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ORGANOPHOSPHORUS CHEMICALS: POTENT INHIBITORS OF THE HUMAN METABOLISM OF STEROID HORMONES AND XENOBIOTICS

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Although it has been known for some time that organophosphate chemicals containing the P = S moiety are irreversible inhibitors of cytochrome P450, this knowledge has not been generally applied to the human metabolism of xenobiotics. Recent studies have demonstrated that organophosphate insecticides containing this moiety are potent inhibitors of the metabolism of both xenobiotics and endogenous substrates by human liver microsomes and by specific human cytochrome P450 isoforms.

Key Words: Carbaryl; Chlorpyrifos; Chlorpyrifos oxon; Cytochrome P450 (CYP); DEET; Estradiol; Human CYP isoforms; Human liver microsomes; Organophosphorus insecticides; Permethrin; Testosterone.

INTRODUCTION

Almost all studies of the human metabolism and metabolic interactions of xenobiotics to date have involved clinical drugs. Chemicals used in public health, in agriculture, and during military deployments, as well as occupational and environmental chemicals, have seldom been the subjects of these studies. Unfortunately, investigations of such chemicals carried out in experimental animals assume relevance only when they can be extrapolated with confidence to humans. Since most exogenous chemicals can be substrates, inhibitors and/or inducers of xenobiotic-metabolizing enzymes (XMEs), these enzymes are potentially an important locus for metabolic interactions. However, human XMEs often differ from those of experimental animals, rendering such extrapolations of reduced value. Based on animal studies, large uncertainty factors, usually undefined and therefore used as default values, are employed in numerical estimates of human health risk. Under these circumstances, it is difficult to be certain that the best estimate of human health risk is being derived. During the last few years we have been involved in studies designed to close this data gap and, thereby, improve the estimation of human health risk from xenobiotic exposure.

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Moreover, certain aspects essential in risk analysis of chemicals can only be examined in human studies. They include the determination of variation within the human population, which is highly outbred. Little of value can be learned about human variation by the use of inbred experimental animals. Most, if not all, XMEs are polymorphic with the distribution of polymorphic variants varying between population sub-groups and individuals, potentially putting some individuals and subpopulations at greater or lesser risk than others.

Toxicity is the endpoint of a cascade of events that starts with exposure and ends with the expression of a toxic endpoint, intermediate steps including absorption, distribution, metabolism, distribution of metabolites, excretion and/or interaction with cellular macromolecules, followed by overt toxicity or repair. Although interactions can occur at any of these loci, metabolism is of critical importance, inasmuch as chemicals may be metabolically detoxified or activated to products more toxic than the parent compound. Furthermore, since XMEs are often not substrate specific, metabolic interactions of importance between different xenobiotics and between xenobiotics and endogenous substrates may occur. Several of these interactions involve inhibition of XMEs and it is this aspect as it relates to organophosphorus xenobiotics that is the subject of this mini-review.

Phase I metabolism generally results in the introduction of a reactive group into the molecule, a reactive group that is subsequently conjugated with an endogenous compound during Phase II metabolism. The most important Phase I enzymes are the isoforms of cytochrome P450 (CYP) and the flavin-containing monooxygenase (FMO) (Hodgson and Smart, 2001).

Our recent studies have examined the role of specific human XMEs on the metabolism of a subset of chemicals important in agriculture, public health, and military deployments. Carbaryl, chlorpyrifos, diethyl toluamide (DEET), fipronil, permethrin, pyridostigmine bromide, sulfur mustard and its degradation products, and other chemicals of interest have been tested as substrates and inhibitors of the most important human XMEs, including cytochrome P450s (CYPs), flavin-containing monooxygenases (FMOs), alcohol and aldehyde dehydrogenases and esterases (Choi et al., 2002, 2004; Dai et al., 2001; Tang et al., 2001, 2002; Usmani et al., 2002, 2003, 2004). A database on Phase I enzymes and the metabolism of insecticides and related chemicals was recently updated (Hodgson, 2003). The ability of these chemicals to act as inhibitors or enhancers of physiological (endogenous) substrates may also be of importance to the health of exposed individuals and effects on testosterone and estradiol metabolism have been described.

Phosphorothioates are activated to their oxons by CYP isoforms. This reaction involves the formation of a phosphooxythirane ring, which spontaneously rearranges to form the oxon, as well as several detoxication products (Fig. 1). While the focus of research in this area has centered on oxon production, since the latter are potent inhibitors of acetylcholinesterase, it has long been known, based on studies of rabbit CYPs, that highly reactive sulfur is also released in the oxidative desulfuration reaction. As a result, CYP-mediated reactions are inhibited during oxon formation (Halpert et al., 1980; Neal, 1980; Neal and Halpert, 1982; Neal et al., 1983). The mechanism of this inhibition is complex, since it involves more than one reaction at different sites in the CYP molecule. However, it is known (Neal et al., 1983) that at least 50% of the loss of monooxygenase activity during parathion metabolism can be attributed to the loss of heme from the CYP isoform involved. There does not appear to be any simple explanation for the remaining loss of activity, but it appears probable that it is due to structural changes in the CYP protein caused by the binding of atomic sulfur to cysteine residues bringing about irreversible inhibition.

More recently, it was demonstrated that, *in vivo*, chlorpyrifos is a CYP inhibitor when administered at a rate of 50 mg/kg to rats (Vodela and Dalvi, 1995). Until recently (Butler and Murray, 1997; Dai et al., 2001; Di Consiglio et al., 2005; Tang et al., 2001, 2002; Usmani et al., 2002, 2003), however, these studies had not been extended to humans.

Organophosphorus Inhibitors of Xenobiotics and Endogenous Substrates: Xenobiotics

Chlorpyrifos. Organophosphorus insecticides, including chlorpyrifos (Fig. 1), are commonly used to control disease vectors, as well as household and agricultural pests. They are potent inhibitors of acetylcholinesterase (AChE), resulting in a variety of symptoms, including increased secretion, hyperactivity, and eventually death by respiratory failure (Ecobichon, 2001). Many are organophosphorothioates, weak anticholinesterase inhibitors that are activated to their oxons, potent acetylcholinesterase inhibitors, by CYP catalyzed desulfuration. The resultant oxons are approximately three orders of magnitude more potent as AChE inhibitors than the parent chemicals. The same enzymes involved in organophosphate activation are also important in the detoxication process. Thus, the ratio of activation to detoxication is dependent upon the individual CYP isoforms present in the tissue at a given time and will determine the amount of oxon which is available to cause toxicity (Levi and Hodgson, 1985).

