
**CONFOUNDING FACTORS: INDIVIDUAL DIFFERENCES
AND INTERACTIONS**

INDIVIDUAL DIFFERENCES IN SUSCEPTIBILITY TO PESTICIDES

This chapter presents and discusses some of the circumstances and conditions that may confer differing susceptibilities on different individuals and populations. Differences at the basic individual level, i.e., genetic differences, are an important source of differences in susceptibility. For example, as discussed in Chapter Four, DEET is especially toxic to individuals with genetic or acquired defects in ammonia metabolism such as carriers of ornithine carbamoyl transferase (OCT) deficiency. In addition to genetic differences, heterogeneity in human populations may be expected to produce differences in susceptibility. However, even homogeneous populations of test animals often display a distribution of effects in response to toxic challenge, which indicates that other individual differences must also be important. These may include differences in exposure, absorption, and resulting effective doses.

Exposure and Absorption Differences

Many factors may affect the rate and magnitude of pesticide absorption. Mode of delivery, of course, affects the primary route of absorption (e.g., dermal or ocular absorption, inhalation), and for each route, individual factors condition the amount absorbed in “comparable” exposures. Protective clothing and individual differences in skin properties and integrity influence dermal exposure (Aprea et al., 1994; Keeble et al., 1993; Kishi et al., 1995; Lander and Hinke, 1992). Inhalational exposure may vary with ventilation and may also be affected by other factors, including properties of airway membranes. Intestinal absorption may be highly variable and is itself in turn affected to varying degrees by AChE-inhibitor exposures themselves (and consequent increased peristalsis). Moreover, the actual position of the individual with respect to the source of the exposure (for inhalational exposure) and differences in handling

(for dermal exposure) may lead to widespread differences in de facto exposure levels among individuals in the same “setting.”

Clearance Differences

Differences in clearance of pesticides depend on the amounts, genotype, and activity of enzymes involved in their metabolism. For example, mammals are protected from OPs by at least two mechanisms: First, BuChE binds these agents, sequestering them from neural tissue; second, paroxonase/arylesterase metabolizes OPs by hydrolysis, rendering them harmless products that are excreted (Haley et al., 1999a; Mutch et al., 1992; Li et al., 1993, 1995; Shih et al., 1998).

There are widespread differences in the genetics and activity of these enzymes from one individual to another. For example, Golomb (1999) noted that genetic polymorphisms in BuChE could have a role in differing clearance rates. The K-variant of BuChE and, more rarely, atypical BuChE lead to reduced clearance rates. There are also differences in the activity of enzymes even when the same genotype is present; moreover, these differences may themselves depend to some degree on prior exposures.

Clearance differences were shown to be important in a study of 1,003 workers exposed to the alkyl phosphate OPs methylparathion (n = 135) and ethylparathion (n = 169), the carbamate propoxur (n = 233), the pyrethroid cyfluthrin (n = 440), methyl-parathion and cyfluthrin (n = 19), or propoxur and cyfluthrin (n = 7) (Leng and Lewalter, 1999). Plasma levels, urine metabolite levels, and activity of plasma ChE and AChE were measured. At the same propoxur plasma concentration, only subjects with low individual AChE activity reported symptoms. Wide variation was present in baseline (pre-employment) AChE levels, with 100 workers having pre-employment ACh activity below the range of the published reference values (2,900 to 4,100 units per liter (U/l)). Inhibition values, ranging from 17 percent to 64 percent in 135 workers exposed to propoxur after an accident, were related to pre-exposure values. Symptoms of tearing, sweating, fatigue, dizziness, and visual disturbances were seen primarily in subjects with baseline AChE activity below 3,000 U/l (Leng and Lewalter, 1999). Among 10 workers who reported symptoms following exposure to cyfluthrin alone or in combination with other agents, the cyfluthrin plasma half-life ranged from 1.5 hours to 14 hours, more than a ninefold range.

In contrast, subjects who metabolized cyfluthrin rapidly reported fewer symptoms than those with a lower rate of metabolism, a tendency also evident with mixed exposure (cyfluthrin and methylparathion) (Leng and Lewalter, 1999), indicating the importance of individual susceptibility in determining the potential for toxic effects.

