Inhibition of the Human Liver Microsomal and Human Cytochrome P450 1A2 and 3A4 Metabolism of Estradiol by Deployment-Related and Other Chemicals

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ABSTRACT:
Cytochromes P450 (P450s) are major catalysts in the metabolism of xenobiotics and endogenous substances such as estradiol (E2). It has previously been shown that E2 is predominantly metabolized in humans by CYP1A2 and CYP3A4 with 2-hydroxyestradiol (2-OHE2) the major metabolite. This study examines effects of deployment-related and other chemicals on E2 metabolism by human liver microsomes (HLM) and individual P450 isoforms. Kinetic studies using HLM, CYP3A4, and CYP1A2 showed similar affinities (Km) for E2 with respect to 2-OHE2 production. Vmax and CLint values for HLM are 0.32 nmol/min/mg protein and 7.5 µl/min/mg protein; those for CYP3A4 are 6.9 nmol/min/nmol P450 and 291 µl/min/nmol P450; and those for CYP1A2 are 17.4 nmol/min/nmol P450 and 633 µl/min/nmol P450. Phenotyped HLM use showed that individuals with high levels of CYP1A2 and CYP3A4 have the greatest potential to metabolize E2. Preincubation of HLM with a variety of chemicals, including those used in military deployments, resulted in varying levels of inhibition of E2 metabolism. The greatest inhibition was observed with organophosphorus compounds, including chlorpyrifos and fonofos, with up to 80% inhibition for 2-OHE2 production. Carbaryl, a carbamate pesticide, and naphthalene, a jet fuel component, inhibited ca. 40% of E2 metabolism. Preincubation of CYP1A2 with chlorpyrifos, fonofos, carbaryl, or naphthalene resulted in 96, 59, 84, and 87% inhibition of E2 metabolism, respectively. Preincubation of CYP3A4 with chlorpyrifos, fonofos, deltamethrin, or permethrin resulted in 94, 87, 58, and 37% inhibition of E2 metabolism. Chlorpyrifos inhibition of E2 metabolism is shown to be irreversible.

The cytochrome P450 (P450) monoxygenase system is composed of a superfamily of heme-containing enzymes, expressed in many mammalian tissues with the highest levels found in liver, and capable of catalyzing the metabolism of a wide range of both endogenous and xenobiotic substrates (Nelson et al., 1996). Human CYP3A4 and CYP1A2 are two of the most important and abundant drug-metabolizing P450 isoforms in human liver microsomes (HLM). On average, CYP3A4 and CYP1A2 account for approximately 40 and 13% of the total P450 in HLM, respectively (Shimada et al., 1994; Lehmann et al., 1998). Human CYP3A4 and CYP1A2 not only metabolize xenobiotics but also are responsible for the metabolism of endogenous compounds, such as steroid hormones. Both human CYP1A2 and CYP3A4 are involved in the metabolism of estradiol (E2) and estrone (Lee et al., 2001, 2003), whereas CYP3A4 plays a predominant role in the metabolism of testosterone (TST), androstenedione, and progesterone (Waxman et al., 1988; Usmani et al., 2003).

In the human female, E2 is the most potent primary circulating estrogen of a group of endogenous estrogen steroids that includes estrone and estriol. During female development, E2 stimulates the growth of female sex organs, regulates and sustains female sexual development and reproductive function, promotes hypertrophy in female breast and male muscle during puberty, initiates the synthesis of specific proteins, and controls fat deposition and distribution in subcutaneous tissues, thereby determining the characteristic female figure. Like TST in males, estrogens are the primary cause of the growth spurt, maturation of long bones, and development of secondary sexual characteristics in females. In the adult, E2 regulates events during the menstrual cycle (growth of endometrial lining), is important during pregnancy and lactation, and contributes to the maintenance of sexual drive and female personality (Constanti et al., 1998; Wilson et al., 1998). Maintaining hormonal balance relies on a number of variables, including the rate of hormone synthesis, interactions among hormones, and rates of secretion, transport, and metabolism by phase I and phase II enzymes. P450s (phase I) are a major element in the maintenance of proper steroid hormone levels in mammalian systems and are the sole subject of this investigation. Exposure to foreign compounds may exert changes in endocrine function both...
directly (hormone agonists or antagonists) or indirectly (altering circulating levels of hormones by influencing rates of hormone synthesis or metabolism) that can severely affect steroid hormone action (Wilson and LeBlanc, 1998). It follows that perturbation of the P450 system by xenobiotics may in turn affect the subsequent metabolism and disposition of E2. Perturbations in E2 metabolism may affect levels of circulating E2 with possible reproductive and other consequences, including further modulation of the expression of some P450 proteins.