Numerous studies have been conducted in experimental animals (see Sultatos, 1991 for references) on the metabolism of organophosphorus pesticides, including chlorpyrifos. Chlorpyrifos is metabolized to the oxon and both chlorpyrifos and its oxon are rapidly hydrolyzed to 3,5,6-trichloro-2-pyridinol (3,5,6-TCP). In humans, low doses of chlorpyrifos are completely metabolized and subsequently eliminated in the urine as 3,5,6-TCP or glucuronide conjugates of 3,5,6-TCP (Nolan et al., 1984). Other metabolites may include deethylated chlorpyrifos (O-ethyl trichloropyridyl phosphorothioate) as well as glutathione conjugates.

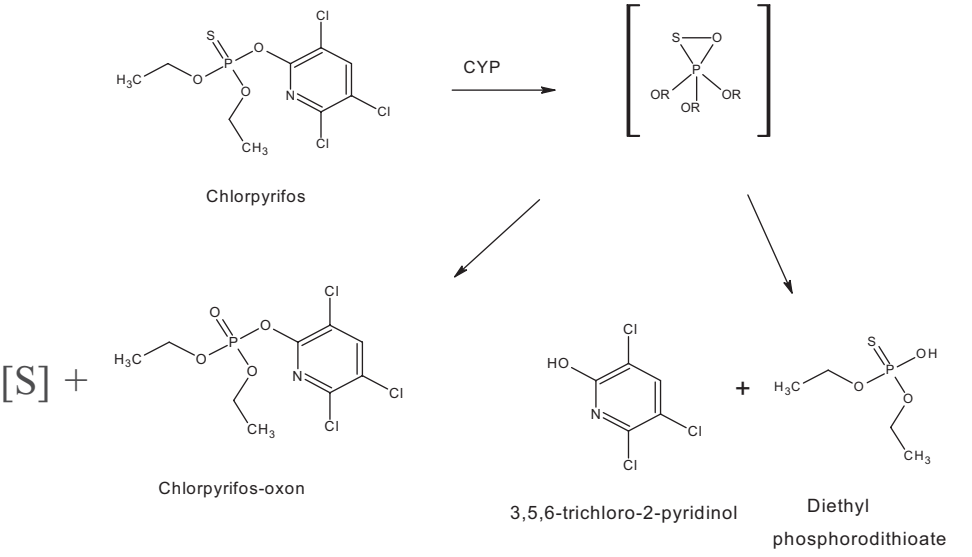

Figure 1 Chlorpyrifos metabolism.

Organophosphorus insecticides are readily metabolized by cytochrome P450. Oxidative metabolism can result in both intoxication as a result of desulfuration or detoxication by several pathways. Esterase metabolism of organophosphates is also an important mechanism of detoxication and a human polymorphic esterase, paraoxonase (PON1), has been studied extensively (Furlong, 2000; Furlong et al., 1993, 1998).

Studies of *in vitro* human chlorpyrifos metabolism (Tang et al., 2001) demonstrate that pooled human liver microsomes, although effective in both desulfuration and oxidative dearylation, are less active toward this substrate than either rat or mouse microsomes. Pooled human female liver microsomes were somewhat more active in chlorpyrifos metabolism than pooled male liver microsomes. Tang et al. (2001) further showed that the ability of human liver microsomes to detoxify chlorpyrifos and to activate it to its oxon was due to CYP isoforms 1A2, 2B6, 2C9, 2C19, and 3A4. The ratio of activation to detoxication varied between CYP isoforms, the ratios of desulfuration to dearylation ranging between 3.38 for the isoform most active in desulfuration (2B6) to 0.14 for the isoform least active in desulfuration (2C19). More recently, Buratti et al. (2003) showed by correlation analysis that human CYP2B6 and CYP3A4 are most active in oxon production from a number of organophosphorus insecticides, including chlorpyrifos.

Potential differences in chlorpyrifos metabolism in the human population based on differences in hepatic enzymes have been examined using microsomes from individual livers. Individuals with higher levels of CYP2B6 and CYP3A4 show higher levels of desulfuration than those with lower levels of these isoforms. CYP3A4 contributes to both desulfuration and dearylation of chlorpyrifos and parathion (Butler and Murray, 1997; Mutch et al., 1999; Sams et al., 2000; Tang et al., 2001).

As previously mentioned, two principle CYP isoforms involved in chlorpyrifos metabolism are CYP3A4 and 2C19. CYP3A4 produces both the desulfuration and dearylation products in approximately equal amounts, while 2C19 produces the dearylation product almost exclusively. Each of three polymorphic forms of CYP2C19 examined had significantly reduced ability to metabolize chlorpyrifos (Tang et al., 2001). In contrast, one polymorphic form of CYP3A4 was more efficient in chlorpyrifos metabolism, two were equivalent to wild type in metabolism, and one produced essentially no metabolites (Dai et al., 2001). No information on the potential impact of polymorphic forms of CYP 1A2 and 2B6 with respect to pesticide metabolism is presently available.

Considerations of metabolic differences between individuals should also consider the contributions of esterases. Unfortunately, with the exception of PON1, the role of the human esterases involved in organophosphorus insecticide detoxication have not been investigated.

N, N-Diethyl-m-toluamide (DEET) metabolism and inhibition by chlorpyrifos. Every year approximately one-third of the US population, over 75 million people, use insect repellent products with DEET concentrations ranging from 10–96% in a variety of liquids, lotions, gels, sprays, sticks, and impregnated materials, some 30 million packages of DEET-containing products being sold annually (Veltri et al., 1994). Given this wide use, DEET must be considered safe when applied properly; although there are accounts of side effects, largely anecdotal, that include toxic encephalopathy, seizure, acute manic psychosis, cardiovascular toxicity, and dermatitis. A few cases of death may have resulted from extensive skin absorption (Schoenig et al., 1996).