Paraoxonase. Paraoxonase (PON or PON1) is a high-density lipoprotein-bound “A-esterase” that is active in metabolizing (via hydrolysis) OPs to varying degrees. For example, it is more active toward chlorpyrifos oxon than paraoxon (Sultatos et al., 1984, 1985; Costa et al., 1990; Li et al., 1993 in Richardson, 1995). It is not thought to play a direct role in metabolism of pyridostigmine bromide (PB) (Furlong et al., 1989), nor does the literature contain information indicating a role in the breakdown of other carbamates.

Paraoxonase is an important factor determining the toxicity of OP pesticides and nerve agents to mammals, presumably including humans (Costa et al., 1990, 1997; Li et al., 1993, 1995; Davies et al., 1996). There are two polymorphic sites at amino acid positions 55 (L → M) and 192 (G → A). The Gln192 form is classically defined as the “A” or “Q” genotype, while the Arg192 is known as the “B” or “R” genotype (Haley et al., 1999; Mackness et al., 1996, 1997). A study of the influence of these polymorphisms on PON activity in 279 healthy human subjects (Mackness et al., 1997) showed that the 55 polymorphism significantly affected PON activity, with the MM homozygotes demonstrating more than 50 percent less activity toward paraoxon than the LL and LM genotypes, irrespective of the 192 genotype (Mackness et al., 1997). Previous studies had already shown that 192 polymorphism had an impact on PON activity. Multiple regression showed that the 192 polymorphism, 55 polymorphism, and serum PON concentration were responsible for 46, 16, and 13 percent of the variation in PON activity, respectively (all $p < 0.001$); no other examined parameters influenced PON activity. This suggests that AA/MM and AB/MM individuals may be more susceptible to OP intoxication; however, between the A and B genotypes, the type that is better at hydrolyzing paraoxon (Type A) is less adept at hydrolyzing sarin (as well as diazinon and soman) (Davies et al., 1996), so susceptibility depends on the OP to which one has been exposed. For additional discussion of PON, see Golomb (1999).

Chlorpyrifos Oxonase. Chlorpyrifos oxonase is an important metabolizing enzyme for certain OPs (such as chlorpyrifos). Individual differences in hydrolyzing efficacy appear to result from differences in activity but not from genetic differences. Following hydrolytic cleavage of chlorpyrifos oxon by chlorpyrifos oxonase to products that are much more water soluble, the OP is eliminated relatively rapidly from species ranging from fish to rats and humans and is considered to have a substantially lower potential to accumulate with repeated exposures at relatively low doses (Richardson, 1995; Eto, 1979; Nolan et al., 1984.; Sunaga et al., 1989; Barron et al., 1991). Brain AChE inhibition in rats dosed with chlorpyrifos oxon is greatly reduced by prior IV injection of paraoxonase (Costa et al., 1990; Richardson, 1995), suggesting the important reduction in toxicity conferred by this enzyme.

Differences across species in the activity of chlorpyrifos oxonase vary with LD₅₀s for chlorpyrifos. Rabbits have 40 times greater chlorpyrifos oxonase activity than do rats, and about sevenfold to twentyfold higher LD₅₀s of chlorpyrifos (rabbit LD₅₀ is 2,000 mg/kg, per os [p.o.] in corn oil; rat LD₅₀ is 118 to 245 mg/kg p.o. in corn oil) (McCollister et al., 1974; Richardson, 1995); and low LD₅₀s for chlorpyrifos in hens (50 mg/kg p.o. in gelatin capsules) (Rowe et al., 1978; Richardson, 1995) are thought to reflect the very low levels of serum A-esterases generally present in bird species (Brealy et al., 1980; Costa et al., 1990).

In contrast to the results for paraoxon, human chlorpyrifos oxonase activity has not been shown to have clear genetic polymorphism, but a four- to fivefold (Furlong et al., 1988) or even a thirteenfold variation in chlorpyrifos oxonase activity has been found in human serum (Richardson, 1995). Chlorpyrifos oxonase is among the enzymes for which individual differences may influence susceptibility to effects of selected pesticides; in this case, the differences are in activity and are not genetic.