The uses of deployment-related chemicals are essential for the health and well being of deployed forces during both peace-keeping and wartime missions. Human hazards associated with chemicals used to protect personnel during peace and wartime are poorly understood because the definition of cause and effect relationships depends on knowledge of the mechanisms of toxic action and interaction in humans. Following the Gulf War, some veterans reported illnesses that might have been the result of chemical exposures. Some studies of these veterans have concluded that significant correlations between perceived illnesses and chemical use exist (Haley and Kurt, 1997). The reported chemical exposures included the insect repellent N,N-diethyl-m-toluamide (DEET), insecticides such as permethrin and chlorpyrifos used to protect against insect-borne diseases, and the neuroprotective agent pyridostigmine bromide used to protect against possible nerve gas attack. It has been reported that chlorpyrifos and DEET are metabolized by human P450s (Tang et al., 2002; Usmani et al., 2002) and that interactions of deployment-related chemicals can inhibit or induce the P450s involved in their metabolism (Usmani et al., 2002). In a recent study examining the effects of various deployment-related chemicals on TST metabolism, Usmani et al. (2003) reported that organophosphorus pesticides are potent noncompetitive and irreversible inhibitors of TST metabolism caused by CYP3A4 inhibition. Other studies have reported that interaction of Gulf War-related chemicals could produce greater than additive toxicity in rats and mice (Chaney et al., 1997; McCain et al., 1997), increased neurotoxicity in hens associated with increased inhibition of brain acetylcholinesterase and neurotoxicity target esterase (Abou-Donia et al., 1996a,b), and neurobehavioral deficits associated with significant inhibition of brainstem acetylcholinesterase activities in rats (Abou-Donia et al., 2001). Possible detrimental interactions as a result of altered estrogen metabolism have not yet been examined relative to these chemicals, nor have studies been carried out to examine the induction or inhibition by these or related compounds of human P450-mediated metabolism of estrogens, such as E2.

The objective of the present study was to study the inhibition or activation by various deployment-related chemicals of the metabolism of E2 by HLM, CYP1A2, and CYP3A4.

### Materials and Methods

**Chemicals.** Chlorpyrifos, fonofos, phorate, DEET, fipronil, imidacloprid, deltamethrin, permethrin, carbosulfan, carbaryl, naphthalene, and pyridostigmine bromide on E2 metabolism were examined using HLM, CYP1A2, or CYP3A4 after preincubation with the test compounds in otherwise complete mixtures lacking only the substrate E2. HLM, CYP3A4, or CYP1A2 was incubated with individual test compounds (final concentration 50 μM) for 5 min at 37°C before adding E2 (final concentration 50 μM). All the inhibitors, except pyridostigmine bromide, were added in 2.5 μl of acetonitrile with an equal volume of acetonitrile being added to the control incubations. Pyridostigmine bromide was added in 2.5 μl of water. Because of the likelihood that phosphorothioates are metabolized to irreversible P450 inhibitors, preincubation is tested as a variable with these chemicals. Preliminary experiments indicated little or no difference between preincubation and coinubcation for other potential inhibitors. Only those chemicals that were capable of greater than 50% inhibition of E2 metabolism at a concentration of 50 μM were studied further.

