In Vitro Human Phase I Metabolism of Xenobiotics I: Pesticides and Related Compounds Used in Agriculture and Public Health, May 2003

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ABSTRACT: This is the first revision of a database covering human phase I enzymes and their isoforms that metabolize pesticides and related compounds. The original version included enzymes that metabolize chloroacetamide and triazine herbicides, and organophosphorus insecticides. This revision also includes carbamate, nicotinoid, and pyrethroid insecticides and insect repellents. © 2003 Wiley Periodicals, Inc. J Biochem Mol Toxicol 17:201–206, 2003; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jbt.10080

KEYWORDS: **Alcohol Dehydrogenase; Aldehyde Dehydrogenase; Carbamates; Cytochrome P450; CYP; Flavin-Containing Monooxygenase; FMO; Human Metabolism; Nicotinoids, Pesticides; Pyrethroids; Repellents**

INTRODUCTION

Although there have been hundreds of communications over the last several decades on the subject of the in vitro metabolism of pesticides and other chemicals used in agriculture and public health, almost without exception they have utilized experimental animals, usually rodents, while studies of in vitro human metabolism have been few. However, with the realization of the role that human studies may play in improving the scientific basis of human health risk assessment and in facilitating the development of new, effective but safer chemicals, this area of study is receiving increased emphasis. The development of methods for the expression of recombinant human xenobiotic-metabolizing enzymes, their isoforms and polymorphisms, has made possible the investigation of the role of these enzymes without invasive procedures. The initial summary [1] included three classes of pesticides, in the current revision this is expanded to seven.

INSECTICIDES

Carbamates

Metabolisms of two quite different carbamate insecticides have recently been published, aldicarb (2-methyl-2-(methylthio)propanol *O*-(methylamino) carbonyl oxime) [2] and carbaryl (1-naphthol *N*-methylcarbamate) [3]. The details are presented in Table 1. It is of interest that while 14 of 16 CYP isoforms tested metabolized carbaryl to three metabolites, 4-hydroxycarbaryl, 5-hydroxycarbaryl, and methylol carbaryl, both the overall activity and the ratio of the metabolites varied dramatically between isoforms [3].

Organophosphorus Compounds

There are several interesting features to be noted concerning the in vitro metabolism of organophosphorus insecticides by human microsomes or CYP isoforms (Table 2). Although dearylation and desulfuration are believed to be the product of common, very unstable, phosphooxythiiran intermediate, the ratio of desulfuration to dearylation products varies widely, up to 24-fold between active isoforms [5]. Furthermore, the variation in oxon production between microsomes from individual human livers varied almost 10-fold [5,9]. It is remarkable that CYP2B6 metabolizes chlorpyrifos primarily to the oxon [5] while metabolizing parathion primarily to *p*-nitrophenol [9]. By using phorate as a substrate, the sulfoxide of the thioether sulfur appears to be formed preferentially over the oxon [12] leaving open the possibility that the active metabolite is the oxon of the sulfoxide or the sulfone rather than phorate oxon.

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TABLE 1. In Vitro Metabolism of Carbamate Insecticides by Human Phase I Xenobiotic-Metabolizing Enzymes

Chemical	*Enzyme Preparation Isoform and/or Polymorphism*	*Reaction*	*References*
Aldicarb	Liver microsomes	Sulfoxidation	[2]
	FMO1	Sulfoxidation	[2]
	FMO3	Sulfoxidation	[2]
Carbaryl	Liver microsomes	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP1A1	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP1A2	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2A6	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2B6	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2C8	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2C9*1	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2C9*2	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2C9*3	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2C18	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2C19	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2D6*1	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP2E1	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP3A4	Ring hydroxylation	[3]
		Methyl oxidation	[3]
	CYP3A5	Ring hydroxylation	[3]
		Methyl oxidation	[3]

TABLE 2. In Vitro Metabolism of Organophosphorus Insecticides by Human Phase I Xenobiotic-Metabolizing Enzymes