Differences in Metabolizing Enzymes Among PGWV

Some studies have shown no significant differences in atypical BuChE between PGWV who have entered registries and those who have not (see Golomb, 1999). However, this very rare polymorphism alone could not readily account for large differences in illness, so this finding is not wholly unexpected. Golomb (1999) suggested that more common polymorphisms, such as paraoxonase and the K-variant of BuChE, receive increased attention and that enzyme activity, not just genetic type, should be examined.

One study found that ill PGWV with neurological symptoms had 3.5 times higher odds of having the R allele of paraoxonase (either QR or RR, vs. the QQ genetic composition) than did healthy controls (95 percent CI = 1.01–12.18, $p < 0.05$) (Haley et al., 1999a). Moreover, low activity of the Q alloenzyme (the alloenzyme that is more efficient at hydrolyzing several OPs, including diazinon, as well as sarin and soman) (Haley et al., 1999b) distinguished ill PGWV from controls, and did so more accurately than the PON1 genotype or the activity levels of the type R alloenzyme, total arylesterase, total paraoxonase, or BuChE. The OR of being in the lowest quartile of Type Q arylesterase activity in ill PGWV compared with controls was 4.5 (95 percent CI = 1.24–16.35, $p = 0.02$), and for those with the comparatively severe symptom complex 2, the OR was 9.0 (95 percent CI = 1.72–46.99) compared with controls. There was a trend toward ill PGWV having lower BuChE activity; the OR for being in the lowest quartile for BuChE activity was 2.67 for all veterans, but this did not reach significance ($p = 0.2$, 95 percent CI = 0.6–11.8). This trend merits evaluation in a larger sample.

Low activity of the Q alloenzyme was also associated with acute toxicity after taking PB (although paraoxonase is not believed to break PB down), and acute symptoms in response to PB had previously been linked to development of either of two of the three main factor-analysis-derived syndromes in PGWV identified by Haley et al. (1999b). The study of paraoxonase alloenzymes was conducted on a sample of 25 ill PGWV meeting criteria for Haley's symptom complex 1 (n = 5), 2 (n = 12), or 3 (n = 5), and one each with symptom complexes 4 to 6; the controls were 20 healthy veterans matched for age, education, and sex. Mean type Q activity was significantly lower in men at or over 45 years of age than in younger men (p = 0.009). There also was a possible trend toward ill PGWV having lower BuChE levels (most of those at the lowest levels were ill rather than healthy), although the allelic variant was not associated with illness in this study.

Nutritional and Other Cofactors

Individual differences in cofactors that modify the effect of pesticides, are essential for metabolism of pesticides, or permit or inhibit toxic effects by pesticides may contribute to individual differences in clinical effects. These differences may depend on exogenously introduced factors (such as antioxidant vitamins C and E, phytochemicals, and cholesterol) and could thus be construed as interaction effects. Vitamins E and C may confer protection not only against OPs and carbamates (Grabarczyk and Kopec-Szlezak, 1992; Khan and Sinha, 1996), but also against other pesticides, such as organichlorines (OCs) (Podstawka et al., 1991) and pyrethroids (Flannigan et al., 1985; Tucker et al., 1984), with which OPs and carbamates may interact.

INTERACTIONS

General Issues

Exposures to pesticides in combination with other agents may exert effects different from those experienced with pesticides alone. Moreover, effects from two pesticides may differ from those expected from each separately. Therefore, this review includes the literature that considers pesticides in combination with other exposures.¹

¹In this section, we explore various other exposures, including heat, dietary factors, and illegal drugs. This is not to suggest that these exposures were known to occur with specific frequency during ODS/DS; rather, the discussion is intended to summarize some of what is known about the interaction of these and other exposures with pesticides. A forthcoming report being prepared by OSAGWI, *Pesticides Environmental Exposure Report* (OSAGWI, 2000), investigates pesticide exposures during ODS/DS and draws conclusions based on all the available evidence.

