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Pesticide metabolism in humans, including polymorphisms

by RL Rose, PhD,[1] J Tang, PhD,[1] J Choi, PhD,[1] Y Cao, BSc,[1] A Usmani, PhD,[1] N Cherrington, PhD,[2] E Hodgson, PhD [1]

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Recent epidemiologic studies involving Gulf War veterans or agricultural workers suggest that pesticide–pesticide or pesticide–drug interactions may be related to Gulf-War-related illnesses or elevated cancer risks, respectively. Metabolic interactions are one of many potential mechanisms requiring exploration in humans. The goal of the studies is to characterize important metabolic profiles of selected pesticides and examine potential interactions to characterize human risks associated with exposure. Pesticides examined using human liver microsomes and cytosolic fractions included chlorpyrifos, carbaryl and permethrin. The metabolic pathways involved include cytochrome P450 monooxygenases (CYP), esterases, and alcohol and aldehyde dehydrogenases. Specific isoforms and some polymorphic enzymes were characterized. Pesticide–pesticide interactions with metabolizing enzymes were demonstrated. Exposure of human hepatocytes to chlorpyrifos and permethrin demonstrated their potential to induce CYP isoforms using the bDNA (branched deoxyribonucleic acid) assay [used to monitor mRNA (messenger ribonucleic acid) levels]. These studies suggest that knowledge of human metabolic pathways will provide information that can aid the risk assessment process.

Key terms carbaryl; chlorpyrifos; cytochrome P450; esterase; induction inhibition; insecticide; permethrin.

The ultimate fate and disposition of pesticides, or any xenobiotic to which an organism is exposed, depends on interactions involving absorption, metabolism, distribution, and excretion. Although most organs and tissues have metabolic capabilities, the liver is the primary site of metabolism. In the liver, and in other organs, chemicals are generally detoxified; however, in some cases, metabolism can significantly increase toxicity. The dynamic processes of activation and detoxification determine the ultimate result of toxicant interactions.

In the process of pesticide registration, animal studies are routinely conducted to verify pesticide safety for nontarget organisms, including humans. During the course of these studies, metabolic routes are often well-characterized in rodent and other species so that potential difficulties with metabolites can be avoided. Perhaps because of conceived ethical considerations, few human studies involving pesticide metabolism have been encouraged or conducted. However, several important aspects of pesticide metabolism can only be elucidated by human studies.

Many epidemiologic studies suggest that high pesticide exposure levels may result in significant adverse effects. For example, farmers are known to have higher than normal rates of Hodgkin's disease, leukemia, multiple myeloma, non-Hodgkin's lymphoma, and cancers of the lip, stomach, prostrate and skin (1). Proximity to commercial pesticide applications has also recently been associated with an elevated risk of fetal death due to congenital abnormalities (2). Recent studies of illness related to the Gulf War indicate that adverse neurological health effects may have resulted from exposure to a variety of chemicals, including organophosphates, chemical nerve agents, the insect repellant N,N-diethyl-3-methyl (DEET), and pyridostigmine bromide (3). Subsequent studies have indicated that veterans with low levels of the paraoxonase gene (PON1) type Q arylesterase activity may have been more susceptible to neurotoxiocologic damage than those with higher levels of this particular polymorphism (4). The type Q isoform is the paraoxonase isoform most involved in the hydrolysis of the nerve agent sarin and some insecticidal organophosphates.

Studies of chemical interactions between deployment-related chemicals, including pesticides, are relatively recent in origin. For example, an acute oral study

1 North Carolina State University, Raleigh, North Carolina, United States.
2 University of Arizona, Tucson, Arizona, United States.

Correspondence to: Randy L Rose, Department of Environmental and Molecular Toxicology, Box 7633, Raleigh, NC, 27695, USA. [E-mail: randy_rose@ncsu.edu]

involving pyridostigmine bromide, permethrin, and DEET in rats indicated that animals fed combinations of these chemicals at lethal dose 16 (LD_{16}) suffered greater than additive mortality (5). The co-administration of binary or tertiary combinations of pyridostigmine bromide, DEET, and chlorpyrifos to hens significantly increased neurotoxicity, with associated increases in the inhibition of brain acetylcholinesterase and neurotoxicity target esterases (6, 7).

