to administer PB) does not preclude injury by conventional weapons, with a subsequent need for surgery and concomitant anesthesia. Some agents used during anesthesia may interact with PB through cholinergic or anticholinergic effects, or through end-organ effects, and these interactions have been reviewed (Keeler, 1990). However, conventional casualties were rare in the PGW and need for surgery during use of PB was not common; PB-anesthetic agent interactions cannot be responsible for the illnesses currently seen in PGW veterans who did not have surgery.

PB and Stress; PB and Heat

Chapter Seven describes one possible interaction between stress and PB—enhanced access of PB to the CNS through increased permeability of the blood-brain barrier due to stress.

This study used a “forced swim” test in rodents as the encountered “stress.” It should be reinforced that the different classes of events that to which the term

“stress” may be applied do not produce uniform consequences. Rather, the many different forms of “stress” (heat stress, cold stress, grief, embarrassment, acute threat to safety, chronic threat to safety, sleep deprivation, hunger, exposure to a given toxin, exertion stress, illness stress, etc.) may have some effects in common but also have distinct biochemical signatures. As one example of differing effects, serum cholesterol typically rises sharply, with a time-course of hours, after certain types of emotional stress, an effect mediated by catecholamine-induced hemoconcentration (Wertlake, Wilcox, et al., 1958; McCabe, Hammarsten, et al., 1959; Dimsdale and Herd, 1982; Muldoon, Bachen, et al., 1992; Patterson, Gottdiener, et al., 1993); on the other hand, serum cholesterol typically plummets, with a time-course of days, after some kinds of major physical stressors such as surgery, childbirth, myocardial infarction, or severe illness (Goodman, Kellogg, et al., 1962; Ryder, Hayes, et al., 1984; Brugada, Wenger, et al., 1996; Ploekinger, Dantendorfer, et al., 1996), perhaps because cholesterol from the blood may be used to repair membranes in injured tissues. Thus, in the absence of detailed knowledge about the mechanisms of an effect, it cannot be presumed that consequences of stress from one type of stressor also occur with a distinct type of stressor (even if both can be broadly described as “physical,” or both as “emotional”). Specifically, it remains to be determined which forms of “stress,” in addition to forced swim, restraint stress, or exposure to heat (the latter two serve a facilitatory role), produce or enhance permeability of the blood-brain barrier.

A second possible interaction may occur through activation of nicotinic receptors on chromaffin cells in the adrenal medulla; increased ACh binding (from increased ACh availability due to PB) may enhance secretion of catecholamines or perhaps potentiate the ability of stress to do so.

Third, the heightened toxicity reported with interactions between PB and adrenergic agents, noted above, may result in part or in whole from effects of PB distinct from those on the adrenal medulla. Studies discussed in Chapter Fourteen report that physostigmine, a carbamate related to PB that readily crosses the blood-brain barrier even in the absence of stress, produces catecholamine surges by a central rather than peripheral mechanism.

A fourth, perhaps speculative mechanism by which PB could interact with stress, leading to death of cells in a region of the brain, is discussed in the footnote.³

³PB could interact with stress to produce chronic neurological change in an area of the brain termed the hippocampus, thought to be involved in memory. Stress produces release of corticosteroids. The hippocampus is uniquely rich in receptors for corticosteroids, and that provides negative feedback regulation for corticosteroids—which holds stress responses in check (Sapolsky, 1992). (Many receptors are also found in an area termed the amygdala that is mentioned in Chapter Eleven, “Multiple Chemical Sensitivity”; this area is also rich in receptors for corticosteroids but

PB and Caffeine

A study previously discussed also examined the effect of caffeine on toxicity from PB and DEET in mice (Chaney, Moss, et al., 1997); see the section on PB and pesticides, above.

PB and Fluoroquinolones

Fluoroquinolones, such as ciprofloxacin (used as an antidiarrheal agent in the PGW), are GABA inhibitors (Committee to Study the Interactions of Drugs, 1996). GABA is the major inhibitory neurotransmitter of the vertebrate nervous system (Saleh, Zied, et al., 1993; Allen and Albuquerque, 1987). GABA released from “interneurons” in the brain activates chloride ion channels in the receiving cell, changing their voltage to make them more resistant to “firing” or sending a signal to other cells. Bromide in PB may influence these chloride channels (see Chapter Ten, “Bromism”); PB may influence the GABA system by this means. Moreover, ACh modulates release of GABA presynaptically (Albuquerque, Boyne, et al., 1995; Lena, Changeux, et al., 1993) (see section on GABA in Chapter Three), so that PB and fluoroquinolones could interact through joint modulation of ACh. (This may be a subtractive interaction, however.)

Fluoroquinolones may also have musculoskeletal and liver effects (Committee to Study the Interactions of Drugs, 1996), offering additional potential sites of interaction.