Studies of absorption and metabolism in humans suggest that a small percentage of topically applied DEET is rapidly absorbed (5–8%) and excreted. As many as six DEET major metabolites were recovered from the urine (Selim et al., 1995). Studies of

the oxidative metabolism of DEET have been conducted using pooled human liver microsomes, rat liver microsomes, and mouse liver microsomes. In this first *in vitro* study of DEET metabolism in humans (Usmani et al., 2002) it was shown that human liver microsomes metabolized DEET primarily to two products, a ring methyl oxidation product, N,N-diethyl-m-hydroxymethylbenzamide and an N-deethylated product, N-ethyl-m-toluamide.

In these studies, the production of the ring methyl oxidation product, *N, N*-diethyl-*m*-hydroxymethylbenzamide was approximately 10-fold higher than that of the *N*-deethylated product, *N*-ethyl-*m*-toluamide. Both the affinities and intrinsic clearance of HLM for ring methyl hydroxylation are greater than those for *N*-deethylation. An interesting observation is that the two products are formed almost exclusively by two different sets of CYP isoforms. Among 15 cDNA-expressed CYP enzymes examined, CYP1A2, 2B6, 2D6*1 (Val_{374}), and 2E1 metabolized DEET to the ring methyl metabolite, while CYP3A4, 3A5, 2A6, and 2C19 produced the N-deethylated metabolite. CYP2B6 is the principal CYP involved in the metabolism of DEET to its major ring methyl oxidation product, while CYP2C19 had the greatest activity for the formation of the N-deethylated product.

To summarize, CYPs 1A2, 2B6, 2D6, and 2E1 produced the hydroxymethyl product and 3A4, 3A5, 2A6, and 2C19 produced the deethylated product with little or no overlap between the two groups of isoforms; that is, there are no isoforms that produce both products. Given the different affinities observed it is probable that at low substrate concentrations only the ring methyl hydroxylation is likely to be observed.

The use of phenotyped HLM demonstrated that individuals with high levels of CYP2B6, 3A4, 2C19, and 2A6 have the greatest potential to metabolize DEET. Mice treated with DEET demonstrated induced levels of the CYP2B family, increased hydroxylation, and a 2.4-fold increase in the metabolism of chlorpyrifos to chlorpyrifos-oxon, a potent anticholinesterase.

The effects on DEET metabolism of chlorpyrifos, permethrin, and pyridostigmine bromide alone or in combination have been investigated in human microsomes (Usmani et al., 2002). The greatest effect shown was the inhibition of the metabolism of DEET to the ring methylol derivative by chlorpyrifos. None of the three compounds tested had any significant inhibitory effect on the production of the N-deethylated metabolite. Human liver microsomes differed from those from rodents in that pyridostigmine and permethrin, alone or in combination, showed an activation effect for the production of both of the major products of DEET metabolism.

Carbaryl metabolism and inhibition by chlorpyrifos. Carbaryl, 1-naphthol N-methylcarbamate, is a broad spectrum carbamate insecticide with a variety of agricultural and public health applications. Due to its wide use, humans may be exposed either occupationally or through food and other routes (Cranmer, 1986). The mechanism underlying the toxicity of carbamate pesticides is its action as a cholinesterase inhibitor (Fukuto, 1990). The inhibition of cholinesterase by carbamates is reversible and less persistent than that by organophosphates.

Early studies of carbamate metabolism focused on hydrolysis because of the assumption that the ester linkage was susceptible to esterase attack as well as limitations of the analytical techniques then available (Dorough, 1970). However, the importance of oxidative pathways had been shown earlier with the demonstration of NADPH-dependent metabolic activity toward carbamates in rat liver microsomes (Hodgson and Casida, 1960, 1961). The major hydroxylation products include 5-hydroxycarbaryl, (5-hydroxy 1-naphthyl N-methylcarbamate), 4-hydroxycarbaryl, (4-hydroxy 1-naphthyl N-methylcarbamate), and carbaryl methylol, (1-naphthyl N-(hydroxymethyl)carbamate) (Dorough et al., 1963; Dorough and

Casida, 1964; Strother, 1972) and it has been suggested that hydroxylation by CYP is the more important route of carbaryl metabolism (Ward et al., 1988; Ehrich et al., 1992).

Because both carbamate and organophosphorus insecticides are metabolized by CYP and act as anticholinesterases it is possible that they may interact in metabolic pathways as well as in target sites. Although the hepatic metabolism of carbaryl in humans has been previously investigated *in vitro* (Strother, 1972; Chin et al., 1974), the contributions of individual CYP isoforms to the metabolic pathways had not been elucidated prior to the studies of Tang et al. (2002). Knowledge of the varying contributions of CYP isoforms to carbaryl metabolism should enable better understanding of differences in metabolism among individuals as well as among sub-populations and will provide important information relative to metabolic interactions of carbaryl with other chemicals.

Four metabolites were detected after incubation of carbaryl with pooled human liver microsomes (Tang et al., 2002), namely, 5-hydroxycarbaryl, 4-hydroxycarbaryl, carbaryl methylol, and 1-naphthol. The first three were generated only in the presence of an NADPH-generating system and are, therefore, the products of CYP-mediated reactions. Only very small amounts of 1-naphthol, an hydrolysis product produced in the presence or absence of the NADPH-generating system, were generated in either the microsomal or the cytosolic fractions of the human liver. Based on kinetic constants and clearance rates, methylol carbaryl was produced most efficiently and 5-hydroxymethylcarbaryl least efficiently by liver microsomes.

Of 16 human CYP isoforms tested (Tang et al., 2002), only CYP2D6*10 and CYP4A11 were without detectable activity toward carbaryl. All other isoforms generated all three oxidative metabolites, although the activities and product ratios varied widely among isoforms. The most active isoforms were CYP1A1, 1A2, 2B6, 2C19, and 3A4. Of these, CYP2B6 had the highest activity and produced primarily (almost exclusively) the methylol derivative. Polymorphic variants of CYP isoforms have not been extensively investigated with respect to carbaryl metabolism. However, CYP2C9*2 is less active than the wild type 2C9, and CYP2D6*10 showed no activity toward carbaryl, although 2D6*1 did, producing primarily 5-hydroxycarbaryl (Tang et al., 2002).

Human liver microsomes (Tang et al., 2002) from five selected individuals showed a 2-fold difference in the generation of either 5-hydroxycarbaryl or 4-hydroxycarbaryl with the activities not correlated with any single CYP isoform. On the other hand, the same microsomes showed a 5-fold difference in the generation of methylol carbaryl, a difference correlated with the level of CYP2B6 activity.