It is not feasible to predict the toxicity of agent mixtures in general, or of pesticide mixtures (or pesticides in combination with other agents) in particular, on the basis of the toxicity of single compounds (Marinovich et al., 1996). Moreover, the number of possible combinations increases exponentially with the number of agents, as 2^n ; thus, 10 compounds have over 1,000 possible combinations that could have different consequences. When agents are experienced together, the effect may be additive, synergistic, or antagonistic, and the character of the interaction may differ for different effects of the compounds. Because of the computational intractability of studying every possible combination, the FDA does not require examination of drug combinations in determining approval for an individual drug; it does not even require examination of combinations that may commonly occur together. Similarly, health consequences of pesticide mixtures, and coexposures to pesticides and other factors, are in general poorly understood, and “testing even most potential mixtures with the classical toxicological protocol is unfeasible” (Marinovich et al., 1996). However, it is conceivable that multiple exposures to pesticides and other compounds occurred during ODS/DS, underscoring the need for further investigation to focus further research. Some of the possible multiple exposures encountered during ODS/DS are discussed below.

Interactions Among DEET, PB, and Pesticides

The Institute of Medicine (IOM, 1996) and the National Research Council (NRC, 1994) have stated that studies are needed to resolve uncertainties about the effects of DEET, PB, and other pesticides in combination. Some studies have been conducted in response to questions raised after the Gulf War concerning whether the combinations of these exposures might be related to health complaints of veterans.

Some data are available concerning mixtures of pesticides with the insect repellent DEET. Because DEET may enhance the dermal penetration of other chemicals and because of its ubiquitous use, several studies have focused on the combination of exposures PGWV may have encountered during ODS/DS. For example, PGWV could have applied DEET and permethrin as prescribed by the DoD Insect Repellent System (Young and Evans, 1998) and at the same time taken PB tablets as a prophylactic treatment for nerve agents.

In two studies by Abou-Donia et al. (1996a,b), adult hens exposed to the combination of DEET, PB, and either permethrin or chlorpyrifos showed greater-than-additive (not synergistic) effects when exposed to two of the three compounds, and there was an even greater effect when all three chemicals were present. The authors suggest that this could mean that agents such as PB,

which ordinarily do not reach the brain, can enhance the neurotoxicity of chlorpyrifos, which is known to do so. Similarly, while DEET does not exhibit cholinergic effects, it may enhance this effect from other chemicals. The authors hypothesize that three mechanisms could be responsible for the interactive effects. First, concurrent exposure to these chemicals may enhance their absorption. Second, the chemicals may be competing substrates, resulting in a decreased catabolism of the combination of chemicals. Because such competition could increase the likelihood that these chemicals are transported to nerve tissue, individual variations in plasma and liver esterases would highlight subpopulations that are more susceptible to neurotoxins. Third, the concurrent exposure to all three chemicals could cause trauma to the cerebrovasculature, thereby increasing vascular permeability or altered blood flow to the brain.

It should be noted, however, that both DEET and permethrin were administered in these studies at high doses (500 mg/kg), and the route of exposure, subcutaneous injection, may limit the relevance of the findings. The exposure values used in this study would be equivalent to a 70-kg person being exposed to 467 tablets of PB, 1,667 cans of permethrin, and 76 tubes of 33 percent DEET. The implications of these studies are unclear when trying to understand the potential for human health effects at lower doses; however, the inference that mixtures can lead to effects exceeding or distinct from the component parts deserves further attention in the context of Gulf War illnesses.

McCain et al. (1997) evaluated the lethal interaction of DEET, PB, and permethrin when given orally to rats by gastric gavage. A significant increase in lethality over expected additive values occurred when all three chemicals were given concurrently. This study also used high doses of these chemicals, sufficient for a single dose to produce a lethal effect. They calculated that to match the lowest doses used in their study (PB = 46 mg/kg, permethrin = 279 mg/kg, DEET = 1,946 mg/kg), a 70-kg person would have to simultaneously ingest 107 PB tablets, 23 cans of permethrin, and 6.6 tubes of 33 percent DEET. As in other such studies, the method of DEET and permethrin exposure (ingestion) also presents difficulties in applying these findings to an understanding of health effects in humans. Baynes et al. (1997) make this point and hypothesize that the exposure route strongly affects the bioavailability of these chemicals, suggesting that significantly less DEET and permethrin would be bioavailable following dermal exposure than following oral or subcutaneous exposure. In their study, no permethrin was absorbed in the skin of either rats, mice, or pigs when it was applied topically with DEET at either 15 or 35 percent concentration and using a number of different solvent mixtures. In fact, DEET appeared to decrease permethrin absorption in mouse skin, which is more permeable than human skin. In addition, the exposures in this study were limited to eight hours, which may limit the applicability of the findings to any Gulf War exposure scenario.

