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Inhibition of Human and Rat CYP1A2 by TCDD and Dioxin-like Chemicals

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Dioxins have been shown to bind and induce rodent CYP1A2, producing a dose-dependent hepatic sequestration *in vivo*. The induction of CYP1A2 activity has been used as a noninvasive biomarker for human exposure to dioxins; while there is a consistent relationship between exposure and hepatic CYP1A2 induction in rodents, this relationship has only been observed in some of the highest exposed human populations. This may be explained by inhibition of CYP1A2 activity by dioxins as some rodent studies demonstrate that rodent CYP1A2 activity can in fact be inhibited by dioxins *in vitro*. CYP1A2 activity was examined using a series of dioxins to inhibit human and rat CYP1A2 activity in species-specific CYP1A2 SUPERSOMES using three common CYP1A2 substrates. Methoxyresorufin was a more efficient substrate than acetanalide or caffeine in this *in vitro* system. Rat and human CYP1A2 enzymatic activity is inhibited by TCDD, PCDD, TCDF, 4-PeCDF, and PCBs 126, 169, 105, 118, and 156 in a concentration-dependent manner. These data demonstrate that the *in vitro* metabolism of prototype substrates is similar between the rat and human CYP1A2 SUPERSOME preparations and that dioxins inhibit CYP1A2 activity in both species. Because of the potential for inhibition of CYP1A2 activity by TCDD and other dioxins, studies examining CYP1A2 induction in dioxin-exposed populations using these substrates should be viewed cautiously.

Key Words: **CYP1A2; dioxins; TCDD; PCDD; TCDF; 4-PeCDF; PCBs 126, 169, 105, 118, and 156.**

Dioxin-like chemicals are a family of ubiquitous environmental contaminants known to produce a broad spectrum of toxic effects in animals, some of which include carcinogenicity, teratogenicity, immunotoxicity, and endocrine toxicity. Dioxin-like chemicals consist, in part, of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs). This subset shares a relatively planar conformation and a characteristic lateral chlorine substitution pattern. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) is the prototype and most potent of the dioxins. Dioxins induce their toxic effects by binding to the Ah receptor (AhR). There is a concordance between the ability to bind AhR and the toxic potency of these chemicals. In addition to binding and activating the AhR, chemicals must also persist and bioaccumulate in the environment and biological systems to be considered dioxin-like for regulatory purposes (van den Berg *et al.*, 1998).

In addition to the AhR, some dioxins bind to an inducible hepatic protein that results in a dose-dependent hepatic sequestration of these chemicals (Abraham *et al.*, 1988; DeVito *et al.*, 1998; Kedderis *et al.*, 1991b). Cytochrome P450 1A2 (CYP1A2) was identified as the binding protein based on several lines of evidence. TCDD and related congeners were shown to bind to immunoprecipitated CYP1A2 (Poland and Glover 1990; Poland *et al.*, 1989; Voorman and Aust 1989). CYP1A2 is induced by dioxins, which is confirmed by dose-dependent increases in mRNA and protein concentrations and enzymatic activity (DeVito *et al.,* 1998; Dey *et al.*, 1999; Kedderis *et al.*, 1991a). CYP1A2 appears predominately inducible in hepatic tissue (Goldstein and Linko, 1984), which is consistent with hepatic-specific sequestration of these chemicals. CYP1A enzymes are also inducible in humans, demonstrated by induction in human hepatocytes (Xu *et al.*, 2000).

The most direct demonstration of the role of CYP1A2 in dioxin sequestration in the liver comes from studies using CYP1A2 deficient mice. TCDD and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) are highly sequestered in the liver of wild-type mice but not in CYP1A2 knockout mice (Diliberto *et al.*, 1997, 1999). In addition, the distribution of TCDD and 4-PeCDF changed dramatically in the knockout mice as the ability of the liver to sequester these chemicals was significantly decreased resulting in increased concentrations of these chemicals in extrahepatic tissues. The distribution of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), a non-dioxin

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like PCB that is not sequestered in hepatic tissue of wild-type mice, was unaltered in the knockout mice. The structure activity relationship for hepatic sequestration appears different than the structure activity relationship for Ah receptor binding and activation (DeVito *et al.*, 1997).