Our recent studies have shown that human metabolism studies can be used effectively in combination with animal studies to determine potential risks of pesticide exposure. Animal studies of the chloroacetamide herbicides previously demonstrated that alachlor is metabolically activated to form a carcinogenic metabolite that causes a rare nasal adenoma in rats (8). Our studies, conducted with humans, confirmed that, for alachlor, the putative carcinogenic metabolite can be formed by human liver microsomes, but at rates significantly less (10-fold) than those formed by rats. These studies also identified cytochrome P450 (CYP) iso-forms CYP3A4 and CYP2B6 as the isoforms most responsible for the formation of the rate-determining metabolite in this pathway (9). We also demonstrated that, of the four most commonly used chloroacetamides, butachlor and metolachlor were the least likely to be metabolized to the carcinogenic product, while acetochlor had the greatest capacity for the formation of this product in humans (10).

Pesticide metabolism studies conducted with humans, such as those conducted with the chloroacetamide herbicides, provide valuable information as to metabolic pathways and the enzymes involved in these pathways and can aid in the identification of individual persons or populations that may have increased risks after exposure events due to polymorphisms in the population. Although the CYP enzymes are extremely important in the metabolism of many chemicals, other enzyme systems, such as esterases, alcohol and aldehyde dehydrogenases and a variety of conjugating enzymes, are also often involved.

Just as with pharmaceuticals, pesticide exposures may result in potential metabolic interactions that may lead to detrimental effects. Although humans do not generally purposefully expose themselves to pesticides, occupational and incidental exposure to pesticides does occur, and the result of such exposure must be fully understood before the potential risks involved can be evaluated. Our preliminary studies suggest that pesticides can significantly inhibit the metabolism of other pesticides, as well as that of endogenous substrates. We have also demonstrated in human hepatocytes that pesticides are capable of inducing several important CYP isoforms, although the biological significance of this observation is yet uncertain.

Materials and methods

Pooled human liver microsomes, cytosol, and the heterologously expressed CYP isoforms 1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2E1 and 3A4 were purchased from BD Biosciences (Woburn, MA, USA). Polymorphic CYP2C19 isoforms were provided by Joyce Goldstein (National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA). Human hepatocytes were generously provided by Xenotech (Kansas City, KS, USA) and Edward LeClyuse (University of North Carolina, Chapel Hill, NC, USA). Chlorpyrifos, chlorpyrifos oxon, 3,5,6-trichloro-2-pyridinol (TCP), permethrin, and carbaryl were purchased from Chem Service (West Chester, PA, USA). 5-Hydroxycarbaryl, 4-hydroxycarbaryl, carbaryl methylol, and desmethylcarbaryl were obtained from the late Dr WC Dauterman. Testosterone and several metabolites were supplied by J LeBlanc (North Carolina State University) or purchased from Steraloids (Newport, RI, USA). HPLC (high-performance liquid chromatography) grade acetonitrile, water, methanol, and tetrahydrofuran came from Fisher Scientific (Fair Lawn, NJ, USA). Other chemicals, if not specified, were purchased through Sigma (St Louis, MO, USA).

In vitro assays of pesticide metabolism. Chlorpyrifos, carbaryl, and permethrin were each incubated with human liver fractions (cytosolic or microsomal) as described previously (11–13). Briefly, these incubations typically involved mixing microsomal or cytosolic protein with appropriate cofactors in Tris [tris(2,3-dibromopropyl) phosphate] or phosphate buffer (pH 7.4) and adding 3 to 5 ml of pesticide stock solutions (10 mM in acetonitrile, final concentration of 200 μM) to the mixture. Incubations involving specific CYP isoforms were initiated by the addition of the ice cold CYP isoforms (final P450 contents 50 pmol/ml) to the prewarmed incubation media. Reactions were conducted at 37°C and typically involved 10–30 minute incubation times. Reactions were terminated by the addition of 250 µl of cold acetonitrile or methanol. After centrifugation at 20 000 *g* for 5 minutes, the supernatant was stored at 4°C until HPLC analysis.