Clearly, longer-term absorption studies are warranted before conclusions regarding the interaction between DEET and other chemicals (especially pesticides) can be drawn. Also, it should be considered that the estimated area of overlapping between DEET-treated skin and permethrin-treated uniforms is small—approximately 5 percent or less—and therefore is probably of little importance in view of the limited amounts of dual exposures (NRC, 1994).

Interactions Among Pyrethroids, Organophosphates, and Carbamates

Pyrethroids have a number of proposed mechanisms of toxicity, chief among which are reduction in Na⁺K⁺-ATPase (which has been documented in mammalian studies in various brain regions, including frontal cortex, hippocampus, and cerebellum) (Husain et al., 1996) and increase in monoamine oxidase activity leading to shifts in polyamines (Husain et al., 1994). Although pyrethroids are not normally thought to exert their effect primarily through AChE inhibition, they have been reported to influence ACh activity by

- Increasing AChE not only in blood and bronchoalveolar lavage fluid (Jian and Tian, 1996), but also in brain regions, including the frontal cortex, striatum (basal ganglia), hippocampus, cerebellum, and pons medulla in mammalian studies (Husain et al., 1996).
- Affecting ACh concentrations in the brain (Aldridge et al., 1978).
- Increasing striatal muscarinic receptors and altering nicotinic receptors (Eriksson and Nordberg, 1990; Eriksson and Fredricksson, 1991; Husain et al., 1996).

Effects through the ACh system constitute one mechanism by which interactions of pyrethroids with OP and carbamate pesticides may be mediated, although many other mechanisms of interaction are possible. (For example, it has been suggested that changes in the physiological concentration of polyamines may produce effects on cholinergic and dopaminergic systems by adversely affecting calcium homeostasis, experience-dependent brain growth, and neurotransmitter uptake, thus leading to derangements in overall synaptic events (Husain et al., 1994).

It should be noted that persistence of pyrethroids in the fat and brain of exposed subjects has been reported in animal studies and in studies of poisoned cotton sprayers (Husain et al., 1996), so the possibility of interactions occurring even with a delay following pyrethroid exposure remains a concern.

Pesticide Formulations

As has been noted, the active ingredient on a pesticide label relates only to activity against the species targeted by the substance. However, many of the inactive ingredients in pesticide formulations (e.g., the petroleum distillates) can also have harmful effects on humans. In most cases of observed human effects of pesticide exposure, the exposure was to a pesticide formulation; however, in many animal or other laboratory studies, only the active ingredient is tested. It is possible that not only might inactive ingredients of pesticide formulations pose a hazard to human health, these ingredients may act in combination with the active ingredient to produce an effect different from that predicted from animal models.

Pesticide Interactions with Drugs and Other Exposures

The IOM issued a report on interactions among drugs and chemicals and proposed a mechanism for evaluating whether presumptive interactions were likely (Committee to Study the Interactions of Drugs, Biologics, and Chemicals in U.S. Military Forces, 1996). The thesis of the report was that if there were similar loci of effect, interactions would be more likely to occur, and concern regarding interactions should be heightened. Although this approach is on its face sensible, it has several limitations (Golomb, 1999). For example, while PB was represented as having effects (and therefore potential interactions) only on the nervous system, in fact PB has documented effects on virtually all of the systems listed. Therefore, use of this approach does little to restrict the domains of consideration, or the exposures with which PB might interact, provided a sufficiently comprehensive examination of the evidence is employed to determine loci of effect.

The report on PB characterizes interactions between it and heat, stress, caffeine, nicotine, and antihistamines. Because other carbamates, as well as OPs, share with PB the major pharmacological effect of AChE inhibition, the data on potential interactions with these agents also have bearing here.

Pyridostigmine Bromide. One hypothesis is that primarily peripheral binding of AChE by PB will occupy peripheral binding sites and potentially drive a higher fraction of OP pesticides to central binding sites. Thus, a synergistic effect on central ACh regulation may ensue.