The studies of Tang et al. (2002) indicate that chlorpyrifos inhibited the metabolism of carbaryl by human liver microsomes, preferentially inhibiting the formation of the methylol derivative. This is correlated with the findings first, that the production of this metabolite is primarily the result of CYP2B6 activity and that this is the same isoform that produces the oxon from chlorpyrifos, and, second, that CYP isoforms are inhibited during oxon production. This interpretation was confirmed by the results of experiments carried out directly on CYP 2B6.

Chlorpyrifos oxon

Permethrin metabolism and inhibition by chlorpyrifos oxon and carbaryl. The discovery of the first photostable pyrethroid, permethrin (Elliott et al., 1973; Elliot, 1976), revolutionized the use of pyrethroids as a class. Permethrin, 3-phenoxybenzyl (±)-*cis, trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate, is a synthetic pyrethroid commonly used as an insecticide. Since its discovery, this class of insecticide

has become one of the mainstays of chemical control due to its high insecticidal efficacy, low mammalian toxicity, and lack of environmental persistence.

Permethrin has been extensively studied with respect to its toxicological properties (Metker et al., 1978; Litchfield, 1985; Ishmael and Litchfield, 1988). At high doses, permethrin produces a syndrome characterized by aggressive sparring, increased sensitivity to external stimuli, and a fine tremor that progresses to whole body tremor and prostration (Verschoyle and Barnes, 1972). At low doses, a dose-dependent decrease in locomotor activity and an increase in startle response to acoustic stimuli are observed (Crofton and Reiter, 1988). Bloomquist (1993) provides an overview of electrophysiological effects and interactions of pyrethroid insecticides with biochemical and physiological target sites in mammals and insects.

Permethrin toxicity is the result of its interaction with the sodium channel, by slowing both the activation and inactivation properties of the channel, leading to a stable hyperexcitable state (Ray, 2001). Although both activation and inactivation of the channel are affected, the hyperexcitable state is primarily a consequence of the prolonged negative afterpotential, which produces abnormal repetitive discharges. Other less important effects of pyrethroids include their ability to antagonize GABA-mediated inhibition, modulate nicotinic cholinergic transmission, and enhance noradrenaline release or actions on calcium ions (Ray, 2001). The low acute mammalian toxicity of permethrin and other pyrethroids is primarily due to their rapid metabolism and excretion (Miyamoto, 1976; Elliott et al., 1976; Gaughan et al., 1977), although there is also evidence that there are differences in the sodium channel sensitivity between insects and mammals (Ray, 2001).

Prior to recent studies (Choi et al., 2002), studies of permethrin metabolism in humans had been limited to the detection of primary metabolites in blood or urine samples (Angerer and Ritter, 1997; Asakawa et al., 1996; Leng et al., 1997; Hardt and Angerer, 2003). The more recent studies have demonstrated that while cis-permethrin is poorly metabolized by human liver fractions, the trans isomer is readily metabolized by both soluble and microsomal esterases (Choi et al., 2002). The resulting phenoxybenzyl alcohol is then readily metabolized to phenoxybenzoic acid by sequential oxidations by alcohol dehydrogenase and aldehyde dehydrogenases. Although mammalian, including human, alcohol and aldehyde dehydrogenases have been studied extensively prior to these studies, the role of alcohol and aldehyde dehydrogenase enzymes in the metabolism of pyrethroids had not been described in either humans or in mammals, although the metabolites detected would have suggested the existence of these metabolic pathways.

Trans-permethrin is metabolized to phenoxybenzyl alcohol and phenoxybenzoic acid in both pooled human liver microsomes and human liver cytosol, while the *cis* isomer does not appear to be metabolized by these preparations. The addition of NADPH to the microsomal preparations was without effect, indicating that the initial step, to phenoxybenzoic alcohol, was a hydrolysis, a finding supported by the observation that cytosol and microsomes produced the same metabolites. As noted following, this hydrolysis can be inhibited by chlorpyrifos oxon and carbaryl. The hypothesis that the metabolism of phenoxybenzyl alcohol to phenoxybenzoic acid was mediated by alcohol dehydrogenase and aldehyde dehydrogenase and that phenoxybenzaldehyde was an intermediate was confirmed by the use of recombinant human enzymes (Choi et al., 2002). All four purified recombinant human alcohol deydrogenases tested metabolized phenoxybenzyl alcohol to phenoxybenzaldehyde with Km values ranging from 4 to 48 μM. These Km values are around two orders of magnitude smaller that those for ethanol, and catalytic efficiencies indicate that phenoxybenzyl alcohol is a preferred substrate for this enzyme. A single isoform of human aldehyde dehydrogenase (ALDH3A1) available for these

studies (Choi et al., 2002) catalyzed the metabolism of phenoxybenzaldehyde to phenoxybenzoic acid.

Because the initial step in the metabolism of trans-permethrin in human liver is hydrolysis, it appeared important to test the ability of other chemicals to inhibit this reaction (Choi et al., 2004). Two inhibitors, chlorpyrifos oxon and carbaryl, were examined in some detail. Trans-permethrin hydrolysis in human liver fractions was inhibited more effectively by chlorpyrifos oxon than by carbaryl. Under similar assay conditions, the IC50s of chlorpyrifos oxon in human liver cytosolic and microsomal fractions were 35 nM and 60 nM, respectively. Above 60 nM (cytosol) or 150 nM (microsomes) levels, *trans*-permethrin hydrolysis was completely inhibited by chlorpyrifos oxon.

When the cytosolic fraction was pre-incubated for 5 min with chlorpyrifos oxon before the addition of permethrin, the Vmax value was significantly reduced while Km values stayed approximately the same. Five minutes of pre-incubation with chlorpyrifos oxon in the microsomal fraction again resulted in a significant decrease in Vmax value with only an insignificant decrease in Km. Based on these observations, non-competitive or irreversible inhibition was assumed for chlorpyrifos oxon inhibition of permethrin hydrolysis in human liver fractions. The inhibition constants (*Ki*) for chlorpyrifos oxon, an indicator of inhibitor affinity for the target enzyme, were 21 nM for the cytosolic fraction and 95 nM in the microsomal fraction as calculated from Vmax and Km values. These values are approximately 100 times lower than those for carbaryl, indicating a higher inhibitory potential of chlorpyrifos oxon.