The inhibition studies involved the addition of varying concentrations of chlorpyrifos, carbaryl, and chlorpyrifos oxon to incubations containing carbaryl [10 or 500 μM (12)] or testosterone [250 μM (14)] in the presence of appropriate cofactors. In some cases, the inhibitors were coincubated with the substrate, while in others they were preincubated prior to the addition of the substrate.

Detailed methods for the HPLC separation and quantitation of chlorpyrifos, carbaryl, and permethrin, testosterone and their metabolites have been described previously (11–14).

Human hepatocyte treatments and branched DNA (deoxyribonucleic acid) assays. Human hepatocytes were cultured on collagen-coated plates in serum-free medium containing 0.1 µM dexamethasone, insulin (6.25 µg/ml), transferrin (6.25 µg/ml), and selenium (6.25 ng/ml), as described by LeCluyse et al (15). The cell medium was changed daily, and treatments were initiated 72 hours after the culture establishment. The prototypical inducers used as positive controls were 3-methylcholanthrene (3-MC) (10 µM), rifampicin (10 µM), and phenobarbital (1 mM). The concentrations of chlorpyrifos and permethrin were 50 and 100 µM. All treatments were compared with an untreated control and a dimethylsulfoxide (solvent) control. These doses were nontoxic to the hepatocytes, as determined by the Trypan Blue exclusion method. After three consecutive doses administered at daily intervals, the hepatocytes were harvested for subsequent gene expression analysis.

Branched DNA assays were conducted as described by Hartley & Klaassen (16), according to the manufacturer's protocol (Bayer Diagnostics, East Walpole, MA, USA). The CYP isoforms tested were CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C, 2D6, 2E1, and 3A4. The data were normalized to glyceraldehyde phosphate dehydrogenase levels and expressed as the ratio of activity to control.

Table 1. Metabolic activities towards chlorpyrifos in CYP2C19 expressed in *Escherichia coli*.[a] (CYP = cytochrome P450, SE = standard error of the mean)

Isoform	Desulfuration [nmol of product/(nmol P450 · min)]		Dearylation [nmol of product/(nmol P450 · min)]	
	Mean [a]	SE	Mean [a, b]	SE
2C19*1B	0.68	0.03	6.11 c	0.28
2C19*8	Not detected		1.66 d	0.07
2C19*6	Not detected		0.69 e	0.05
2C19*5	Not detected		0.68 e	0.06

[a] N=3 determinations.
[b] Means in the same column followed by the same letter (c, d or e) are not significantly different, P<0.01. [Taken from Tang et al (11)]

Table 2. Chlorpyrifos metabolism by individual human liver microsomes. (CYP = cytochrome P450, SE = standard error of the mean)

CYP	Desulfuration [nmol/(mg protein · min)]		Dearylation [nmol/(mg protein · min)]		Desulfuration/ dearylation [nmol/ (mg protein · min)]
	Mean activity [a]	SE	Mean activity [a, b]	SE	
HG6	0.09 c	0.01	0.16 c	0.03	0.56
HG23	0.16 c	0.01	0.31 c	0.04	0.50
HG42	0.74 d	0.10	0.67 c, d	0.07	1.10
HG43	0.08 c	0.01	0.61 c, d	0.04	0.13
HG112	0.67 d	0.08	0.91 d	0.10	0.74