Limited data is available to support hepatic sequestration in humans (Iida *et al.*, 2004; Schecter *et al.*, 1994a,b; Thoma *et al.*, 1990). In an accidental poisoning incident in Yusho, concentrations of 4-PeCDF were higher in the liver than in adipose tissue in several highly exposed patients (Ryan, 1987). Quantitative understanding of the dose response relationship has been suggested by several investigators using a physiologically based model to describe the kinetics of distribution and absorption of PCDDs and PCDFs in mammalian species (Andersen *et al.*, 1997; Carrier *et al.*, 1995; Emond *et al.*, 2004). However, these models, as well as current human health risk assessments, assume that the distribution of dioxins is similar between species.

Several studies have attempted to use probe substrates, such as caffeine, to examine the relationship between dioxin exposure and induction of CYP1A2. These studies tend to show marginal relationships between serum TCDD concentration and altered caffeine metabolism (Abraham *et al.*, 2002, Halperin *et al.*, 1995, 1998). One possible explanation for the discrepancy between dioxin exposure and CYP1A2 induction as measured by caffeine metabolism is that dioxins may inhibit CYP1A2 catalytic activity.

Hepatic sequestration of dioxins is due to both their induction and binding of CYP1A2. Thus species differences could be due to either differences in induction or differences in binding of these chemicals to CYP1A2. Human and rat CYP1A2 have similar substrate specificity. Both of these enzymes metabolize caffeine, acetanilide, and methoxyresorufin. However, there is no comparative data on the ability of dioxins to inhibit rat and human CYP1A2 activity. The present study uses *in vitro* approaches to compare the inhibition of rat and human CYP1A2 by dioxins. This study offers insight into apparent species differences in the dose-dependent disposition of dioxins and the influence of CYP1A2 on the distribution of these chemicals.

The objective of this *in vitro* study is to investigate the assumption that rodent and human CYP1A2 function similarly by comparing their ability to metabolize several probe substrates and the ability of dioxins to inhibit these CYP1A2 activities. Human and rat CYP1A2 specific SUPERSOMES were tested using three substrates (methoxyresorufin, acetanalide, and caffeine), and activity was measured in the presence of a series of dioxins. The use of CYP1A2 SUPERSOMES allows for investigation of one specific P450 isoform, providing the opportunity to use these results as an indirect measure of binding as well as a direct measure of inhibition of catalytic activity; thus allowing for a more accurate extrapolation of CYP1A2 sequestration effects between species.

MATERIALS AND METHODS

Microsomes. Rat and human CYP1A2 + P450 reductase SUPERSOMES (rat and human CYP1A2 cDNA expressed in baculovirus infected insect cells for preparation of microsomes) were obtained from GenTest Corporation (Woburn, MA). SUPERSOMES were kept at −80°C until use, then rapidly thawed at 37°C and stored on ice. A 1:10 dilution of both human or rat SUPERSOMES were prepared daily using .05M (pH 8) TRIS buffer.

Chemicals. All of the test chemicals were obtained from AccuStandard Inc. (New Haven, CT). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-pentachloro-p-dioxin (PCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) were 98% or greater purity. 3,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB 169), 3,3′4,4′,5-pentachlorobiphenyl (PCB 126), 2,3,3′4,4′,5-hexachlorobiphenyl (PCB 156), 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118), 2, 2′, 4, 4′, 5, 5′-hexachlorobiphenyl (PCB 153), and 2,3,3′4,4′-pentachlorobiphenyl (PCB 105) were 99% or greater purity (impurities were not AhR ligands). All chemicals were dissolved in DMSO and stored at 4°C. β-Nicotinamide Adenenine Dinucleotide Phosphate (β-NADPH), methoxyresorufin, and resorufin were purchased through Sigma Chemical Co. (St. Louis, MO) and prepared daily.