In range finding assays, carbaryl showed IC50 values of 10 μM in both microsomal and cytosolic fractions. The most noticeable difference from chlorpyrifos oxon was that *trans*-permethrin hydrolysis in either the microsomal and the cytosolic fractions was not completely inhibited by a wide range of carbaryl concentrations. This observation had led to an assumption that the esterases involved in *trans*-permethrin hydrolysis in both the microsomal and the cytosolic fractions are composed of at least two different entities, which have differential susceptibilities to carbaryl and chlorpyrifos oxon inhibition.

Ki values for carbaryl were 2.49 μM in the cytosolic fraction and 11.08 μM in the microsomal fraction. In contrast to chlorpyrifos oxon, in the assay to determine the inhibition type, carbaryl appeared to act as a non-competitive inhibitor.

These studies demonstrate that there are potentially important interactions between permethrin and chlorpyrifos in humans. Chlorpyrifos, sometimes used in conjunction with permethrin, is a very potent inhibitor of *trans*-permethrin hydrolysis after metabolic activation to chlorpyrifos oxon. This observation implies that co-exposure to chlorpyrifos could potentiate the toxicity of permethrin by deactivating the metabolic detoxification pathway for permethrin. In a related study, co-exposure of a small number of human volunteers to both cyfluthrin and methyl parathion appeared to have significantly increased the half-life of cyfluthrin (Leng et al., 1999). Other chemicals, including the insect repellent (N,N-diethyl-*m*-toluamide, DEET) and a nerve gas prophylactic (pyridostigmine bromide) did not cause the inhibition of *trans*-permethrin hydrolysis, regardless of the presence of an NADPH regeneration system.

Chlorpyrifos oxon completely inhibits *trans*-permethrin hydrolysis in both human liver microsomal and cytosolic fractions with very low *Ki* values, indicating that B-esterases are responsible for *trans*-permethrin hydrolysis in human liver fractions. Compared to chlorpyrifos oxon, the parent compound, chlorpyrifos, and the other major chlorpyrifos metabolite (3,5,6-trichloro-2-pyridinol) show minimal levels of inhibition in either fraction. The observation that pre-incubation with NADPH in the microsomal

fraction substantially increased chlorpyrifos inhibition capability confirmed that chlorpyrifos oxon is the chemical species responsible for the inhibition of *trans*-permethrin hydrolysis.

The mechanism of chlorpyrifos oxon inhibition of esterases is trans-esterification, in which a covalent bond is formed between the oxon and the alcohol functional group of a serine residue in the active site of the esterase. With a normal substrate, a transient bond is formed in place of the covalent bond and readily cleaved by deacylation (Chambers and Levi, 1992). The observed inhibition kinetics (reduced Vmax and constant Km) and the irreversible nature of inhibition strongly implies that the inhibition of the human liver esterases hydrolyzing permethrin is mediated by the same mechanism previously described.

Carbaryl shows a different pattern of inhibition from chlorpyrifos oxon, typical noncompetitive inhibition. This result is in an accord with the fact that carbamate compounds are reversible and less persistent inhibitors compared to organophosphorus compounds, and that carbamate compounds can be hydrolyzed by esterases. This also explains why *Ki* values for carbaryl are two orders of magnitude higher than those for chlorpyrifos oxon.

Another important observation is that, in contrast to chlorpyrifos oxon, carbaryl cannot completely inhibit *trans*-permethrin hydrolysis even at high concentrations. Incomplete inhibition at high concentrations of carbaryl suggests that there are multiple hydrolytic enzymes involved in *trans*-permethrin hydrolysis, a finding that was not revealed by chlorpyrifos oxon inhibition. It is deduced that in *trans*-permethrin hydrolysis in human liver fractions, at least two species (or groups) of B-esterases are involved, both sensitive to chlorpyrifos oxon inhibition, but one with higher sensitivity to carbaryl inhibition and the other with lower or no sensitivity to carbaryl.

Organophosphorus Inhibitors of Xenobiotics and Endogenous Substrates: Clinical Drugs

Drug–drug interactions have been a matter of concern for some considerable time; however, the interaction between clinical drugs and other xenobiotics has seldom been addressed. Given the fact that it has been shown that OPs are potent inhibitors of the human CYP-mediated metabolism of both other xenobiotics and endogenous metabolites, this question assumes considerable importance, since individuals may be exposed environmentally or occupationally to OPs while using clinical drugs. In this regard, the recent publication of Di Consiglio et al. (2005) is important since it demonstrates significant inhibition of the metabolism of the antidepressant, imipramine, by three OPs (chlorpyrifos, azinphosmethyl, and parathion) in human liver microsomes and by the human CYP isoforms, 2C19, 1A2, and 3A4.

Organophosphorus Inhibitors of Xenobiotics and Endogenous Substrates: Endogenous Substrates

Inhibition of Testosterone Metabolism. Many pesticides are known to have significant endocrine disrupting effects. There are several avenues by which endocrine disruption may occur, including interference with synthesis, secretion, transport, binding, or elimination of natural hormones that are responsible for homeostasis and reproductive development. Although some pesticides are known to interact directly with the hormone receptors, the methods by which many cause endocrine disruption is still poorly

understood. It is suspected that many pesticides with endocrine disrupting potential may do so by interfering with the normal hormone synthesis and degradation. In this regard, we have demonstrated in mice that subchronic administration of some polychlorinated biphenyl compounds can significantly increase metabolism of testosterone and estradiol (Gillette et al., 2002). These changes in hormone metabolism as observed in mice were mediated primarily by induction of CYP isoforms.

Cytochrome P450 monoxygenases are not only major catalysts involved in the metabolism of xenobiotics, but also in the oxidative metabolism of endogenous substrates such as testosterone. Major testosterone metabolites formed by human liver microsomes include 6β-hydroxytestosterone, 2β-hydroxytestosterone, and 15β-hydroxytestosterone. A screen of 16 cDNA expressed human CYPs demonstrated (Usmani et al., 2003) that 94% of all testosterone metabolites are produced by members of the CYP3A subfamily with 6β-hydroxytestosterone accounting for 84% of all testosterone metabolites. While similar Km values were observed with human liver microsomes, regardless of which metabolite is measured, Vmax and CLint were much higher for 6β-hydroxytestosterone than for any other metabolite.