Heat. In addition to its possible impact on the blood brain barrier, heat affects acetylcholinergic nerve terminal function. The effects of heating and cooling on electrophysiological testing and acetylcholinergic function are complex and have long been studied (Adrian, 1914; Delbeke et al., 1978; Denys, 1991; Gasser, 1931; Hofmann et al., 1966; Lang and Pusa, 1980; Lass and Fischback, 1976;

Lowitzsch et al., 1977; Rutchik and Rutkove, 1998; Rutkove et al., 1997; Stegeman and Weerd, 1982; Tasaki, 1949). Cooling prolongs the refractory period of nerve and muscle, increases facilitation, decreases AChE activity, decreases the amount of ACh released, and increases the amplitude of the excitatory endplate potential (Rutchik and Rutkove, 1998). Insofar as heat may produce effects tending in the opposite direction, it may be linked to comparatively increased amounts of ACh released, potentially exacerbating the effect of AChE inhibitors (although the net impact of this and changes in AChE activity are difficult to ascertain). Heating is associated with reductions in the amplitude of spontaneous repetitive motor action potentials with OP poisoning (analogous to the finding seen in patients with myasthenia gravis) (Rutchik and Rutkove, 1998).

The net impact of any of these effects on the clinical impact of AChE-inhibitor exposure is not well defined, although heat has been reported to exacerbate the effect of AChE-inhibitor exposures (Richter et al., 1992). For instance, depression in ChE activity has been found to be increased with heat stress and dehydration (Baetjer, 1983), and heat stress and heat strain were found to be hazards for pesticide-spray pilots (Gribetz et al., 1980; Richter et al., 1992). Not only may heat modify the impact of AChE-inhibitor exposure, AChE inhibitors may conceivably modify the impact of heat by increasing body temperature. In a study of 70 cases of acute carbamate and OP poisoning in Jordan (where 58 percent of the subjects were intoxicated with carbamates), 47 percent had low-grade fever (37.5°C to 38.5°C) with no evidence of infection, and all resolved spontaneously within five days (Saadeh et al., 1996). However, the report does not discuss whether lower doses produce an effect on body temperature.

An additional concern is that increased body temperature (e.g., to 39°C) is associated with increased ischemia-induced blood brain barrier permeability. Cooling attenuates post-ischemic blood brain barrier consequences and the rise in extracellular glutamate that accompanies this attenuation (Dietrich et al., 1992).

Antihistamines. Some AChE inhibitors have been shown to influence histamine function. For example, malathion metabolites cause rapid release of histamine from basophilic cells, and with prolonged incubation malathion itself does, suggesting that these cells may metabolize malathion (Xiong and Rodgers, 1997).

Antihistamines also have potential cholinergic effects. Histamine modulates heat-stress-induced increases in blood brain barrier permeability, which may be enhanced by histamine H1 receptor blockers and reduced by histamine H2 receptor blockers (Sharma et al., 1992). Histamine H2 receptor blockers have been shown to have AChE-inhibiting effects that are stronger for ranitidine and

nizatidine than for cimetidine (Laine-Cessac et al., 1993). Moreover, histamine H1 blockers have been shown to increase central ACh and ACh action in animal studies (Dringenberg and DeSouza-Silva, 1998). Thus, H1 blockers at the time of OP or carbamate exposure might worsen effects by heightening brain penetration and augmenting the excess of ACh available centrally. The possible treatment impact of antihistamines in ill PGWV or post-AChE-inhibitor-exposure subjects remains to be determined.

Because antihistamines have effects on cholinergic function, and AChE inhibitors have effects on histamine, interactions between antihistamines and OP/carbamate pesticide exposure might be anticipated.

Diet, Alcohol, and Dietary Supplements. Studies in rats have demonstrated that diet can affect susceptibility to the adverse effects of pesticides, including inhibition of serum aliesterase and AChE activity in liver microsomes. Rats on low-protein diets were found to be more markedly affected by pesticide exposure, both acute and chronic OP, as well as OC (Casterline and Williams, 1969, 1971; Baron et al., 1964; Baron et al., 1966; Boyd, 1969; Lee et al., 1964; Weatherholtz et al., 1969).