PB and Nicotine

Interactions with nicotine have played little role in existing investigations into chemical interactions with PB. Because nicotine is a drug well known to affect the acetylcholinergic function—specifically, to amplify action at nicotinic receptors (discussed further in Chapter Thirteen, “Neurotransmitter Dysreg-

provides positive rather than negative feedback regulation to the corticosteroid system.) Particularly in an area of the hippocampus termed “CA1,” it has been shown that corticosteroids may lead to cell death under certain circumstances—for instance, when there is reduced blood flow or reduced oxygen (Sapolsky and Pulsinelli, 1985; Morse and Davids, 1990; Sapolsky, 1992). But if PB successfully enters the brain, it would engender excessive activity of ACh neurons (as it has been shown to do in studies using mice (Friedman, Kaufer, et al., 1996)), which would be expected to result in increased need for oxygen and bloodflow. If the needs for blood and oxygen exceed the ability to supply it, death of hippocampal neurons may ensue, potentially leading to problems with memory—and perhaps with other functions to which the hippocampus contributes—as well as increased subsequent stress responses. However, this speculation hinges on the supposition first that PB is able to access the brain; and second that the resulting need for blood and oxygen in this region is exceeded. At present these assumptions have not been assessed empirically, although studies in other species could be done. (Exertion, via exercise, might be anticipated to further divert flow of blood.)

ulation”)—it is imperative that future research related to PB interactions include potential interactions with nicotine.

PB and Vaccines

Anthrax vaccine was given to an estimated 150,000 individuals in the PGW, while botulinum toxoid vaccine was given to an estimated 8,000. There is evidence that some personnel, particularly special forces and perhaps some on the front lines, received both (see Golomb, forthcoming).

PB and vaccines might interact to increase permeability of the blood-brain barrier. Aluminum, contained in vaccine adjuvants for botulinum toxoid and anthrax vaccines, and cholinergic effects such as those anticipated with PB have both been demonstrated to enhance permeability of the blood-brain barrier (see Chapter Seven, “Blood-Brain Barrier Passage”). Whether this effect of aluminum takes place at the low doses employed in vaccines is not known. Moreover, other exposures may further interact with PB and vaccines to enhance permeability and promote breach of the protection of the blood-brain barrier. These include chemical cocktails involving PB, stress, and pesticides or nerve agent chemical combinations, all of which have also been implicated in increases in blood-brain barrier permeability in animal models (Chapter Seven).

It has also been suggested that at least some vaccines may influence the metabolism of other drugs, perhaps by interfering with the liver cytochrome P450 metabolizing system (Committee to Study the Interactions of Drugs, 1996; Kramer and McClain, 1981). Since the P450 system has been implicated in the metabolism of several drugs that interact with PB (see Chapter Eight, “Individual Differences in Reactions to PB”), the addition of vaccines could amplify the effects of drug interactions among agents that compete for metabolism through this system and through other systems, such as BChE. Finally, aluminum (in vaccines) may reduce the activity of both choline acetyltransferase and AChE, thus affecting the ACh system, another avenue for possible interaction with PB.

There is no direct evidence for an interaction between PB and vaccines used in the PGW. However, there are common sites at which both PB and vaccines may exert an effect, such as the blood-brain barrier and drug-metabolizing systems. Thus, interactions between PB and vaccines are “possible” in accordance with the IOM model, which recognizes possible interactions when there are common sites of effect. However, the presence of such interactions is purely speculative.

PB and Antihistamines

Antihistamines might be speculated to influence central effects of PB by several mechanisms. First, histamine modulates heat-stress-induced increases in blood-brain barrier permeability in animals (resulting from exposure to four hours at 38° C) (Sharma, Nyberg, et al., 1992): H-2 receptor blocker appear to protect against the increase in permeability, while H-1 receptor blockers may enhance it. Second, antihistamines may increase central ACh, possibly potentiating any central effects of PB. Histamine H-2 receptor blockers have been shown to have AChE-inhibiting effects, stronger for ranitidine and nizatidine than for cimetidine (Laine-Cessac, Turcant, et al., 1993); and histamine H-1 receptor blockers have been shown to increase central ACh and ACh action in animal studies (Dringenberg, De Souza-Silva, et al., 1998). The net effect for H-2 blockers is unclear; they may be protective if given before heat is encountered. However histamine H-1 receptor blockers might be expected to doubly heighten the effect of PB, both enhancing PB penetration in conditions of heat and further increasing the excess ACh available centrally. Personnel may have received H-2 blockers for GI complaints and H-1 blockers for allergic problems and perhaps sleep complaints.