Our study of effects on endocrine metabolism (Usmani et al., 2003) indicates effects, often dramatic, of organophosphorus and other chemicals on the oxidative metabolism of testosterone. Pre-incubation of human liver microsomes with a variety of ligands, including the deployment-related test chemicals used throughout this project, resulted in varying levels of inhibition or activation of testosterone metabolism. The greatest inhibition of testosterone metabolism in human liver microsomes was seen following pre-incubation with organophosphorus compounds, including chlorpyrifos, phorate, and fonofos, with up to 80% inhibition of the formation of several metabolites, including 6β-hydroxytestosterone. Pre-incubation of CYP3A4 with chlorpyrifos, but not chlorpyrifos oxon, resulted in 98% inhibition of testosterone metabolism. Kinetic analysis indicated that chlorpyrifos is one of the most potent inhibitors of testosterone metabolism to be discovered to date and that phorate and fonofos were also potent inhibitors. In all cases, the inhibition is noncompetitive and irreversible. Conversely, pre-incubation of CYP3A4 with pyridostigime bromide increased the metabolism of testosterone to the 6β- and 2β- derivatives. Pre-incubation of aromatase (CYP19) with the test chemicals had no effect on the production of the endogenous estrogen, 17β-estradiol.

Inhibition of Estradiol Metabolism. To date, similar studies have not been carried out on estradiol metabolism. We are currently carrying out such studies (Usmani et al., 2005, in preparation) using human preparations and the methods developed during studies involving perturbations of estradiol metabolism by PCBs in rodents (Gillette et al., 2002)

Cytochrome P450 (CYP) enzymes are major catalysts involved in the metabolism of xenobiotics and endogenous substrates such as estradiol (E_2). The major E_2 metabolite formed by human liver microsomes (HLM) is 2-hydroxyestradiol (2-OHE_2). It has previously been shown that E_2 is predominantly metabolized by CYP1A2 and 3A4 with 2-OHE_2 being the major metabolite (Lee et al., 2001, 2003).

The purpose of our study was to examine possible effects of OPs and other xenobiotics on the metabolism of E_2 by HLM and individual human CYP isoforms. Comparative *in vitro* metabolism kinetic studies using HLM, CYP3A4, and CYP1A2 demonstrated similar enzyme affinities (K_m) for the substrate with respect to 2-OHE_2 production. However, V_{max} and CL_{int} were significantly higher for CYP1A2 (~60-fold) and CYP3A4 (~30-fold) than HLM. The use of phenotyped HLM demonstrated that individuals with high levels of CYP1A2 and CYP3A4

have the greatest potential to metabolize E_2. Pre-incubation of HLM with a variety of ligands resulted in varying levels of inhibition of E_2 metabolism. The greatest inhibition was observed with organophosphorus compounds, including chlorpyrifos and fonofos, with up to 80% inhibition observed for 2-OHE_2 production. Carbaryl, a carbamate pesticide, and naphthalene, a jet fuel component, inhibited approximately 40% of E_2 metabolism.

Pre-incubation of CYP1A2 with chlorpyrifos, fonofos, carbaryl, and naphthalene resulted in 96, 59, 84, and 87% inhibition of E_2 metabolism, respectively. Similarly, pre-incubation of CYP3A4 with chlorpyrifos, fonofos, deltamethrin, or permethrin under the same conditions resulted in 94, 87, 58, and 37% inhibition of E_2 metabolism, respectively. The K_i values indicated that chlorpyrifos, carbaryl, 4-hydroxycarbaryl, naphthalene, and 1-naphthol were the most potent inhibitors of E_2 metabolism and inhibited E_2 metabolism non-competitively.

SUMMARY

As indicated previously, there are numerous metabolic interactions between OPs and other xenobiotics and endogenous metabolites that are based on the inhibition, by one chemical, of the metabolism of another. For example, chlorpyrifos is a potent inhibitor of DEET, carbaryl, testosterone, and estradiol metabolism. Because the mechanism of this inhibition is almost certainly due to the formation of highly reactive sulfur during the oxidative desulfuration of chlorpyrifos followed by the interaction of this sulfur with the heme iron of cytochrome P450, this interaction will occur in the presence of chlorpyrifos whenever another chemical is metabolized by a CYP isoform that carries out the desulfuration reaction. It should also be a general interaction of any organophosphorus compound that has a P=S group in the molecule and our studies have shown that, although chlorpyrifos is the most potent inhibitor, other organophosphorus chemicals act in the same way. Other interactions noted are the inhibition of permethrin metabolism by chlorpyrifos oxon and by carbaryl.

DEDICATION

This article is written in memory of David Kupfer, a good friend and an ardent toxicologist.

REFERENCES

Angerer, J., Ritter, A. (1997). Determination of metabolites of pyrethroids in human urine using solid phase extraction and gas chromatography-mass spectrometry. *J. Chromatogr.* 695:217.

Asakawa, F., Jitsunari, F., Miki, K., Choi, J., Takeda, N., Kitamado, T., Suna, S., Manabe, Y. (1996). Agricultural worker exposure to and absorption of permethrin applied to cabbage. *Bull. Environ. Toxicol.* 56:42.

Bloomquist, J. R. (1993). Neuroreceptor mechanisms in pyrethroid mode of action and resistance. *Rev. Pestic. Toxicol. Vol 2*. In: Roe, R. M., Kuhr, R. J., eds. *Toxicology Communications.* Raleigh, NC.

Buratti, F. M., Volpe, M. T., Meneguz, A., Vitozzi, L., Testai, E. (2003). CYP-specific bioactivation of four prosphorothioate pesticides by human liver microsomes. *Toxicol. Appl. Pharmacol.* 186:143–154.

Butler, A. M., Murray, M. (1997). Biotransformation of parathion in human liver: participation of CYP3A4 and its inactivation during microsomal parathion oxidation. *J. Pharmacol. Exp. Therap.* 280:966–973.

Chambers, J. E., Levi, P. E. (1992). *Organophosphates: chemistry, Fate and Effects.* San Diego, CA: Academic Press.