MROD enzyme assay. Methoxyresorufin, a classic CYP1A2 substrate (Burke *et al.*, 1994) was used to examine the inhibition of CYP1A2 by a series of dioxin-like chemicals. Methoxyresorufin is metabolized to resorufin by CYP1A2, which can be assayed fluorometrically. Reactions were carried out in a 96 well plate and the production of resorufin was recorded for 5 min at 37°C (within the linear range of activity) using a Spectromax Gemini XS plate reader. The reaction mixture included 50 μl microsome solution, 110 μl of 0.05M (pH 8) TRIS buffer, 5 μl of test chemical or DMSO, and 50 μl methoxyresorufin (0.21, 0.6, 2.1, 6.0, or 21.0 μM) solution. Following a 10-min incubation at 37°C, 25 μl NADPH (prepared fresh, pH 8.0 at 37°C) was added to begin the reaction. MROD data were analyzed using Lineweaver-Burke plots (Prism Software, CA). Inhibitor constant (K_I) approximations were calculated using the median inhibitor concentration and average K_{Mobs} and K_M values according to the following equation (GraphPad Software, 1999): K_I = [Inhibitor]/{($K_{M,obs/}$ K_M)-1}. Calculations were based on linear reaction results.

ACOH enzyme assay. Acetanilide 4-hydroxylase (ACOH), a common marker for CYP1A2, was determined by the method of Lui *et al.* (1991) as modified by DeVito *et al.* (1993). Briefly, SUPERSOMES were incubated for 20 min at 37°C in a buffer containing 50 mM Tris, 0.3 mM $MgCl_2$, 0.6 mM NADPH, and 1 mg bovine serum albumin/ml. The reaction was initiated by the addition of 20 μl 20 nM acetanilide in acetone. 4-Hydroxyacetanilide was extracted by the addition of 2.5 ml acetate containing 0.1 μg 3-hydroxyacetanilide. Recovery of 4-hydroxyacetanilide was estimated based on the recovery of 3-hydroxyacetanalide. Reverse phase HPLC was used to separate and quantify 4-hydroxyacetanilide (4-AC) using a Beckman Instruments Model System Gold Liquid Chromatograph with an autosampler and UV detector and ODS column (4.6 × 25 cm).

Caffeine enzyme assay. Caffeine is metabolized by CYP1A2 (Berthou *et al.*, 1991) to three metabolites: theophylline, paraxanthine, and theobromine. SUPERSOMES were incubated for 5 min at 37°C in a buffer containing 0.1 M sodium phosphate buffer at pH 7.4 and caffeine ranging in concentrations from 0.1 mM to 64 mM. The reaction was initiated by 50 μl of 25 mg/ml NADPH (40 min. incubation at 37°C), stopped by the addition of 350 μl of cold zinc sulphate (2% w/v) followed by the addition of 25 μl of internal standard (300 μM β-hydroxyethyltheophyline in acetone) and 25 μl of HCl to acidify the solution. Caffeine, theophylline (1,3 dimethylxanthine), paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethyxanthine), and the internal standard were extracted twice by ethyl acetate/isopropanol (98/2; v/v) using a total of 4.5 ml. After each extraction, the mixture was centrifuged at 2500 × *g* for 5 min (4°C) and the upper organic layer was evaporated to dryness using a AES2010 Savant Speedvac. The residue was reconstituted with 300 μl of mobile phase (0.05% Acetic Acid/Methanol [88:12]); 100 μl of this mixture was injected onto the HPLC system at a flow rate of 1.3 ml/min with UV

detection set at 273 nm. The HPLC system consists of auto-sampler (Beckman model 105), a dual pump (Beckman model 126) and a UV detector (Beckman model 166). Reverse phase HPLC was used to separate and quantify the metabolites using a Beckman ODS column (4.6 × 25 cm) and Beckman ODS guard column (4.6 × 4.5). Each sample was eluted for 40 min. Recovery of caffeine and metabolites was estimated based on the recovery of the internal standard. Kinetic parameters were determined using Michaelis-Menton equations described above.