**Inhibition Kinetics of Deployment-Related Chemicals on E2 Metabolism by CYP3A4 and CYP1A2.** The chemicals for which IC50 values were below 25 μM as determined by preliminary incubation studies were analyzed further for inhibitory potential. Range-finding assays were conducted for chlorpyrifos, fonofos, phorate, deltamethrin, and permethrin (0.39–200 μM) by incubating with CYP3A4 for 5 min at 37°C before adding E2 (final concentration 50 μM). The kinetic study of E2 using varying E2 concentrations (4.31–200 μM) was performed using CYP3A4, which was preincubated with chlorpyrifos or fonofos (0.78, 1.56, or 3.125 μM) for 5 min at 37°C.

For CYP1A2, range-finding assays were conducted for chlorpyrifos and fonofos as described above. Concentrations of chlorpyrifos (0.5 and 2.0 μM) and fonofos (12.5 and 50.0 μM) were preincubated with CYP1A2 for 5 min at 37°C before adding E2 (final concentration 3.125–100 μM) to measure their inhibition of CYP1A2 metabolism of E2. In all the inhibition studies involving preincubation with the inhibitor, the NADPH-generating system was present during the preincubation.

**Preincubation Time- and Concentration-Dependent Inactivation by Chlorpyrifos of CYP3A4 Metabolism of E2.** These studies were conducted using the same conditions as described in the assay conditions (50 μM E2; 20-min incubation at 37°C) but varying concentrations (0, 1, 2, 5, 10, and 50 μM CPS) and preincubation times (0, 1, 2, 3, 4, and 5 min). The generation of 2-OHE2 (the major metabolite of E2) was compared in this assay and conducted in duplicate. Inactivation parameters (Kinact and KI) were estimated as described in previous studies (Silverman, 1995; Heydari et al., 2004). The rate constant for initial inactivation (kinact) at each concentration of inhibitor (CPS) was calculated from the slopes in the regression plots (kinact)” natural logarithm of remaining activity as percentage of control activity versus preincubation time). The inactivation values (kinact and KI) were calculated as described in Heydari et al. (2004): kinact = kinact×[I]/[K + I], where [I] is the
inhibitor concentration, $k_{max}$ is the maximum rate constant for inactivation, and $K_i$ is the inhibitor concentration that produces half-maximal rate of inactivation.

Inhibition Kinetics of Carbaryl and Its Metabolites on E2 Metabolism by CYP1A2. Before the $K_i$ (inhibition constant) determination was done, range-finding assays were conducted for the inhibition by carbaryl and its metabolites of the metabolism of E2 by CYP1A2. Varying concentrations (1.56–50 μM) of carbaryl, 4-hydroxycarbaryl, 5-hydroxycarbaryl, and carbaryl methylol were preincubated or coincubated with CYP1A2. Preincubation experiments with inhibitors were conducted similarly to those described above. For coincubation experiments, carbaryl or its metabolites were incubated with E2 (final concentration 50 μM). The reactions were initiated by the addition of ice-cold CYP1A2. For Michaelis-Menten plots, carbaryl (6.25 and 12.5 μM), 4-hydroxycarbaryl (6.25 and 12.5 μM), and carbaryl methylol (12.5 and 25 μM) were coincubated with E2 (final concentration 3.125–100 μM). The reactions were initiated by the addition of ice-cold CYP1A2.

Inhibition Kinetics of Naphthalene and Its Metabolites on E2 Metabolism by CYP1A2. Before the $K_i$ determination was done, range-finding assays were conducted for the inhibition by naphthalene and its metabolites of the metabolism of E2 by CYP1A2. Varying concentrations of naphthalene (3.125–25 μM), 1-naphthol (0.78–25 μM), 2-naphthol (25 μM), trans-1,2-dihydro-1,2-naphthalenediol (25 μM), 1,2-naphthoquinone (25 μM), and 1,4-dihydroxynaphthalene (25 μM) were preincubated or coincubated with CYP1A2. The preincubation and coincubation experiments with naphthalene and its metabolites were conducted similarly as described above. For Michaelis-Menten plots, naphthalene (1.56, 3.125, and 6.25 μM) was coincubated with E2 (final concentration 3.125–100 μM). The reactions were initiated by the addition of ice-cold CYP1A2.