Chemical	*Enzyme Preparation Isoform and/or Polymorphism*	*Reaction*	*References*
Azinphos-methyl	CYP1A2	Desulfuration	[4]
	CYP2B6	Desulfuration	[4]
	CYP2C19	Desulfuration	[4]
	CYP3A4	Desulfuration	[4]
Chlorpyrifos	Liver microsomes	Desulfuration	[5]
		Dearylation	[5]
	CYP1A2	Desulfuration	[5]
		Desulfuration	[4]
		Dearylation	[5]
	CYP2B6	Desulfuration	[5]
		Dearylation	[5]
		Desulfuration	[4]
	CYP2C9	Desulfuration	[5]
		Dearylation	[5]
	CYP2C19	Desulfuration	[5]
		Dearylation	[5]
		Desulfuration	[4]
	CYP2C19*1B	Desulfuration	[5]
		Dearylation	[5]
	CYP2C19*8	Dearylation	[5]
	CYP2C19*6	Dearylation	[5]
	CYP2C19*5	Dearylation	[5]
	CYP3A4	Desulfuration	[4–6]
		Dearylation	[5,6]
	CYP3A4-F189S	Desulfuration	[6]
		Dearylation	[6]
	CYP3A4-L293P	Desulfuration	[6]
		Dearylation	[6]
	CYP3A4-M445T	Desulfuration	[6]
		Dearylation	[6]
	CYP3A4-P467S	Desulfuration	[6]
		Dearylation	[6]
Diazinon	Liver microsomes	Desulfuration	[7]
		Dearylation	[7]
	CYP1A2	Desulfuration	[4]
	CYP2B6	Desulfuration	[4]
	CYP2C19	Desulfuration	[4,8]
	CYP2D6	Desulfuration	[7]
		Dearylation	[7]
	CYP3A4	Desulfuration	[7,8]
		Dearylation	[7]
		Desulfuration	[8]
Parathion	Liver microsomes	Desulfuration	[7,9–11]
		Dearylation	[7,9–11]
	CYP1A2	Desulfuration	[7–11]
		Dearylation	[7,9–11]
	CYP2B6	Desulfuration	[7–11]
		Dearylation	[7,9–11]
	CYP2C8	Desulfuration	[9]
		Dearylation	[9]
	CYP2C19	Desulfuration	[4,9]
		Dearylation	[9]
	CYP2D6	Desulfuration	[9]
	CYP2E1	Desulfuration	[9]
		Dearylation	[9]
	CYP3A4	Desulfuration	[7–11]
		Dearylation	[7,9–11]
	CYP3A5	Desulfuration	[9]
		Dearylation	[9]
Phorate	Liver microsomes	Sulfoxidation	[12]
	CYP1A2	Sulfoxidation	[12]
	CYP2C8	Sulfoxidation	[12]
	CYP2C9	Sulfoxidation	[12]
	CYP2C18	Sulfoxidation	[12]
	CYP2C19	Sulfoxidation	[12]
	CYP2E1	Sulfoxidation	[12]
	CYP3A4	Sulfoxidation	[12]
	FMO1	Sulfoxidation	[12]

TABLE 3. In Vitro Metabolism of Nicotinoid Insecticides by Human Phase I Xenobiotic-Metabolizing Enzymes

Chemical	*Enzyme Preparation Isoform and/or Polymorphism*	*Reaction*	*References*
Imidacloprid	CYP2A6	Imidazolidine oxidation	[13]
	CYP2C9	Imidazolidine oxidation	[13]
	CYP2C19	Imidazolidine oxidation	[13]
	CYP3A4	Imidazolidine oxidation	[13]
	CYP1A2	Nitroimine reduction	[13]
	CYP2B6	Nitroimine reduction	[13]
	CYP2D6	Nitroimine reduction	[13]
	CYP2E1	Nitroimine reduction	[13]

Nicotinoids

The first communication on the in vitro human metabolism of a nicotinoid, imidacloprid, has recently been published [13] (Table 3). It is of interest that of the two principal pathways, one is oxidative, the other reductive and that two different sets of CYP isoforms catalyze the two reaction pathways.

Pyrethroids

The in vitro human metabolism of pyrethroids has not been widely studied. A recent study [14] of the human metabolism of permethrin has demonstrated that following an initial hydrolysis of *trans*-permethrin, one of the hydrolysis products, phenoxybenzyl alcohol, is metabolized in sequence by alcohol dehydrogenase and aldehydrogenase to yield phenoxybenzoic acid (Table 4).

HERBICIDES

Chloroacetamides

Chloroacetamides are metabolized by a series of reactions involving CYP isoforms and arylamidase to yield either 2,6-diethyldiethylbenzoquinone amine or 2-methyl-6-ethylbenzoquinone imine, the presumptive DNA reactive metabolites [15,16]. In the case of metolachlor this occurs in rodents but may not occur in humans since the initial step is an O-demethylation rather than an N-demethylation [15] (Table 5).