PB's Effect on the Immune System

There is no direct evidence for an influence of PB on susceptibility to infection. However, several characteristics of PB would make such an interaction possible. During acute PB administration, enhanced mucociliary clearance due to ACh might be experienced. This acute effect might be reversed on discontinuation of PB if cholinergic downregulation occurs (see Chapter Thirteen). It is thus conceivable that infections transmitted through the "respiratory" route might be facilitated by prior administration of PB. Whether this effect occurs (and if so, what duration of treatment is required and for what duration the downregulation would occur) is not known, but is amenable to scientific inquiry.

The existence of ACh receptors located on immune cells (Schwarz, Glick, et al., 1995; Sastry and Sadavongvivad, 1979) suggests a role for cholinergic activity in the immune system. But the role of these receptors is not known. Heightened cholinergic activity with active treatment, and suppressed cholinergic activity due to downregulation following treatment, could influence these cells. The nature of the effect, and the consequences of a putative effect, are not known. Rates of infectious diseases (indeed, of all disease and nonbattle injury) among U.S. military personnel in Operation Desert Storm/Operation Desert Shield were quite low relative to wartime expectation (Hyams, Wignall, et al., 1995). Improved data on who received PB might allow evaluation of

infectious illness requiring hospitalization in those who did and those who did not receive PB. However, if an unidentified infectious agent were responsible for symptoms that do not lead to hospitalization in PGW veterans (see *A Review of the Scientific Literature As It Pertains to Gulf War Illnesses*, Vol. 1: *Infectious Diseases* (Hilborne and Golomb, 1998)), this approach might not uncover a connection.

In combination with other agents, PB may affect the blood-brain barrier (through its cholinergic effect and possibly through other effects) so that it more readily allows passage of infectious agents (see “Interactions,” below). Cholinergic action is believed to enhance the permeability of the blood-brain barrier; and studies have demonstrated that neuropathic viruses that are normally denied access to the CNS may cause fatal disease in animals when the blood-brain barrier is breached by administration of PB; aluminum in vaccines could, theoretically, participate in enhancing the permeability of the blood-brain barrier (see Chapter Seven). However, such neurovirulent but non-neuroinvasive viruses are rare, and there is no evidence of increased CNS infection in PGW veterans.

In summary, there are theoretical reasons PB could affect the immune system or enhance susceptibility to selected infectious diseases. However, there is no evidence that enhanced susceptibility to any class of infection was present in PGW veterans (but see Golomb, forthcoming, chapter on “Mycoplasma.”)

Interactions and Individual Differences

Individual differences occur in the properties of enzymes used to metabolize certain drugs, in the levels of such enzymes, in past exposures, and in other endogenous and exogenous factors. These differences might control which individuals experience effects with drug combinations, just as these factors influence which individuals experience benefit and adverse effects with individual drugs. Thus, for instance, individual differences in the enzyme “PON” may influence the rate at which certain cholinesterase inhibitors (other than PB) are broken down—such as certain pesticides and nerve agents. The resulting differences in amount of nerve agent or pesticide in the body may in turn influence the degree to which synergistic toxicity may be produced with the “same” dose of PB. (See Chapter Eight.)⁴

⁴For instance, one form of PON is relatively good at metabolizing sarin but relatively bad at metabolizing protein pesticides (Davies, Richter, et al., 1996). For the other form, the reverse is true. Thus, which form of PON is present will determine relative susceptibility to pesticide exposures (that is, levels of pesticide after a given exposure); and pesticide levels in turn condition the degree of synergistic toxicity seen with PB. Thus, individual differences determine differences in sensitivity to other exposures, which in turn influence response to PB.

Unidentified Interactions

PB appears to have many possible interactions. Many such interactions have only recently been identified, as scientific work has just begun to approach this issue. It is likely that as such work continues, additional potential interactions will be identified. Identification of interactions becomes more complex when multiple exposures are considered together, and the full effects of PB interactions may never be known. (This is particularly true because high-quality work on such interactions cannot readily be done in humans, so that data from animal studies must be relied upon.)

STRATEGY FOR TESTING DRUG INTERACTIONS

There is no ideal strategy for testing of long-term effects of PB with other chemicals, for reasons including the following:

1. The number of possible combinations increases rapidly with the number of agents considered, and there are many agents to be considered. (There are $2^n - 1$ interactions when n interacting drugs are considered, counting the effect of each drug given individually.) This effectively eliminates the option of testing all possible interactions. (Even more combinations are obtained when possible timing of interactions is taken into account. For instance, PB may enhance the toxicity of soman if given after soman, but protect against its toxicity if given in advance.)
2. Some agents may protect against the effects of others (or counteract their toxicity), rather than producing synergistic or additive toxicity. Because some agents could mask interactions among others, the strategy of testing all possible interacting agents together and weeding down to a smaller number if an effect is seen, could in theory miss important interactions, interactions that would be seen if the "correct" subset of chemicals were examined. Nonetheless, this may be a reasonable first approach.
3. Human studies designed exclusively to identify adverse effects, such as those from interactions among possible toxins, are usually unacceptable for ethical reasons; however, no animal model can accurately reflect the human physiology and the constants and the diversity that will influence toxic interactions.