[a] N=3–4 determinations).
[b] Means in the same column followed by the same letter (c or d) are not significantly different, P<0.01. Individual human liver microsomes (protein concentration, 20 mg/ml). CYP2B6, 2C19, 2D6, and 3A4 activities [pmol/(mg of protein · min)], represented by (S)mephenytoin *N*-demethylase, (S)-mephenytoin 4'-hydroxylase, bufarolol 1'-hydroxylase, and testosterone 6α-hydroxylase catalytic activities, respectively, were 3.1, 36, not detectable, and 2990 for HG6 (16-year-old male), 12.2, 78.1, 160, and 4050 for HG23 (25-year-old male), 140, 3.5, 110, and 14530 for HG42 (48-year-old female), 7.4, 212, 10.6, and 3408 for HG43 (23-year-old female), and 59.1, 260, 23, and 17519 for HG 112 (2-year-old female) (data provided by GenTest). [Taken from Tang et al (11)]

Results

Chlorpyrifos. A comprehensive in vitro study of chlorpyrifos metabolism in humans showed that human liver microsomes both detoxify and activate chlorpyrifos (11). The specific isoforms involved in metabolism (CYPs 1A2, 2B6, 2C9, 2C19 and 3A4) produce both metabolic products, although the metabolic ratio of activation to detoxication varied between the iso-forms. CYP2B6 and 3A4 (both of which are polymorphic) were responsible for the formation of most of the active desulfuration metabolite. An examination of two polymorphic alleles of CYP3A4 showed that one was incapable of chlorpyrifos metabolism while the other was less active than the wild type allele (17).

The detoxication product, TCP, was formed the most readily by CYP2C19, followed by 2C9 and 3A4. Like CYP3A4, 2C19 is also polymorphic. Tests of chlorpyrifos metabolism showed that the CYP2C19 wild type produced both dearylation and desulfuration products, while the three other polymorphic forms produced only the dearylation product, although in significantly smaller amounts (table 1).

Because the concentrations of the CYP isoforms can vary widely from person to person, studies were initiated to determine whether differences in the composition of the CYP isoforms could contribute to differences in human susceptibility to chlorpyrifos based on metabolism. Persons with varying levels of CYP2B6, 2C19, 2D6, and 3A4 were selected to represent contrasting levels of predicted metabolic activity. Thus persons possessing high levels of CYP2B6 and 3A4 would be expected to possess a greater ability to form the desulfuration product than those with lower levels of these isoforms. Similarly, persons with lower levels of CYP2B6, but with high levels of CYP2C19 and 3A4, can be expected to produce more dearylation products than those with significantly reduced levels of these isoforms. Although it was difficult to find persons with highly contrasting levels of both CYP2B6 and 3A4, in general, the predictions of metabolite production among five persons with contrasting levels of this group of enzymes were consistent with expectations based upon the phenotyped isoform levels (table 2). These and other studies have shown that the ratio of activation to detoxication is dependent upon

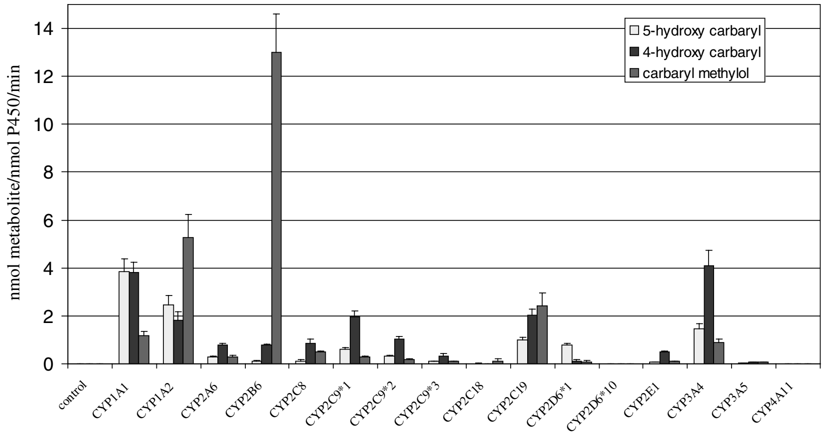

Figure 1. Distribution of carbaryl metabolites by different human CYP isoforms. Data are reported as mean metabolic activities [nmol of product/(nmol cytochrome P450 · min) + standard error of the mean] (N=3 determinations) using GenTest supersomes™. [Taken from Tang, et al (12)]

the individual isoforms present in the tissue at a given time, and it will determine the amount of oxon available to cause toxicity (11,18).