TABLE 4. In Vitro Metabolism of Pyrethroid Insecticides by Human Phase I Xenobiotic-Metabolizing Enzymes

Chemical	*Enzyme Preparation Isoform and/or Polymorphism*	*Reaction*	*References*
Permethrin	Liver microsomes	Hydrolysis	[14]
Phenoxybenzyl alcohol	Alcohol dehydogenase α	Oxidation	[14]
	Alcohol dehydogenase β-I	Oxidation	[14]
	Alcohol dehydogenase β-II	Oxidation	[14]
	Alcohol dehydogenase γ	Oxidation	[14]
Phenoxybenzaldehyde	Aldehyde dehydrogenase ALDH3A1	Oxidation	[14]

TABLE 5. In Vitro Metabolism of Chloroacetamide Herbicides and Selected Metabolites by Human Phase I Xenobiotic-Metabolizing Enzymes

Chemical	*Enzyme Preparation Isoform and/or Polymorphism*	*Reaction*	*References*
Alachlor	Liver microsomes	N-Dealkoxylation	[12,15,16]
		Aliphatic hydroxylation	[12,15,16]
	CYP3A4	N-Dealkoxylation	[12,15,16]
		Aliphatic hydroxylation	[12,15,16]
	CYP2B6	N-Dealkoxylation	[15]
Butachlor	Liver microsomes	N-Dealkoxylation	[15]
	CYP3A4	N-Dealkoxylation	[15]
	CYP2B6	N-Dealkoxylation	[15]
Acetachlor	Liver microsomes	N-Dealkoxylation	[15]
	CYP3A4	N-Dealkoxylation	[15]
	CYP2B6	N-Dealkoxylation	[15]
Metolachlor	Liver microsomes	O-Demethylation	[15]
	CYP2B6	O-Demethylation	[15]
Diethylaniline	Liver microsomes	Ring hydroxylation	[15]
Methylethylaniline	Liver microsomes	Ring hydroxylation	[15]

TABLE 6. In Vitro Metabolism of Triazine Herbicides by Human Phase I Xenobiotic-Metabolizing Enzymes

Chemical	*Enzyme Preparation Isoform and/or Polymorphism*	*Reaction*	*References*
Atrazine	Liver microsomes	N-Deethylation	[17]
		N-Deisopropylation	[17]
		Isopropyl 1-hydroxylation	[17]
	CYP1A1	N-Deethylation	[18]
		N-Deisopropylation	[18]
	CYP1A2	N-Deethylation	[18]
		N-Deisopropylation	[18]
	CYP2B6	N-Deisopropylation	[18]
	CYP2C19	N-Deethylation	[18]
		N-Deisopropylation	[18]
	CYP2D6	N-Deethylation	[18]
	CYP2E1	N-Deethylation	[18]
		N-Deisopropylation	[18]
	CYP3A4	N-Deethylation	[18]
		N-Deisopropylation	[18]
Terbuthylazine	Liver microsomes	*t*-Butyl hydroxylation	[17]
		N-Deethylation	[17]
	CYP1A1	N-Deethylation	[18]
	CYP1A2	N-Deethylation	[18]
	CYP2C19	N-Deethylation	[18]
	CYP3A4	N-Deethylation	[18]
Ametryne	Liver microsomes	N-Deethylation	[17]
		N-Deisopropylation	[17]
		Isopropyl 1-hydroxylation	[17]
		Sulfoxidation	[17]
	CYP1A1	N-Deethylation	[18]
		N-Deisopropylation	[18]
		Sulfoxidation	[18]
	CYP1A2	N-Deethylation	[18]
		N-Deisopropylation	[18]
		Sulfoxidation	[18]
	CYP2B6	Sulfoxidation	[18]
	CYP2C8	N-Deisopropylation	[18]
	CYP2C9	N-Deisopropylatiion	[18]
		Sulfoxidation	[18]
	CYP2C19	N-Deethylation	[18]
		N-Deisopropylation	[18]
	CYP3A4	N-Deethylation	[18]
		N-Deisopropylation	[18]
		Sulfoxidation	[18]
Terbutryne	Liver microsomes	*t*-Butyl 1-hydroxylation	[17]
		N-Deethylation	[17]
		Sulfoxidation	[17]
	CYP1A1	N-Deethylation	[18]
		Sulfoxidation	[18]
	CYP1A2	N-Deethylation	[18]
		Sulfoxidation	[18]
	CYP2B6	Sulfoxidation	[18]
	CYP3A4	N-Deethylation	[18]
		Sulfoxidation	[18]

TABLE 7. In Vitro Metabolism of Insect Repellents by Human Phase I Xenobiotic-Metabolizing Enzymes