Chambers, H., Brown, B., Chambers, J. E. (1990). Noncatalytic detoxication of six organophosphorus compounds by rat liver homogenates. *Pest. Biochem. Physiol.* 36:308–316.

Chin, B. H., Eldridge, J. M., Sullivan, L. J. (1974). Metabolism of carbaryl by selected human tissues using an organ-maintenance technique. *Clin. Toxicol.* 7:37–56.

Choi, J., Rose, R. L., Hodgson, E. (2002). In vitro human metabolism of permethrin: the role of human alcohol and aldehyde dehydrogenases. *Pest. Biochem. Physiol.* 73:117–128.

Choi, J., Rose, R. L., Hodgson, E. (2004). Inhibition of trans-permethrin hydrolysis in human liver fractions by chlorpyrifos oxon and carbaryl. *Drug Metabol. Drug Interact.* 20:233–246.

Cranmer, M. F. (1986). Carbaryl: a toxicological review and risk analysis. *Neurotoxicology* 7:247–332.

Crofton, K. M., Reiter, L. W. (1988). The effects of type-1 and type-II pyrethroids on motor-activity and the acoustic startle response in the rat. *Fund. Appl. Toxicol.* 10:624–634.

Dai, D., Tang, J., Rose, R. L., Hodgson, E., Bienstock, R. J., Mohrenweiser, H. W., Goldstein, J. A. (2001). Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. *J. Pharmacol. Exptl. Therap.* 299:825–831.

Di Consiglio, E., Meneguz, A., Testai, E. (2005). Organophosphorothionate pesticides inhibit the bioactivation of imipramine by human hepatic cytochrome P450s. *Toxicol. Appl. Pharmacol.* 205:237–246.

Dorough, H. W. (1970). Metabolism of insecticidal methylcarbamates in animals. *J. Agric. Food Chem.* 18:1015–1022.

Dorough, H. W., Leeling, N. C., Casida, J. E. (1963). Nonhydrolytic pathway in metabolism of N-methylcarbamate insecticides. *Science* 140:170–171.

Dorough, H. W., Casida, J. E. (1964). Nature of certain carbamate metabolites of the insecticide Sevin. *J. Agric. Food Chem.* 12:294–304.

Ecobichon, D. J. (2001). Toxic effects of pesticides. In: Klaassen, C. D., ed. *Cassarett and Doull's Toxicology: The Basic Science of Poisons*. 6th ed. New York: McGraw-Hill. pp. 763–816.

Ehrich, M., Correll, L., Strait, J., McCain, W., Wilcke, J. (1992). Toxicity and toxicokinetics of carbaryl in chickens and rats: a comparative study. *J. Toxicol. Environ. Health* 36:411–423.

Elliott, M. (1976). In: Metcalf, R. L., McKelvey, Jr., J. J., eds. *Insecticides for the Future: Needs and Prospects.* New York: Wiley.

Elliott, M., Farnham, A. W., Jones, N. F., Needham, P. H., Pulman, D. A., Stevenson, J. H. (1973). A photostable pyrethroid. *Nature* 246:169–170.

Elliott, M., Janes, N. F., Pulman, D. A., Gaughan, L.. C., Unai, T., Casida, J. E. (1976). Radiosynthesis and metabolism in rats of the 1R isomers of the insecticide permethrin. *J. Agric. Food Chem.* 24:270–276.

Fukuto, T. R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. *Environ. Health Perspect.* 87:245–254.

Furlong, C. E. (2000). PON1 status and neurologic symptom complexes in Gulf War veterans. *Genome Res* 10:153–155.

Furlong, C. E., Costa, L. G., Hassett, C., Richter, R. J., Sundstrom, J. A., Adler, D. A., Disteche, C. M., Omiecinski, C. J., Chapline, C., Crabb, J. W., Humbert, R. (1993). Human and rabbit paraoxonases: purification, cloning, sequencing, mapping and role of polymorphism in organophosphate detoxication. *Chem-Biol. Interact.* 87:35–48.

Furlong, C. E., Li, W. F., Costa, L. G., Richter, R. J., Shih, D. M., Lusis, A. J. (1998). Genetically determined susceptibility to organophosphorus insecticides and nerve agents: developing a mouse model for the human PON1 polymorphism. *Neurotoxicology* 19:645–650.

Gaughan, L. C., Unai, T., Casida, J. E. (1977). Permethrin metabolism in rats. *J. Agric. Food Chem.* 25:9–17.

Gillette, J. S., Hansen, L. G., Rose, R. L. (2002). Metabolic effects of episodic polychlorinated biphenyl (PCB) congeners. *Rev. Toxicol.* 4:129–159.

Halpert, J. A., Hammond, D., Neal, R. A. (1980). Inactivation of purified rat liver cytochrome P-450 during the metabolism of parathion. *J. Biol. Chem.* 255:1080–1089.

Hardt, J., Angerer, J. (2003). Biological monitoring of workers after the application of insecticidal pyrethroids. *Internatl. Arch. Occ. Environ. Hlth.* 76:492–498.

Hodgson, E. (2003). In vitro human phase I metabolism of xenobiotics I: pesticides and related compounds used in agriculture and public health. *J. Biochem. Mol. Toxicol.* 17:201–206.

Hodgson, E., Casida, J. E. (1960). Biological oxidation of N,N-dialkyl carbamates. *Biochim. Biophys. Acta* 42:184–186.

Hodgson, E., Casida, J. E. (1961). Metabolism of N,N-dialkyl carbamates and related compounds by rat liver. *Biochem. Pharmacol.* 8:179–191.

Hodgson, E., Smart, R. C. (2001). *An Introduction to Biochemical Toxicology.* New York: John Wiley and Sons.

Ishmael, J., Litchfield, M. H. (1988). Chronic toxicity and carcinogenic evaluation of permethrin in rats and mice. *Fundam. Appl. Toxicol.* 11:308–322.

Lee, A. J., Cai, X. M., Thomas, P. E., Conney, A. H., Zhu, B. T. (2003). Characterization of the oxidative metabolites of 17β-estradiol and estrone formed by 15 selectively expressed human cytochrome P450 isoforms. *Endrocrinology* 144:3382–3398.

Lee, A. J., Kosh, J. W., Conney, A. H., Zhu, B. T. (2001). Characterization of the NADPH-dependent metabolism of 17β-estradiol to multiple metabolites by human liver microsomes and selectively expressed human cytochrome P450 3A4 and 3A5. *J. Pharmacol. Exp. Ther.* 298:420–432.