Carbaryl. Two major metabolites, 4-hydroxy carbaryl and carbaryl methylol, and one minor metabolite, 5-hydroxy-carbaryl, were formed by human liver microsomes (12). Each metabolite was dependent on the presence of a nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system, indicative of the role of CYP-catalyzed oxidation.

A survey of 13 human liver CYP isoforms identified five isoforms with carbaryl metabolizing ability (figure 1). CYP1A1 and 1A2 had the greatest ability to form 5-hydroxy carbaryl, while CYP3A4 and 1A1 were the most active in the production of 4-hydroxy carbaryl. The production of carbaryl methylol was primarily the result of metabolism by CYP2B6.

As with chlorpyrifos, variations in carbaryl metabolism among persons with contrasting amounts of CYP isoforms were also noted (12). Variations among persons in the production of the 4- and 5-hydroxy metabolites were relatively small, while there was a fivefold difference in the production of the methylol metabolite. The larger difference observed for the methylol metabolite was probably the result of the combined effect of wide variations in the amount of CYP2B6 and the lack of metabolism by multiple isoforms.

Permethrin. Our 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 (13). The resulting phenoxybenzyl alcohol is then readily metabolized to phenoxybenzoic acid through sequential oxidations by alcohol dehydrogenase and aldehyde dehydrogenases. Prior to these studies, the role of alcohol and aldehyde dehydrogenase enzymes in the metabolism of pyrethroids had not been described for either humans or mammals; although the metabolites detected would have suggested the existence of these metabolic pathways.

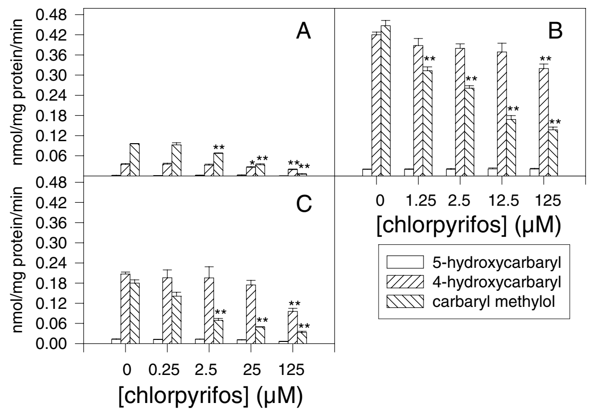

Figure 2. Activities of carbaryl metabolism in the presence of chlorpyrifos: co-incubation of varying chlorpyrifos concentrations with 10 μM carbaryl (A); co-incubation of varying chlorpyrifos concentrations with 500 μM carbaryl (B); pre-incubation of varying chlorpyrifos concentrations before incubation with 500 μM carbaryl (C). Activities are expressed as nmol product/(mg microsomal protein · min) ± standard error of the mean (N=3 determinations). Significant differences from corresponding controls (ie, 0 μM chlorpyrifos) is indicated by P<0.05* or P<0.01**. [Taken from Tang et al (12)]

Pesticide inhibition studies. The realization that both chlorpyrifos and carbaryl were metabolized by a common CYP isoform led to experiments to determine potential interactions. The co-incubation or pre-incubation of chlorpyrifos with human liver microsomes resulted in significant inhibition of carbaryl metabolism at doses as low as 2.5 μM (figure 2). The predominant metabolite inhibited was carbaryl methylol, although higher doses of chlorpyrifos also inhibited the formation of 4-hydroxycarbaryl. These data implicated the specific inhibition of CYP2B6, since this isoform is primarily responsible for the formation of carbaryl methylol. The dissociation constants calculated for the chlorpyrifos inhibition of carbaryl methylol and 4-hydroxycarbaryl formation were 2 μM and 150 μM, respectively (12).