Chemical selection. Chemicals were chosen based on their reported dioxin-like activities and relative potencies. The PCDDs, PCDFs, and some co-planar PCBs are potent dioxin-like chemicals and sequester in hepatic tissues (DeVito *et al.*, 1998). PCBs 126 and 169 were chosen because of their high dioxin-like potency (Safe, 1990; Van den Berg *et al.*, 1998). In contrast, the mono-ortho PCBs are less planar, and hence, less dioxin-like. They still have the ability to cause dioxin-like effects via activation of the AhR; however, it is not their sole mode of action. Previous studies demonstrate that the mono-ortho PCBs are thousands of times less potent that TCDD (Van den Berg *et al.*, 1998). In addition, the mono-ortho PCBs do not sequester in hepatic tissues (DeVito *et al.*, 1998).

RESULTS

Substrate Comparison

Human and rat CYP1A2 SUPERSOMES were used to examine species differences in metabolism of various substrates and to investigate potential inhibition of CYP1A2 activity by TCDD and other dioxin-like chemicals. Methoxyresorufin was used as an initial probe substrate for CYP1A2 (Table 1). Human and rat CYP1A2 SUPERSOMES had similar kinetic parameters with K_M values of 2.5 ± 0.4 and 1.9 ± 0.4 μM, and V_{max} values of 1.8 ± 0.3 and 2.8 ± 0.5 pmol resorufin/min/pg P450, respectively.

The metabolism of acetanilide to 4-hydroxy acetanilide has been used as a prototype substrate reaction for measuring CYP1A2 activity *in vitro*. Although both the human and rat CYP1A2 specific microsomes were able to metabolize acetanilide, the human K_M value was approximately three times lower than the rat, ~15 mM and 50 mM, respectively. The human V_{max} value was slightly lower than the rat, ~97 and 160 nmol/min/pg P450, respectively.

Caffeine metabolism was also determined because it has been used in clinical studies as a biomarker of CYP1A2 activity in humans (Kalow and Tang, 1991). The two species create different caffeine metabolite profiles (data not shown); however, paraxanthine was the dominate metabolite in both species and was therefore the metabolite used for analysis. The estimates of K_M were similar between the human and rat CYP1A2 SUPERSOMES, ~12 mM and ~11 mM, respectively. The V_{max} was approximately five times higher in the human compared to the rat (~0.5 and 0.09 pmol/min/pg P450, respectively). In both species, CYP1A2 has greater affinity for methoxyresorufin than either acetanilide or caffeine.

TABLE 1
K_M (μM) and Vmax (pmol/min/pg P450) Values of Three Prototype Substrates by Human and Rat CYP1A2 SUPERSOMES

	Vmax		K_M	
Substrate	Human	Rat	Human	Rat
MROD	1.8	2.8	2.5	1.9
ACOH	98	160	15,000	50,000
Caffeine	0.5	0.09	12,000	11,000

Inhibition

Human and rat SUPERSOMES were used to test the potential for dioxin and dioxin-like chemicals to inhibit CYP1A2 activity. TCDD can inhibit caffeine demethylation and the hydroxylation of acetanilide in CYP1A2 SUPERSOMES. High concentrations (7.3 and 5.0 μM in caffeine and ACOH assays, respectively) were required to produce a 10–50% inhibition in CYP1A2 activity (data not shown). While caffeine is commonly used as the substrate for noninvasively detecting CYP1A2 induction in humans, acetanilide and methoxyresorufin have been commonly used in rodents. In this study, methoxyresorufin was to compare inhibition by dioxin-like compounds as it has the highest affinity for CYP1A2 and was the most consistent substrate (Tables 1 and 2). Results show that each chemical tested, except the negative control PCB 153, inhibited MROD activity in a concentration-dependent manner in both human and rat CYP1A2 SUPERSOMES. The majority of chemicals tested exhibited competitive inhibition. Figure 1 shows Lineweaver-Burke plots of representative chemicals from each of the subtypes of PHAHs tested (dibenzodioxins, dibenzofurans, and PCBs). K_I values (Table 2) were derived using classical Michaelis-Menton kinetic equations.