Analysis of 2-OHE2 and 6β-Hydroxytestosterone by HPLC. Analysis of the E2 metabolite 2-OHE2 was performed with an HPLC system coupled with in-line UV detection as described previously (Suchar et al., 1995). The HPLC system consisted of a Waters 2690 separation module and a Waters UV photodiode array detector (model 2996). All the system components were controlled through the Waters Powerline firmware. Data were collected via a Waters system controller and analyzed using Waters Empower software. The solvent system for separation of E2 and 2-OHE2 consisted of acetonitrile (solvent A), 0.1% acetic acid in water (solvent B), and 0.1% acetic acid in methanol (solvent C). The solvent gradient (solvent A/solvent B/solvent C) used for eluting E2 and 2-OHE2 was as follows: 8 min of isocratic at 16:68:16, 7 min of a concave gradient (curve number 9) to 18:64:18, 13 min of a concave gradient (curve number 8) to 20:59:21, 10 min of a convex gradient (curve number 2) to 22:57:21, 13 min of a concave gradient (curve number 8) to 58:21:21, followed by a 0.1-min step to 92:5:3 and a 3.9-min isocratic period at 92:5:3. The gradient was returned to the initial condition (16:68:16) for 2 min and held for 3 min before analyzing the next sample. The flow rate was 1.2 ml/min. E2 and 2-OHE2 were separated by an Ultracarb 5 ODS (octade-cylsilane) column (150 × 4.6 mm, Phenomenex, Rancho Palos Verdes, CA) at 30°C and detected at 280 nm. The limit of detection for 2-OHE2 was approx-
immediately 0.04 μM. Concentrations of 2-OHE₂ were obtained from the chromatographic peak area from a standard curve (0.15–20.0 μM).

TST and its metabolite, 6β-hydroxytestosterone, were separated using the method described by Usmani et al. (2003). E₂ and its metabolites eluted after 6β-hydroxytestosterone and TST.

**Data Analysis and Statistics.** The apparent $K_M$ and $V_{max}$ parameters were calculated using the SigmaPlot Enzyme Kinetics Module for Windows, version 8.0 (SPSS Inc.). The $K_M$ values were estimated by nonlinear regression analysis by fitting different models of enzyme inhibition to the kinetic data using SigmaPlot Enzyme Kinetics Module for Windows, version 1.1 (SPSS Inc.). The mode of inhibition was established by comparing the statistical results including the $r^2$ information criterion values of different inhibition models and by selecting the one with the best fit. Furthermore, the mode of inhibition was established by comparing the 95% confidence intervals that did not overlap for $K_M$ and $V_{max}$ values. Significant differences between data sets were determined by Student’s $t$ test using SigmaPlot for Windows, version 8.0 (SPSS Inc.).

**Results**

**Enzyme Kinetics.** HLM, CYP3A4, and CYP1A2 all displayed similar $K_M$ values for metabolism of E₂ to 2-OHE₂ (Table 1). $V_{max}$ and CLint values for HLM were 0.32 nmol/min/mg protein and 7.5 μl/min/mg P450; those for CYP3A4 were 6.9 nmol/min/mmol P450 and 291 μl/min/mmol P450; and those for CYP1A2 were 17.4 nmol/min/mmol P450 and 633 μl/min/mmol P450.

**E₂ Metabolism in Single-Donor HLM.** Incubations of E₂ with individual donor HLM (6 male and 11 female) showed as much as 36-fold variability in metabolism among individuals (Fig. 2). Pheno-type data based on metabolic activities of these individuals was used to derive correlations between P450 isoform content and E₂ metabolic activity. CYP3A4 was the isoform with the best correlation ($r^2 = 0.87$). All the other correlations between E₂ hydroxylation activity and specific P450 isoforms were less than 0.40. To determine the CYP1A2 contribution to E₂ metabolism among individual HLM, ketoconazole (2 μM), a specific CYP3A4 inhibitor, was used to inhibit CYP3A4 activity. Preliminary observations with ketoconazole indicated that 2 μM inhibited less than 5% of CYP1A2 activity (data not shown). In the presence of ketoconazole, variation among individuals was reduced to 12-fold, and the correlation of CYP1A2 with E₂ metabolism increased to 0.81 (Fig. 2).