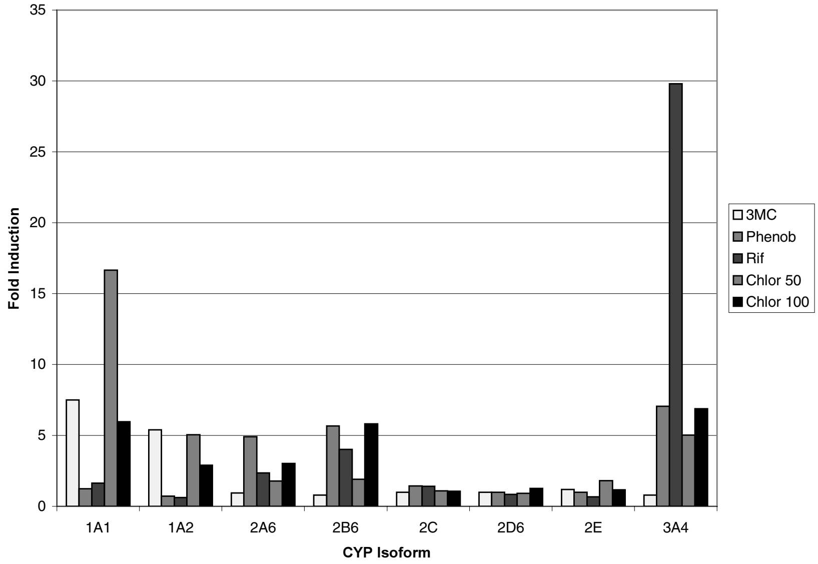

Figure 3. Human hepatocyte induction by chlorpyrifos and prototypical CYP inducers. Results of the bDNA assay are expressed as the ratio of activity relative to the untreated controls. (CYP = cytochrome P450, bDNA = branched deoxyribonucleic acid)

The discovery that ester hydrolysis was the first step in permethrin metabolism led to studies of potential interactions with pesticides and pesticide metabolites known to inhibit esterases. In these studies, varying concentrations of chlorpyrifos oxon or carbaryl were added to microsomal or cytosolic human liver fractions and preincubated for 5 minutes prior to the addition of 200 µM trans-permethrin (19). In both fractions, incubations of chlorpyrifos-oxon produced nearly linear inhibition curves. The calculated 50% inhibition concentrations (IC_{50}) were 60 and 35 nM for microsomes and cytosol, respectively. For both fractions, concentrations of approximately twice the IC_{50} values resulted in complete inhibition. Incubations with chlorpyrifos did not lead to inhibition unless microsomes were co-incubated with an NADPH-regenerating system that would lead to the production of chlorpyrifos-oxon.

Incubations of permethrin with varying concentrations of carbaryl also inhibited permethrin metabolism, although there were substantial differences in both the potency and type of inhibition (19). In contrast with the inhibition of chlorpyrifos-oxon, that of permethrin metabolism by carbaryl produced a biphasic inhibition pattern in both fractions. Although up to 60% of the hydrolysis could be inhibited by concentrations as low as 10 µM, doses as high as 200 µM did not significantly increase inhibition. This pattern was observed in both the microsomal and the cytosolic fractions. The combination of the results obtained from chlorpyrifos-oxon and carbaryl inhibition studies suggest that the permethrin hydrolyzing esterases are two or more B-esterases inhibited by chlorpyrifos-oxon but with varying susceptibility to carbaryl inhibition.

Endocrine effects. Our recent study of testosterone oxidation by human microsomes indicated that pesticides are capable of dramatically altering this metabolism (14). Using human liver microsomes, we confirmed the multiple sites of oxidative metabolism of testosterone, of which 6 • -hydroxylation is the most important, and the primary role of CYP3A4 in this metabolic attack. Of more importance in the present context is the observation that chlorpyrifos and other organophosphorus chemicals almost completely inhibit the 6 • -hydroxylation by human liver microsomes and by CYP3A4.