The two chlorinated dibenzo-*p*-dioxins tested inhibited MROD activity in both species, but were generally better inhibitors of rat CYP1A2 as compared to human. TCDD inhibited CYP1A2 activity in a concentration dependent manner in both species. Rat CYP1A2 yielded a lower K_I than human, 0.06476 ± 0.0098 μM and 0.63 ± 0.035 μM, respectively. PCDD was a more potent inhibitor in the rat CYP1A2 microsomes as compared to human, as shown by a K_I of 0.060 ± 0.027 μM in the rat and 0.51 ± 0.16 μM in the human SUPERSOMES.

The dibenzofurans were the most potent inhibitors of MROD activity in both species. Previous studies have shown 4-PeCDF to have high sequestration in rodent liver (Diliberto *et al.*, 1997, 1999). Of all chemicals tested, 4-PeCDF inhibited human and rat activity to the greatest extent (K_I 0.042 ± .0009 and 0.027 ± 0.0099 μM, respectively). This chemical also provided the best indication of a non-competitive or mixed type of inhibition. TCDF was the second most effective inhibitor of rat CYP1A2 (K_I = 0.035 ± .017 μM), and was also a potent inhibitor of human CYP1A2 activity (K_I = 0.059 ± 0.021 μM).

The co-planar PCBs in this study, 169 and 126, have been shown to bind CYP1A2 and sequester in rodent liver (DeVito *et al.*, 1998). As expected, we saw a concentration-dependent

TABLE 2
Approximate K_I Values Determined by the Inhibition of Methoxyresorufin Metabolism in CYP1A2 SUPERSOMES for All Chemicals Tested (μM)

Chemical	K_I Human	K_I Rat
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	0.63 (± 0.035)	0.076 (± 0.0098)
1,2,3,7,8-Pentachlorodibenzo-p-dioxin (PCDD)	0.51 (± 0.16)	0.060 (± 0.027)
2,3,4,7,8-Pentachlorodibenzofuran (4-PeCDF)	0.042 (± 0.0009)	0.027 (± 0.0099)
2,3,7,8-Tetrachlorodibenzofuran (TCDF)	0.059 (± 0.021)	0.035 (± 0.017)
3,3′,4,4′,5,5′-Hexachlorobiphenyl (169)	0.32 (± 0.21)	0.090 (± 0.039)
3,3′,4,4′,5-Pentachlorobiphenyl (126)	0.064 (± 0.0087)	0.070 (± 0.027)
2,3,3′,4,4′,5-Hexachlorobiphenyl (156)	1.3 (± 0.38)	3.8 (± 1.5)
2,3′,4,4′,5-Pentachlorobiphenyl (118)	1.25 (± 0.18)	2.71 (± 2.4)
2,3,3′,4,4′-Pentachlorobiphenyl (105)	2.13 (± 0.54)	4.1 (± 2.3)
2,2′4,4′,5,5′-Hexachlorobiphenyl (153)	No inhibition	No inhibition

Note. PCB 153 did not inhibit CYP1A2 metabolism of methoxyresorufin.

inhibition of MROD activity in both human and rat microsomes exposed to PCBs 169 and 126. K_I values were essentially the same for PCB 126 in both the human and rat (0.064 ± 0.0087 μM and 0.070 ± 0.027 μM, respectively), but were several times higher with PCB 169 (0.32 ± 0.21 μM and 0.09 ± 0.039 μM, respectively).