**Inhibition of E₂ Metabolism.** The effects of various deployment-related and other chemicals on E₂ (50 μM) metabolism were investigated by preincubating 50 μM concentrations of each chemical with pooled HLM (Fig. 3). The organophosphorus compounds, chlorpyrifos fonofos, and phorate all significantly inhibited E₂ metabolism to 2-OHE₂, although inhibition by chlorpyrifos and fonofos was profound (ca. 80%) compared with phorate (ca. 16.5%). Preincubation of pooled HLM with carbaryl, a carbamate, and naphthalene, a jet fuel component, also resulted in significant inhibition (ca. 40%) of E₂ metabolism to 2-OHE₂. Fipronil, a phenyl pyrazole insecticide, also significantly inhibited E₂ metabolism, although to a lesser degree (ca. 18%).

To further explore the inhibition of E₂ metabolism observed in HLM, assays were also conducted using CYP3A4 and CYP1A2. Preincubation of CYP3A4 with a variety of chemicals resulted in varying levels of activation and inhibition of E₂ metabolism (Fig. 4). E₂ metabolism was significantly inhibited by the organophosphorus compounds chlorpyrifos and fonofos, with up to ca. 90% inhibition of E₂ metabolism to 2-OHE₂. Preincubation of CYP3A4 with the pyrethroids deltamethrin and permethrin resulted in ca. 58 and 37% inhibition of E₂ metabolism to 2-OHE₂, respectively. In contrast, preincubation of CYP3A4 with DEET, an insect repellent, and carbfo-uran, a carbamate, resulted in the production of small, but not significantly greater, levels of 2-OHE₂.

Similarly, preincubation of CYP1A2 resulted in varying levels of activation and inhibition of E₂ metabolism (Fig. 5). Preincubation of chlorpyrifos and fonofos resulted in ca. 96 and 59% inhibition of E₂ metabolism, respectively. In contrast, preincubation of CYP3A4 with DEET, an insect repellent, and carbo-uran, a carbamate, resulted in the production of small, but not significantly greater, levels of 2-OHE₂.

**Mechanism of Inhibition.** To investigate the type of inhibition of CYP3A4 by chlorpyrifos and fonofos in the production of 2-OHE₂, a

**TABLE 1**

**Kinetic parameters for 2-hydroxylation of E₂ by pooled HLM, CYP3A4, and CYP1A2**

<table>
<thead>
<tr>
<th></th>
<th>$K_M$</th>
<th>$V_{max}$</th>
<th>$V_{max}/K_M$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLM</td>
<td>42.8 ± 9.40</td>
<td>0.32 ± 0.02</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>6.9 ± 0.35</td>
<td>291</td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td>23.7 ± 3.00</td>
<td>17.4 ± 0.54</td>
<td>633</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>27.5 ± 2.16</td>
<td>17.4 ± 0.54</td>
<td>633</td>
</tr>
</tbody>
</table>

Fig. 2. Metabolism of E₂ to 2-OHE₂ by single-donor HLM in the presence and absence of ketoconazole. Solid bars, no ketoconazole; open bars, 2 μM ketoconazole.
major E\textsubscript{2} metabolite, different concentrations of chlorpyrifos and fonofos were preincubated for 5 min with CYP3A4 before adding varying concentrations of E\textsubscript{2}. Michaelis-Menten plots showed that the \(V_{\text{max}}\) values were significantly reduced without affecting \(K_m\) values, indicative of a non-competitive inhibition of E\textsubscript{2} metabolism by chlorpyrifos and fonofos (Fig. 6).

In a similar manner, inhibition of CYP1A2 metabolism of E\textsubscript{2} by chlorpyrifos and fonofos involved a 5-min preincubation before the addition of varying concentrations of E\textsubscript{2}. As shown for CYP3A4 (Fig. 5), Michaelis-Menten plots for CYP1A2 also indicated non-competitive inhibition (Fig. 7).