Possible mechanisms of interaction among pesticides that are related to diet and alcohol intake include membrane effects. Some classes of pesticides, including pyrethroids (Moya-Quiles et al., 1995), OCs (Suwalsky et al., 1997a,b; Verma and Singhal, 1991; Antunes-Madeira et al., 1993) and perhaps DEET, may also have membrane effects including fluidization of membranes. The OP pesticides influence and may fluidize membranes (Wysocki et al., 1987), which in turn affects membrane function, including neurotransmitter receptor expression, and the effect is aggravated by low cholesterol. Moreover, dietary fatty acid composition also appears to influence membrane fluidity and function (Block and Edwards, 1987; Clandinin et al., 1991; Clandinin et al., 1982; Gould and Ginsberg, 1985; Greenwood et al., 1989; Heron et al., 1980; Johnson et al., 1979; Scott et al., 1989; Shinitzky and Inbar, 1974). Thus, dietary fat composition may interact with pesticides in exacerbating membrane fluidization.

Alcohol also fluidizes membranes (Johnson et al., 1979) and cannot be excluded as an exacerbating factor. Studies have shown antioxidant vitamins to have protective effects on lipid peroxidation and oxidative damage, which OP agents have been shown to cause (Barja et al., 1994); however, it should be noted that the dose of vitamin may determine whether it has primarily an oxidant or an antioxidant effect. In addition, paraoxonase, which helps to break down some OP pesticides, is an HDL-cholesterol-associated enzyme, and paraoxonase activity is related to HDL-cholesterol levels (Mackness et al., 1996), which in turn are increased with saturated and monounsaturated fat consumption and reduced with polyunsaturated and partially hydrogenated “trans” fat consump-

tion (the latter are commercially modified fats that do not occur in nature and are present in most packaged baked goods).

Cannabis (Marijuana). Two studies, one in humans and one in rats, suggest that marijuana may interact with carbamates (specifically physostigmine) to exaggerate the reaction produced—profound depression in humans and lethality in rats.

A study performed in rats showed that marijuana, or delta-9-tetrahydrocannabinol (Δ^9 -THC) appeared to increase the toxicity of physostigmine (a carbamate and close analog of PB that more readily crosses the blood brain barrier) (Rosenblatt et al., 1972). Thirteen of 17 rats treated with 2 mg/kg of Δ^9 -THC (in propylene glycol serum complex) and 0.4 mg/kg physostigmine salicylate died, compared with two of 17 given physostigmine salicylate (and propylene glycol serum complex) without THC ($p < 0.0002$). (Note that this was chosen to be the LD₁₀ for physostigmine in Sprague Dawley rats, i.e., the dose at which 10 percent were expected to die.) It had been speculated that THC might have anticholinergic actions and thus that it might reduce the lethality of physostigmine. But instead, it augmented the lethality. Rosenblatt et al. noted that another researcher reported (in a personal communication) that THC produces a small inhibition of AChE in vivo and in vitro. Rosenblatt et al. reported that the ante-mortem and post-mortem findings were compatible with peripheral cholinergic crisis. The lethality was antagonized by atropine (two of 10 that were pretreated with 0.8 mg/kg atropine, $p = 0.006$, died) or methylscopolamine (three of 10 that were pretreated with 1 mg/kg methylscopolamine, $p = 0.02$, died).

Another study examined the impact of physostigmine in two marijuana-intoxicated patients; in both cases, physostigmine induced a profound clinical depressive reaction that was antagonized by administration of 1 mg atropine (El-Yousef et al., 1973). While physostigmine normally produces a depressive syndrome, the reaction in these two subjects “far exceeded any observed in 28 subjects of various diagnostic categories who received equal or greater amounts of physostigmine,” suggesting that the THC or contaminants therein may have interacted with physostigmine, potentiating the consequent depressive state.

Cocaine. Inhibition of pseudocholinesterase (plasma cholinesterase) delays the hydrolysis of cocaine, slowing its detoxification and thus increasing the duration and magnitude of its effect. A single case report cites an episode of extreme unprovoked savage aggression, culminating in the murder of two friends, by a person who had concomitant exposures to OP and carbamate pesticides (which he sprayed as a landscaper) and cocaine (Devinsky et al., 1992). Clearly, a causal role for AChE-inhibitor exposure alone or in combination with cocaine is difficult to draw from a single case, but the existence of a mechanism of

interaction and the reported ability of each agent individually to contribute to aggressive behavior suggest that a causal interaction should be considered.