All of the mono-ortho PCBs (105, 118, and 156) tested in this study do not appear to sequester in rodent livers *in vivo* (DeVito *et al.*, 1998). All three of the mono-ortho PCBs inhibited MROD activity in both human and rat SUPERSOMES; however, very high concentrations were needed to produce these results (assay concentrations that were several times higher than liver concentrations found *in vivo* after 90 days of dosing). K_I values for these chemicals are listed in Table 2, but these compounds were generally 10- or 100-times less potent inhibitors of CYP1A2 activity than other dioxin like chemicals tested. PCB 153, a non-dioxin like chemical, was used as a negative control; it did not inhibit CYP1A2 activity in rat or human SUPERSOMES (Fig. 1). This is in concordance with data showing no hepatic sequestration of PCB 153 and limited metabolism *in vivo* (Van den Berg *et al.*, 1998).

DISCUSSION

In order to improve the ability to extrapolate rodent data to humans in the risk assessment of dioxins, it is essential to understand the relationship between human and rodent response to these environmental contaminants. The ability of human and rat CYP1A2 to bind these chemicals influences the disposition and body burdens of these chemicals. It is therefore essential to gain an understanding of similarities and differences in metabolism of these chemicals between species. In the present study, human and rat CYP1A2 had similar substrate specificity for three probe substrates. It should be noted that there were some differences between rodent and human CYP1A2 caffeine metabolism, which is in agreement with other studies. While paraxanthine was the predominate metabolite of caffeine in both species, some of the minor metabolites differed, consistent with other reports (Fuhr *et al.*, 1992).

In this study, the ability of a series of dioxins to inhibit human and rat CYP1A2 activity was examined using methoxyresorufin as the probe substrate. Results show that rat and human CYP1A2 enzymatic activity is inhibited by TCDD, PCDD, TCDF, 4-PeCDF, and PCBs 126, 169, 105, 118, and 156 in a concentration-dependent manner. The dibenzofurans were the most potent inhibitors of both human and rat MROD activity. The majority of the chemicals tested appeared to competitively inhibit CYP1A2 except 4-PeCDF, which acted as a mixed inhibitor with both competitive and uncompetitive components based on examination of the Lineweaver-Burke plots. This observation is consistent with other literature investigating inhibition of CYP1A2 activity in rat liver microsomes (Chen *et al.*, 2003). It is interesting to note that 4-PeCDF is sequestered to a much greater extent in liver than is TCDD or other dioxin-like chemicals. For example, liver to fat ratios of the concentration of dioxins is often used as a measure of sequestration. The liver to fat ratio of 4-PeCDF can be as high as 40 in mice, while under similar conditions the ratio for TCDD is approximately 6 (DeVito *et al.*, 1998).

The mono-ortho PCBs 105, 118, and 156 were all shown to inhibit MROD activity in SUPERSOMES from both species. These chemicals do not appear to sequester in hepatic tissue in rodents (DeVito *et al.*, 1998). It is possible that the inhibition of CYP1A2 activity suggests that they could be substrates for this enzyme.

The relative potency of dioxins is thought to be related to their binding affinity to the AhR and their persistence as compared to TCDD (Van den Berg *et al.*, 1998). In the mouse, CYP1A2 binding and hepatic sequestration also appears to impact the relative potency of dioxins. For example, the relative potency of 4-PeCDF compared to TCDD for inducing skin and liver EROD activity in mice is equivalent when estimated based on administered dose (DeVito *et al.*, 1998). However, when the relative potency of 4-PeCDF is based on tissue dose, skin EROD induction increases and the relative potency for hepatic EROD induction decreases. This due to the greater hepatic sequestration of the 4-PeCDF compared to TCDD and, based on the present study, is most likely due to the greater binding of the 4-PeCDF to CYP1A2 as compared to TCDD. The K_I for inhibition of CYP1A2 is approximately ten