The production of 2-OHE\textsubscript{2} by CYP3A4 was significantly inhibited by CPS in a time- and concentration-dependent manner (Fig. 8A). Inactivation parameters (\(k_{\text{inact}}\) and \(K_I\)) determined for CYP3A4 metabolism of estradiol by CPS were 0.3 min\(^{-1}\) and 4.9 \(\mu\)M, respectively (Fig. 8B). Even at a preincubation of 0', the metabolic activity of CYP3A4 for estradiol was apparently significantly decreased by CPS in a dose-dependent manner, indicating a very rapid initial rate of inhibition.

For IC\textsubscript{50} values, varying concentrations of carbaryl and its metabolites were preincubated and coincubated with CYP1A2. Under preincubation conditions but not with coincubation, carbaryl, 4-hydroxycarbaryl, and carbaryl methylol caused small increases in the production of 2-OHE\textsubscript{2} at the low concentrations (1.56, 3.125, and 6.25 \(\mu\)M), whereas at high concentrations (12.5–50 \(\mu\)M) inhibition of E\textsubscript{2} metabolism was observed (data not shown). Under preincubation conditions but not with coincubation, 5-hydroxycarbaryl caused small increases in the production of 2-OHE\textsubscript{2} at all the concentrations (1.56–50 \(\mu\)M) (data not shown). Under coincubation conditions, carbaryl, 4-hydroxycarbaryl, 5-hydroxycarbaryl, and carbaryl methylol all caused inhibition of E\textsubscript{2} metabolism at all the concentrations tested (1.56–50 \(\mu\)M) (data not shown). To determine the \(K_I\) value and investigate the type of inhibition of CYP1A2 metabolism of E\textsubscript{2} by carbaryl and its metabolites, different concentrations of carbaryl, 4-hydroxycarbaryl, and carbaryl methylol were coincubated with CYP1A2 and varying concentrations of E\textsubscript{2}. Michaelis-Menten plots showed that the \(V_{\text{max}}\) values were significantly reduced without af-
fecting $K_m$ values, indicative of a noncompetitive inhibition of E2 metabolism by carbaryl and its metabolites (Fig. 9). The inhibition constants ($K_i$) indicated that carbaryl is the most potent inhibitor of E2 metabolism with a $K_i$ value of 7.7 μM; 4-hydroxycarbaryl is the second best inhibitor with a $K_i$ value of 10.2 μM, whereas carbaryl methylol is a weak inhibitor with a $K_i$ value of 29.0 μM.

In the determination of IC50 values, varying concentrations of naphthalene and its metabolites were preincubated and coincubated with CYP1A2. Preincubation of naphthalene increased the inhibition of E2 metabolism by nearly 2-fold compared with the coincubation of naphthalene with E2, indicating that a reactive metabolite is involved (data not shown). No differences were observed in the IC50 values when 1-naphthol was preincubated or coincubated with E2 (data not shown). 2-Naphthol, 1,2-naphthoquinone, 1,4-naphthoquinone, and 1,4-dihydronaphthalene (25 μM) inhibited E2 (50 μM) metabolism ca. 85 to 100%, whereas trans-1,2-dihydro-1,2-naphthaledioli had no inhibitory effect on E2 metabolism (data not shown). To determine the $K_i$ value and to investigate the type of inhibition of CYP1A2 by naphthalene on E2 metabolism, naphthalene was preincubated for 5 min with CYP1A2 before adding varying concentrations of E2. The Michaelis-Menten plot showed that the $V_{max}$ values were significantly reduced without affecting $K_m$ values, indicative of a noncompetitive inhibition of E2 metabolism by naphthalene (Fig. 10). To investigate the type of inhibition of CYP1A2 by 1-naphthol on E2 metabolism, 1-naphthol was co-incubated with CYP1A2 and varying concentrations of E2. 1-Naphthol, a predominant naphthalene metabolite, was shown to be a noncompetitive inhibitor of E2 metabolism (Fig. 11). Further investigation of noncompetitive reversible or nonreversible inhibition data revealed that the inhibition of 2-OHE2 by 1-naphthol is nonreversible (data not shown).