Pesticide induction studies. The branched DNA (bDNA) assay is a novel technique that amplifies signals produced when mRNA (messenger ribonucleic acid) binds to specific oligonucleotides in a microplate format. Preliminary assays conducted using human hepatocytes and the bDNA assay suggests that several CYP isoforms are induced by chlorpyrifos, including CYP1A1, 1A2, 2B6 and 3A4 (figure 3). Permethrin has also been shown to induce CYP 2A6, 2B6, and 3A4 (data not shown).

Discussion

Few studies have thoroughly examined the metabolic enzymes involved in the human metabolism of pesticides. Our studies of chlorpyrifos metabolism using human liver microsomes showed that humans metabolize chlorpyrifos to both detoxication and activation products. Calculated clearance rates based on in vitro experiments for chlorpyrifos were lower for humans than for rodents (11). We also showed that 5 of the 12 human CYP isoforms examined (CYP1A2, 2B6, 2C9*1, 2C19, and 3A4) were capable of chlorpyrifos metabolism, each producing varying amounts of both metabolites. Butler & Murray (20) demonstrated that CYP1A2, 2B6, and 3A4 have high desulfuration activities towards parathion.

Although all five isoforms produced both metabolites, CYP2B6 and 3A4 (both of which have polymorphic isoforms) were responsible for most of the oxon, the active desulfuration metabolite. A limited study of people with widely varying levels of CYP2B6, 2C19, 2D6, and 3A4 showed that those with the highest expression of 2B6 and 3A4 produced the greatest concentrations of chlorpyrifos-oxon. Not only do isoform concentrations vary within persons, but some isoforms, including 2B6, vary more widely between persons than others. Therefore, studies examining the individual variation of xenobiotic metabolism must not only account for relative amounts of each isoform within persons but also for variations of specific isoforms between persons as well.

The formation of the chlorpyrifos detoxication product, TCP, was best catalyzed by the wild type of polymorphic CYP2C19. Each of the three polymorphic forms of CYP2C19 we examined was significantly less active than the wild type (table 1). People with the poor metabolizing forms of CYP2C19 represent approximately 3–5% of Caucasians and African Americans with 12–100% of Asian groups (21). Related studies also showed that one polymorphic CYP3A4 allele is defective in activating chlorpyrifos when compared with the normal allele, while a second allele is less active (17).

Our studies of carbaryl metabolism showed the formation of three metabolites, formed by five different CYP isoforms (CYP1A1, 1A2, 2B6, 2C19, and 3A4) (12). The formation of the two predominant metabolites, 4-hydroxy carbaryl and carbaryl methylol, was the result of metabolism by CYP1A1 and 3A4 for the former and 2B6 for the latter. As had been observed for chlorpyrifos, the proportion of metabolites formed by microsomes for persons with known concentrations of important isoforms involved in the metabolism of these pesticides was predictable (table 2).

Prior to our recent studies, metabolic studies of permethrin in humans had been limited to the detection of primary metabolites in blood or urine samples (22–24). Although these studies provided information on permethrin persistence and potential routes of metabolism, the actual metabolic pathways and specific enzymes involved were not available. Our studies showed that permethrin metabolism is primarily the result of esterase hydrolysis, followed by alcohol and aldehyde dehydrogenase oxidations (13). This is the first demonstration of the role that alcohol and aldehyde dehydrogenase enzymes play in permethrin metabolism in humans, although metabolites detected in previous studies suggested the possibility of these pathways.