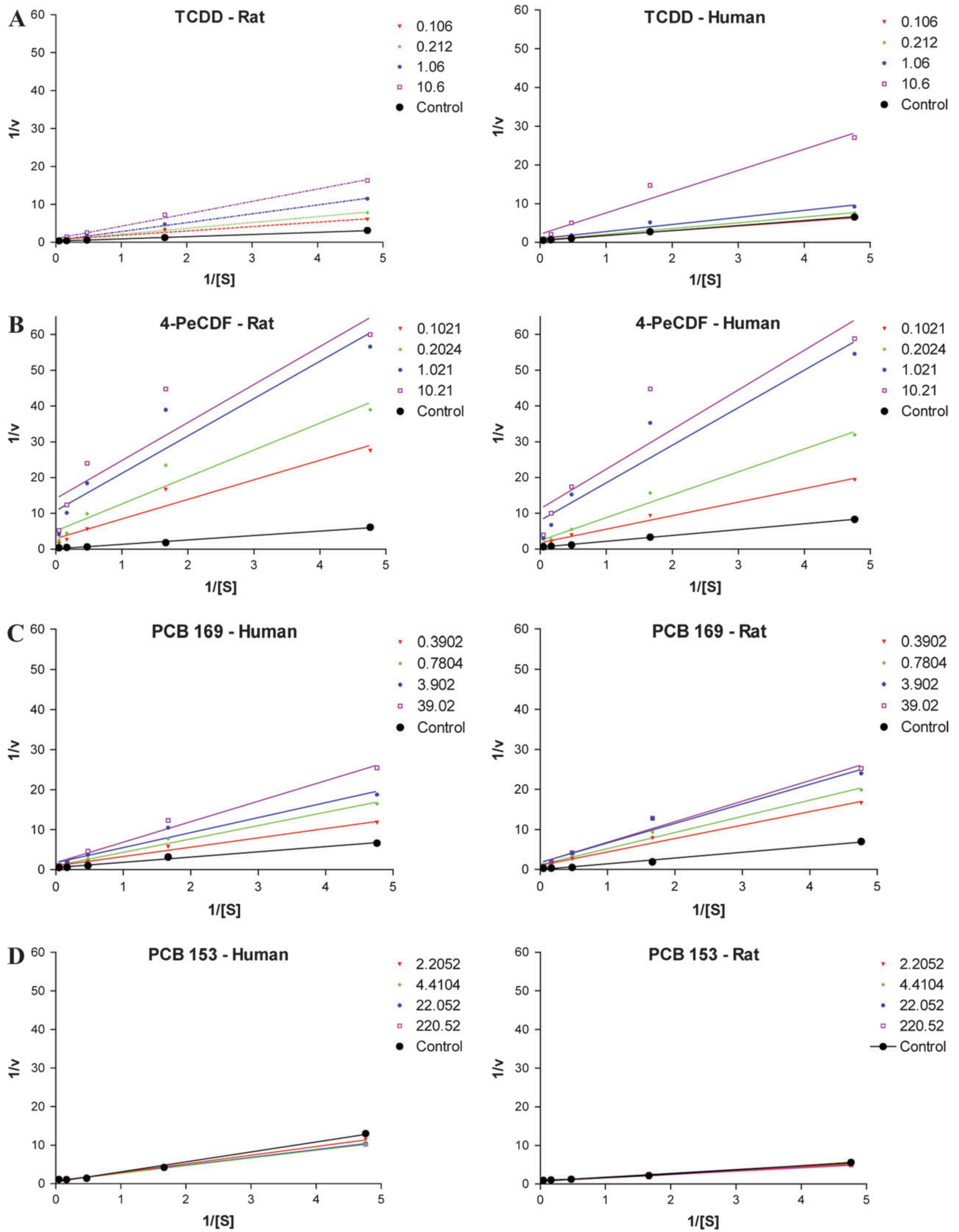

FIG. 1. Lineweaver-Burke plots of human and rat MROD activity following exposure to varying concentrations of (A) TCDD, (B) 4-PeCDF, (C) PCB 169, (D) PCB 153 ([S] = mM, v = pmol resorufin/min/pg P450).

times less for the 4-PeCDF than TCDD in both rat and human SUPERSOMES, suggesting that the influence of CYP1A2 binding on the relative potency of 4-PeCDF is similar between rats and humans. In contrast, the K_I for inhibition of PCB 126 is approximately ten times less in the rat compared to TCDD, but is equivalent in the human, suggesting there may be differences in the relative potencies across species for this chemical. It should be noted that the overall relative potency of a dioxin compared to TCDD is dependent on a variety of parameters and the quantitative role of CYP1A2 binding in the relative potency of dioxins remains to be further examined.