Discussion

P450-dependent hydroxylation is a major pathway of oxidative metabolism of E2 in mammalian liver. The studies carried out using
human P450 isoforms provided further insight into the range of E2 hydroxylation reactions that can be catalyzed by human P450 enzymes. Human CYP1A2 and CYP3A4 are the major isoforms responsible for E2 metabolism, and 2-OHE2 is the major E2 metabolite, as previously shown (Yamazaki et al., 1998; Lee et al., 2001, 2003). The phenotyped HLM data and the mean metabolic intrinsic clearance rates, as estimated by $V_{\text{max}}/K_m$, corroborate earlier findings (Yamazaki et al., 1998; Lee et al., 2001, 2003) that both CYP3A4 and CYP1A2 play major roles in the metabolism of E2 in human liver, and the content of these two P450s in HLM determine which isoform is more important. However, CYP3A4 may play a more important role than CYP1A2 because on average CYP3A4 accounts for approximately 40% of the total P450 in HLM. Our kinetic data presented in this study for CYP1A2 and CYP3A4 are in general agreement with the previous reports showing that CYP1A2 is more active than CYP3A4 in E2 metabolism (Yamazaki et al., 1998; Badawi et al., 2001).

CYP3A4 is one of the most important and abundant P450 isoforms in human liver and has broad substrate specificity. CYP3A4 not only metabolizes xenobiotics but also is responsible for the metabolism of endogenous compounds such as steroid hormones, including TST and E2 (Lee et al., 2003; Usmani et al., 2003). Because TST and E2 are predominantly metabolized by CYP3A4, both can potentially compete for the same catalytic site of CYP3A4. The usual interaction between two different substrates for the same enzyme is competitive inhibition. But because the $K_m$ values for TST and E2 are different ($K_m = 108$ and $24 \mu M$ for TST and E2, respectively), interactions between these substrates may be complicated because of the allosteric characteristics of CYP3A4 (Shimada and Guengerich 1989; Lee et al., 1995; Usmani et al., 2003; Williams et al., 2004). The recent demonstration of a relatively large substrate binding catalytic site by crystal structure elucidation is consistent with the capacity of CYP3A4 to accommodate large molecules and possibly more than one substrate (Yano et al., 2004).

Endogenous steroids, such as E2, always exist in vivo, and considerable amounts of these steroids are metabolized by the P450s expressed in the human liver, where foreign compounds are mainly metabolized. Because TST and E2 both have important, and different, effects in vivo, the inhibition of the metabolism of both of these steroid hormones must be considered in detail. If xenobiotics substantially alter enzymes such as CYP3A4 and CYP1A2, they may affect the rate of E2 metabolism, ultimately disrupting E2 homeostasis. Preincubation of pooled HLM, CYP3A4, and CYP1A2 with organophosphorus compounds, such as chlorpyrifos and fonofos, resulted in the extensive inhibition of the production of 2-OHE2, a major E2 metabolite. Chlorpyrifos and fonofos inhibited 2-OHE2 formation noncompetitively and are among the most potent inhibitors of the P450-dependent oxidation of E2 yet described. Organophosphorus pesticides, such as chlorpyrifos and fonofos, are activated by a P450-catalyzed...
desulfuration reaction (Fukuto, 1990). The sulfur atom released from these pesticides in this reaction is highly reactive and is believed to bind immediately to the heme iron of P450 and inhibit its activity (Norman et al., 1974, Halpert et al., 1980, Neal, 1980; Neal and Halpert, 1982; Butler and Murray, 1997). Our previous study showed that organophosphorus pesticides are potent noncompetitive and irreversible inhibitors of TST metabolism by HLM and CYP3A4 (Usmani et al., 2003).