The discovery that CYP2B6 was a major isoform in the metabolism of both chlorpyrifos and carbaryl led to studies exploring potential metabolic interactions between these two pesticides. The activation of chlorpyrifos, by metabolism to chlorpyrifos-oxon, occurs through a CYP-catalyzed desulfuration reaction. The sulfur released from chlorpyrifos in this reaction is highly reactive and is believed to bind immediately to the heme iron of CYP and inhibit its activity (25). Thus, because CYP2B6 is a major isoform involved in this reaction, it was suspected that incubation with chlorpyrifos should inactivate the metabolism of carbaryl by CYP2B6. Results of this study showed that chlorpyrifos significantly inhibited carbaryl metabolism, primarily by inhibiting the methyl hydroxylation pathway, which is catalyzed principally by CYP2B6.

Endocrine disruption by pesticides remains poorly understood. Although some pesticides are known to interact directly with hormone receptors, others can interact with the endocrine system by interfering with hormone synthesis, secretion, transport, binding, or the elimination of natural hormones that are responsible for homeostasis and reproductive development. Using mice, we have previously showed that the subchronic administration of some pesticides and polychlorinated biphenyl compounds can significantly increase the metabolism of testosterone and estradiol (26, 27). These changes in hormone metabolism, as observed in mice, were mediated primarily by the induction of CYP isoforms by the pesticides.

Since CYP3A4 is the predominant CYP involved in the human metabolism of testosterone (14, 28, 29), and was also shown to be important in the activation of chlorpyrifos to chlorpyrifos-oxon (11), we examined potential interactions between these substrates. Human liver microsomes pre-incubated with chlorpyrifos prior to the addition of testosterone were significantly inhibited in the formation of the primary testosterone product, 6β-hydroxytestosterone. This inhibition was shown to be primarily the result of the nearly complete inhibition of CYP3A4 by chlorpyrifos (14). Although this inhibition may not have biological significance due to the ability of the body to compensate via hormonal feedback mechanisms, potential implications of CYP3A4 inhibition may have relevance in relation to therapeutic drugs or other chemicals to which humans may be exposed.

Many pesticides and drugs are known to induce the metabolism of other coadministered drugs and to induce their own metabolism. Elaborate screening protocols are often used by pharmaceutical companies to verify the absence of harmful interactions between drug candidates. Human hepatocyte cultures are often used in these studies, since cultured hepatocytes retain many aspects of liver function, including the CYP-mediated oxidation of drugs and CYP induction (30). Human hepatocytes also offer the opportunity to explore both the phase I and phase II enzymes involved in metabolism, providing a more complete picture of metabolism than can be obtained using subcellular fractions.

Our preliminary hepatocyte studies utilized the bDNA assay to monitor changes in mRNA expression after the in vitro treatment of human hepatocytes with pesticides and prototypical CYP inducers. The bDNA assay resembles the well-established enzyme-linked immunsorbent assay (ELISA) in principle, but uses multi-oligonucleotides not only to capture the mRNA of interest, but also to link it to an enzyme that produces a chemiluminescent signal on the addition of substrate (16). The primary value of the bDNA assay lies in its ability to assess the differential expression of a chosen set of genes in response to a chemical stimulus. For a targeted gene sequence, such as a series of metabolizing enzymes, one total RNA sample can be split among several different probe sets for the quantitative analysis of many genes.

The results of the bDNA assay demonstrated that chlorpyrifos induces CYP isoforms 1A1, 1A2, 2B6, and 3A4 to levels of fivefold or greater. Since we showed that chlorpyrifos is metabolized by several of these isoforms, it was not particularly surprising that some of these isoforms were induced, since substrates often induce their own metabolism. Using mice, we had previously shown that CYP2b10, the mouse phenobarbital-inducible isoforms analogous to CYP2B6, was also inducible by chlorpyrifos. However, the isoform that was induced to the greatest extent in these studies was CYP1A1, an isoform that did not show any metabolic activity in our metabolism screening assays. We were also surprised by the apparent ability of permethrin to induce CYP2A6, 2B6, and 3A4 because our studies using human liver microsomes and human CYP isoforms did not implicate any involvement of CYP in permethrin metabolism. Additional studies will be necessary to confirm the results of the bDNA assay, particularly in the in vivo situation, since the implications of significant inducing potential by pesticides may be important.

Acknowledgments

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