Bearing in mind these significant limitations, it is necessary to accept some approach to systematically evaluating drug interactions as possible causes of illnesses in PGW veterans. One approach might consist of conducting a first-step screen for interactions by starting with multiple agents and paring down contributing factors, as follows:

- Select the set of exposures of interest. This set may include PB, low-level nerve agent, the anthrax and botulinum vaccines, caffeine, nicotine, petrochemical products, and some form of stress. Use per-kg doses somewhat higher than those employed in humans, to take into account differences in native sensitivity and differences in the sensitivity of available outcome measures (animals cannot be queried regarding whether they feel fatigue or experience discomfort). Employ routes of exposure similar to those experienced by PGW veterans, or justify the use of other routes.
- Select several animal models that have desirable characteristics: No model will be ideal, and interactions uncovered in any suitable model may merit further evaluation. For example, the hen might be selected for the sensitivity to OPIDN that it shares with primates, but mammals should also be tested.
- Select the outcome variables of interest. These should, where possible, include variables that reflect systems prominent in complaints of PGW veterans, who commonly report joint symptoms, cognitive symptoms, sleep symptoms, and reduced energy and/or fatigue. Thus, one might choose a complex learning task, a test of movement strength, sleep studies (including circadian rhythms of endogenous hormones), and measures of activity.
- Test a set of animals with all exposures concurrently. Note that this approach will not be effective if some exposures negate the side effects of others. Where such instances can be identified, additional testing might be performed excluding each such exposure.
- If a relevant effect is seen, undertake binary partitioning of exposures for repeat testing. (That is, test half the exposures, then the other half. If one “daughter” half maintains the adverse interaction, repartition that set. If neither does, recombine exposures and select a different partition.) If at any point the effect is lost, perform a repartition, recombining elements that have been separated in the partitioning. PB might, for instance, reduce the side effects of soman.

This approach, like others, has significant limitations, and other approaches may be preferred.

CONCLUSION

Drug-drug and drug-chemical interactions involving PB cannot be excluded as a contributor to illnesses in PGW veterans: The effect of PB may be enhanced by the presence of other agents. For instance, if metabolism of PB is interfered with or if PB is assisted in gaining access to the brain, PB may, analogously, enhance the effect or toxicity of other agents; PB and chemically similar agents,

or those affecting similar processes, may affect those processes additively or synergistically; or new effects may occur that would not be predicted from each drug individually. Thus, toxic effects of PB could be enhanced through interactions with other drugs or exposures, toxic effects of other drugs could be enhanced through interactions with PB; or new unexpected effects could ensue.

SCIENTIFIC RECOMMENDATIONS

- Current information on exposure to PB in individual veterans is weak, making it difficult to use epidemiological evidence to assess the possibility that PB, alone or in concert with other agents, is tied to illnesses in PGW veterans. Self-report information may be the best information available. Further attempts should be made to evaluate the presence of other exposures in ill and well PGW veterans. Where objective or record information is available, it should be used, but information on these exposures may be gauged, if necessary, by self-report. This exposure data should be evaluated against outcome information. Thus, an effort should be made to evaluate whether PB exposure in the PGW is associated with symptoms in PGW veterans and whether co-administration of PB with other agents, exposures, or individual factors was associated with heightened risk. (Some such efforts have been made; see Chapter Fourteen, “Chronic Effects.”)
- Continued efforts should be made to evaluate the interaction among PB, pesticides, and insect repellents used in the PGW. Evidence is now available in widely divergent species to suggest interactions between PB and (high doses of) DEET, chlorpyrifos, and permethrin. Further work should be performed to examine interactions using dosing schedules and routes of delivery closer to what may have been experienced by veterans and outcome measures more reflective of complaints of PGW veterans (along with physiologic measures). This includes long-term follow-up data after exposure is discontinued. Because nicotine also influences (heightens) activity at (nicotinic) ACh receptors, consideration should be given to testing interactions that include nicotine.
- Further effort should be made to examine, using controlled experimental studies in animals, the effects of various exposures, alone and in combination, that might be hypothesized to compromise the blood-brain barrier, enhancing its permeability to PB and other substances. These exposures include PB itself, stress, heat, caffeine, antihistamines, pesticides and insect repellents, nerve agents, and aluminum from vaccines.
- Consideration should be given to testing a “cocktail” of PGW exposures against a set of carefully devised outcomes. If outcomes are positive, the test should be repeated following successive binary splitting. (Clearly, the

split need not be exactly binary and will not be unless the number of candidate exposures is a power of two.) If no effect is found in either split even though an effect was seen in the parent, then an adding-back process can be initiated until the effect is reconstituted. This process may be iterated to identify the pertinent interaction(s).