Chemical	*Enzyme Preparation Isoform and/or Polymorphism*	*Reaction*	*References*
DEET	Liver microsomes	N-Deethylation	[19]
		Ring methyl oxidation	[19]
	CYP1A1	Ring methyl oxidation	[19]
	CYP2B6	Ring methyl oxidation	[19]
	CYP2D6*1	Ring methyl oxidation	[19]
	CYP2E1	Ring methyl oxidation	[19]
	CYP2A6	N-Deethylation	[19]
	CYP2C19	N-Deethylation	[19]
	CYP3A4	N-Deethylation	[19]
	CYP3A5	N-Deethylation	[19]

TABLE 8. Pesticide and Repellent Substrates for CYP and Other Phase I Human Isoforms[a]

Phase I Isoform	*Substrate*
Alcohol dehydrogenase α	Permethrin metabolite: Phenoxybenzyl alcohol
Alcohol dehydrogenase β-I	Permethrin metabolite: Phenoxybenzyl alcohol
Alcohol dehydrogenase β-II	Permethrin metabolite: Phenoxybenzyl alcohol
Alcohol dehydrogenase γ	Permethrin metabolite: Phenoxybenzyl alcohol
Aldehyde dehydrogenase ALDH3A1	Permethrin metabolite: Phenoxybenzyl aldehyde
CYP1A1	Insecticides: Carbaryl Herbicides: Ametryne; atrazine; terbuthylazine; terbutryne Insect repellent: DEET
CYP1A2	Insecticides: Azinphos-methyl; carbaryl; chlorpyrifos; diazinon; imidacloprid; parathion; phorate Herbicides: Ametryne; atrazine; terbuthylazine; terbutryne
CYP2A6	Insecticides: Carbaryl; imidacloprid Insect repellent: DEET
CYP2B6	Insecticides: Azinophos-methyl; carbaryl; chlorpyrifos; diazinon; imidacloprid; parathion Insect repellent: DEET Herbicides: Acetachlor; alachlor; ametryne; atrazine; butachlor; metolachlor; terbutryne
CYP2C8	Insecticides: Carbaryl; phorate; parathion Herbicide: Ametryne
CYP2C9	Insecticide: Chlorpyrifos; imidacloprid; phorate Herbicide: Ametryne
CYP2C9*1	Insecticide: Carbaryl
CYP2C9*2	Insecticide: Carbaryl
CYP2C9*3	Insecticide: Carbaryl
CYP2C18	Insecticides: Carbaryl; phorate
CYP2C19	Insecticides: Azinphos-methyl; carbaryl; chlorpyrifos; diazinon; imidacloprid; parathion; phorate Insect repellent: DEET Herbicides: Ametryne; atrazine; terbuthylazine
CYP2C19*1B	Insectidie: Chlorpyrifos
CYP2C19*8	Insecticide: Chlorpyrifos
CYP2C19*6	Insecticide: Chlorpyrifos
CYP2C19*5	Insecticide: chlorpyrifos
CYP2D6*1	Insecticide: Carbaryl Insect repellent: DEET
CYP2D6	Insecticides: Diazinon; parathion; imidacloprid Herbicide: Atrazine
CYP2E1	Insecticides: Carbaryl; imidacloprid; parathion; phorate Insect repellent: DEET Herbicide: Atrazine
CYP3A4	Insetcicide: Azinphos-methyl; carbaryl; chlorpyrifos; diazinon; imidacloprid; parathion; phorate Insect repellent: DEET Herbicide: Acetachlor; alachlor; ametryne; atrazine; butachlor; terbuthylazine; terbutryne
CYP3A4-F189S	Insecticide: Chlorpyrifos
CYP3A4-L293P	Insecticide: Chlorpyrifos
CYP3A4-M445T	Insecticide: Chlorpyrifos
CYP3A4-P467S	Insecticide: Chlorpyrifos
CYP3A5	Insecticide: Carbaryl; parathion Insect repellent: DEET
CYP2E1	Herbicide: Atrazine
FMO1	Insecticides: Aldicarb; phorate
FMO3	Insecticide: Aldicarb

[a] For references see Tables 1–7.

Triazines

Although many CYP isoforms are active in the metabolism of triazine herbicides, CYP1A2 is probably the most important in the case of all four triazines used in these studies [17,18] since it is active at low substrate concentrations whereas the other active isoforms appear to be active at high substrate concentration only (Table 6).

REPELLENTS

To date, there has been only a single communication on the in vitro human metabolism of DEET, the principle insect repellent used throughout the world [19]. DEET is either N-deethylated or the ring methyl substituent is oxidized, each reaction being catalyzed by a different set of CYP isoforms (Table 7).

SUMMARY OF THE KNOWN PESTICIDE AND RELATED CHEMICAL SUBSTRATES FOR HUMAN CYP AND OTHER PHASE I ISOFORMS

Table 8 lists all of the known pesticide and related chemical substrates for each human CYP isoform.

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