The CYP1A2-inhibition structure activity relationship (SAR) presented in this study is different from an AhR binding SAR (Safe, 1990). For example, TCDD has the greatest affinity for the Ah receptor in both rats and humans (Safe, 1990), while the 4-PeCDF has a ten times lower K_I for CYP1A2 as compared to TCDD in rats and humans. Differences between the rank order are also apparent for the CYP1A2 inhibition K_I in humans and rats (Table 2). This is consistent with the species differences in substrate specificity for CYP1A2.

Although caffeine metabolism has been used as a biomarker for CYP1A2 activity in humans, the use of a caffeine test as a biomarker of AhR-mediated induction of CYP1A2 following exposure to TCDD has often been questioned for its specificity (Walton *et al.*, 2001). A study by Abraham and coworkers (2002) has addressed this issue *in vivo*. Using a small subject pool ($n = 2$ highly exposed, 1 moderately exposed, and 50 controls), they have shown that hepatic CYP1A2 activity is inducible by approximately 10-fold following high TCDD exposure, but that moderate exposure (up to 1000 ppt in blood lipid) did not cause induction of CYP1A2 activity as measured by caffeine metabolism. The lack of induction of caffeine metabolism observed in a moderately exposed individual could be due to CYP1A2 inhibition by dioxins at low levels of exposure and induction. Alternatively, Dorne *et al.* (2001) found a large variability in CYP1A2 activity as measured by caffeine metabolism in healthy adults. The large variability in background caffeine metabolism would most likely require high exposures in order to discriminate induction of CYP1A2 activity.

In the present study, it is clearly shown that TCDD and other dioxin-like compounds inhibit CYP1A2 activity *in vitro*. The results of this study could have implications for risk assessment and interpretation of epidemiological studies. One of the assumptions of risk assessment for dioxins is that these chemicals distribute similarly in humans and rodents. The present data are consistent with this in that dioxins will bind CYP1A2 in both human and rats, resulting in hepatic sequestration. In addition, studies involving highly exposed populations have not demonstrated an increase in caffeine metabolism in these cohorts (Halperin *et al.*, 1995). The use of probe substrates to estimate CYP1A2 induction in populations exposed to TCDD may be confounded by the fact that dioxins inhibit CYP1A2 activity. The negative results from these epidemiological studies should be viewed cautiously.

A number of recent studies have demonstrated that some PCBs inhibit ethoxyresorfin-O-deethylase (EROD) activity and that in these studies there is not a good correlation between induction of EROD activity and CYP1A protein or mRNA levels in mammals and birds (Kennedy *et al.*, 2003; Petrulis *et al.*, 1999, 2001). Similar studies in fish indicate that some PCBs are potent inhibitors of CYP1A activity and this inhibition results in less induction of EROD activity than in CYP1A mRNA (Besselink *et al.*, 1998). The present study extends these observations to CYP1A2. In rats there are data suggesting that induction of CYP1A2 protein does not necessarily correlate with CYP1A2 activity as measured by MROD activity (DeVito *et al.*, 1996). It is possible that the lack of correlation between CYP1A2 activity and protein may be due to the inhibition of CYP1A2 activity by TCDD and other dioxins. Because of the potential for inhibition of CYP1A2 activity by TCDD and other dioxins, studies using CYP1A2 enzymatic activity as a biomarker of exposure to dioxins in rodents, humans, and possibly wildlife populations should be viewed cautiously.

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