Leng, G., Kuhn, K. H., Idel, H. (1997). Biological monitoring of pyrethroids in blood and pyrethroid metabolites in urine: applications and limitations. *Sci. Total Environ.* 199:173.

Leng, G., Lewalter, J., Rohrig, B., Idel, H. (1999). The influence of individual susceptibility in pyrethroid exposure. *Toxicol. Lett.* 107:123–130.

Levi, P. E., Hodgson, E. (1985). Oxidation of pesticides by purified cytochrome P450 isozymes from the mouse liver. *Toxicol. Lett.* 24:221–228.

Litchfield, M. H. (1985). Toxicity to mammals. In: Leahy, J. P., ed. *The Pyrethroid Insecticides.* London/Philadelphia: Taylor and Francis. pp. 99–150.

Metker, L., Angerhofer, R. A., Pope, C. R., Swentzel, K. C. 1978. Toxicological evaluation of *3-(phenoxyphenyl)methyl (±)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate (Permethrin)*. Report No. 51-0831-78. U.S. Army Environmental Hygiene Agency.

Miyamoto, J. (1976). Degradation, metabolism, and toxicity of synthetic pyrethroids. *Environ. Health Perspect.* 14:15–28.

Mutch, E., Blain, P. G., Williams, F. M. (1999). The role of metabolism in determining parathion toxicity in man. *Toxicol. Lett.* 107:177–187.

Neal, R. A. (1980). Microsomal metabolism of thiono-sulfur compounds: mechanisms and toxicological significance. *Rev. Biochem. Toxicol.* 2:131–171.

Neal, R. A., Halpert, J. (1982). Toxicology of thiono-sulfur compounds. *Ann. Rev. Pharmacol. Toxicol.* 22:321–339.

Neal, R. A., Sawahata, T., Halpert, J., Kamataki, T. (1983). Chemically reactive metabolites as suicide enzyme inhibitors. *Drug Metabol. Rev.* 14:49–59.

Nolan, R. J., Rick, D. L., Feshour, N. L., Saunders, J. H. (1984). Chlorpyrifos: pharmacokinetics in human volunteers. *Toxicol. Appl. Pharmacol.* 73:8–15.

Purdon, M. P., Lehman-McKeeman, L. D. (1997). Improved high-performance liquid chromatographic procedure for the separation and quantification of hydroxytestosterone metabolites. *J. Pharmacol. Toxicol. Methods* 37:67–73.

Ray, D. E. (2001). Pyrethroid insecticides: mechanisms of toxicity, systemic poisoning syndromes, paresthia, and therapy. In: Krieger, R., ed. *Handbook of Pesticide Toxicology.* Vol. 2 San Diego, CA: Academic Press. pp. 1289–1303.

Sams, C., Mason, H. J., Rawbone, R. (2000). Evidence for the activation of organophosphate pesticides by cytochromes P450 3A4 and 2D6 in human liver microsomes. *Toxicol. Lett.* 116:217–221.

Schoenig, G. P., Hartnagel, R. J., Jr., Osimitz, T.G., Llanso, S. (1996). Absorption, distribution, metabolism and excretion of N,N-diethyl-m-toluamide in the rat. *Drug. Metabol. Disp.* 24:156–163.

Selim, S., Hartnagel, R. J., Osimitz, T. G., Garbriel, K. L., Schoenig, G. P. (1995). Absorption, excretion and metabolism of N,N-diethyl-m-toluamide following dermal application to human volunteers. *Fund. Appl. Toxicol.* 25:95–100.

Strother, A. (1972). In vitro metabolism of methylcarbamate insecticides by human and rat liver fraction. *Toxicol. Appl. Pharmacol.* 21:112–129.

Sultatos, L. G. (1991). Metabolic activation of the organophosphorus insecticides chlorpyrifos and fenitrothion by perfused rat liver. *Toxicology* 68:1–9.

Tang, J., Cao, Y., Rose, R. L., Brimfield, A. A., Dai, D., Goldstein, J. A., Hodgson, E. (2001). Metabolism of chlorpyrifos by human cytochrome P450 isoforms and human, mouse and rat liver microsomes. *Drug Metabol. Disp.* 29:1201–1204.

Tang, J., Cao, Y., Rose, R. L., Hodgson, E. (2002). In vitro metabolism of carbaryl by human cytochrome P450 and its inhibition by chlorpyrifos. *Chem.-Biol. Interact.* 141:229–241.

Usmani, K. A., Rose, R. L., Goldstein, J. A., Taylor, W. G., Brimfield, A. A., Hodgson, E. (2002). In vitro human metabolism and interactions of the repellent, N, N-diethyl-m-toluamide (DEET). *Drug Metab. Disp.* 30:289–294.

Usmani, K. A., Rose, R. L., Hodgson, E. (2003). Inhibition and activation of the human liver and human cytochrome P450 3A4 metabolism of testosterone by deployment-related chemicals. *Drug. Metab. Disp.* 31:384–391.

Usmani, K. A., Karoly, E. D., Hodgson, E., Rose, R. L. (2004). In vitro sulfoxidation of thioether compounds by human cytochrome P450 and flavin-containing monooxygenase isoforms with particular reference to the CYP2C subfamily. *Drug Metab. Disp.* 32:333–339.

Veltri, J. C., Osimitz, T. G., Bradford, D. C., Page, B. C. (1994). Retrospective analysis of calls to poison control centers resulting from exposure to the insect repellent N, N-diethyl-m-toluamide (DEET) from 1985 to 1989. *Clin. Toxicol.* 32:1–16.

Verschoyle, R. D., Barnes, J. M. (1972). Toxicity of natural and synthetic pyrethrins to rats. *Pest. Biochem. Physiol.* 2:308–311.

Vodela, J. K., Dalvi, R. R. (1995). Comparative toxicological studies of chlorpyrifos in rats and chickens. *Veterin. Human Toxicol.* 37:1–3.

Ward, S. A., May, D. G., Heath, A. J., Branch, R. A. (1988). Carbaryl metabolism is inhibited by cimetidine in the isolated perfused rat liver and in man. *Clin. Toxicol.* 26:269–281.