Enzyme activity is often modulated by inhibition or induction, either condition modifying the extent to which xenobiotics or endogenous substrates are metabolized (Guengerich, 1997; Szklarz and Halpert, 1998). Inhibition may, in some interactions, be more serious than enzyme induction because inhibition occurs more rapidly than induction (Guengerich, 1997). Preincubation of CYP3A4 with chlorpyrifos and fonofos resulted in almost complete inhibition of E₂ metabolism to 2-OHE₂. Preincubation of CYP1A2 with chlorpyrifos resulted in almost complete inhibition of 2-OHE₂ formation, whereas fonofos was not as potent an inhibitor. The kinetics of inactivation of E₂ metabolism by CPS confirms that CPS is a mechanism-based inactivator of CYP3A4 in these studies. Preincubation of CPS in the presence of an NADPH-generating system increased the inhibitory effect in a time- and concentration-dependent manner. The significant reduction of CYP3A4 activity toward E₂ metabolism by CPS at time 0 suggests that the binding affinity of the inhibitor (CPS) is higher than that of the substrate (E₂) with the CYP3A4 isoform and that the initial rate of inhibition is rapid. The relatively low Kᵢ value (high inhibitory potency) of CPS toward CYP3A4 metabolism of E₂ appears to confirm the strong binding affinity of CPS with this isoform. Therefore, the possibility exists that inhibition of CYP3A4 and CYP1A2 by these chemicals could lead to higher levels of E₂ and alter hormonal properties. The in vivo importance of these observations will require further studies.

Carbaryl is a widely used anticholinesterase carbamate insecticide. In an in vitro study, 5-hydroxycarbaryl, 4-hydroxycarbaryl, and carbaryl methylol were identified as the major metabolites of carbaryl produced...
by HLM (Tang et al., 2002). P450 isoform data indicated that CYP1A1, CYP1A2, CYP2B6, CYP2C19, and CYP3A4 are the most active isoforms in human metabolism of carbyl (Tang et al., 2002). In our preliminary inhibition studies, we found that preincubation of pooled HLM and CYP1A2 with carbyl significantly inhibited E2 metabolism. Because carbyl is metabolized by CYP1A2 while also showing significant inhibition of E2 metabolism, we expected competitive interactions between E2 and carbyl or its metabolites. However, carbyl and its metabolites inhibited formation of 2-OHE2 noncompetitively in our co-incubation studies. The K_i values indicated that carbyl and 4-hydroxy-carbyl were much better inhibitors of 2-OHE2 than carbyl methylol. Although there is no obvious explanation of the slight activation of E2 at low concentrations of carbyl, 4-hydroxy-carbyl, and carbyl methyol, it may be noted that this is a common phenomenon in the oxidation of TST by HLM (Usmani et al., 2003).

The polycyclic aromatic hydrocarbon napthalene is an industrial chemical, an environmental pollutant, and a component of jet fuel and has considerable toxicological importance because of widespread human exposure and its potential to form toxic and carcinogenic metabolites in humans (Wilson et al., 1996). We recently showed that CYP1A2 is the most efficient of the 15 different isoforms tested for their ability to metabolize napthalene to its two major metabolites in HLM, 1-naphthol and 1,2-dihydro-1,2-naphthalenediol (Cho et al., 2006). In this study, preincubations of napthalene with pooled HLM and CYP1A2 significantly inhibited E2 metabolism. Because napthalene is metabolized by CYP1A2 and also significantly inhibited E2 metabolism, we expected a competitive interaction between napthalene and E2 or its metabolite. However, this study showed noncompetitive inhibition of E2 metabolism. Coincubation of 1-naphthol, a well known cytototoxic metabolite of napthalene, resulted in noncompetitive and irreversible inhibition of E2 metabolism.

In conclusion, the hydroxylation of E2 by CYP3A4 and CYP1A2 isoforms indicates important functions for these enzymes other than detoxification of xenobiotics. The deployment-related and other chemicals used in this study, including pesticides, caused a marked modification of P450-mediated E2 metabolism in vitro. Organophosphorus pesticides were very potent inhibitors of the production of 2-OHE2 and inhibited E2 metabolism noncompetitively. Carbyl and some of its metabolites inhibited E2 metabolism noncompetitively. Napthalene inhibited E2 metabolism noncompetitively, an effect probably caused by 1-naphthol, a naphthalene metabolite. 1-Naphthol inhibits E2 metabolism noncompetitively and irreversibly. It should be noted that in vivo toxicokinetic data are not available for these chemicals in humans, and thus the effects noted indicate only the potential for in vivo effects.

References
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