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PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING OF CAFFEINE AND THEOPHYLLINE IN NEONATES AND ADULTS: IMPLICATIONS FOR ASSESSING CHILDREN'S RISKS FROM ENVIRONMENTAL AGENTS

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Children's risks can differ from those in adults for numerous reasons, one being differences in the pharmacokinetic handling of chemicals. Immature metabolism and a variety of other factors in neonates can affect chemical disposition and clearance. These factors can be incorporated into physiologically based pharmacokinetic (PBPK) models that simulate the fate of environmental toxicants in both children and adults. PBPK models are most informative when supported by empirical data, but typically pediatric pharmacokinetic data for toxicants are not available. In contrast, pharmacokinetic data in children are readily available for therapeutic drugs. The current analysis utilizes data for caffeine and theophylline, closely related xanthines that are both cytochrome P-450 (CYP) 1A2 substrates, in developing PBPK models for neonates and adults. Model development involved scale-up of in vitro metabolic parameters to whole liver and adjusting metabolic function for the ontological pattern of CYP1A2 and other CYPs. Model runs were able to simulate the large differences in half-life and clearance between neonates and adults. Further, the models were able to reproduce the faster metabolic clearance of theophylline relative to caffeine in neonates. This differential between xanthines was found to be due primarily to an extra metabolic pathway available to theophylline, back-methylation to caffeine, that is not available to caffeine itself. This pathway is not observed in adults exemplifying the importance of secondary or novel routes of metabolism in the immature liver. Greater CYP2E1 metabolism of theophylline relative to caffeine in neonates also occurs. Neonatal PBPK models developed for these drugs may be adapted to other CYP1A2 substrates (e.g., arylamine toxicants). A stepwise approach for modeling environmental toxicants in children is proposed.

The health risk children face from a given xenobiotic exposure may differ from that experienced by adults because of several factors. Children's exposure

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per body weight may be greater than adults (U.S. EPA, 2000), pharmacokinetic factors in children may lead to a different internal dose as compared to adults (Ginsberg et al., 2002; Renwick, 1998), and pharmacodynamic factors may cause certain periods of development to be key susceptibility windows for effects on endocrine and nervous systems or for the induction of cancer (Faustman et al., 2000; Ginsberg, 2003; Anderson et al., 2000). While each of these areas merits careful analysis in developing a framework for assessing children's risks (Daston et al., in press), this article focuses on the question of child/adult differences in pharmacokinetics. Two xanthine medications used in children and adults, caffeine and theophylline, are used in this analysis to evaluate pharmacokinetic differences across age groups and the implications of these differences for environmental toxicants that are cleared by similar mechanisms.

A wide variety of xenobiotic metabolizing systems, including cytochrome P-450 (CYP) enzymes, phase II conjugation systems, serum esterases such as paraoxonase-1 (PON1), and epoxide hydrolase, are immature at birth and develop at varying rates during infancy (Cresteil, 1998; Hines & McCarver, 2002; McCarver & Hines, 2002; Clewell et al., 2002). These immaturities have been associated with slower clearance of a wide array of therapeutic drugs (Renwick, 1998; Renwick et al., 2000; Ginsberg et al., 2002) and in certain cases have been implicated in unique adverse drug reactions in young children (Vest, 1965; Mulhall et al., 1983; Dreifuss et al., 1987; Bryant & Dreifuss, 1996; Cote et al., 2000). This suggests that early life immaturities can affect chemical metabolism and children's susceptibility to a variety of xenobiotics including environmental toxicants.

One can speculate on the degree to which this may occur based on the magnitude of child/adult differences in the function of key enzymes involved in the clearance or activation of a given xenobiotic. However, immaturity in a single pathway may influence internal dosimetry to a greater or lesser extent than expected based on other pharmacokinetic factors such as (a) the presence of compensating metabolic or clearance pathways; (b) blood flow limitations to the key metabolizing organ; and (c) influence of differences in body composition or protein binding capacity (Kedderis, 1997; Clewell et al., 2002). Therefore, it is important to evaluate child/adult differences in internal dosimetry with physiologically based pharmacokinetic (PBPK) models. These models integrate many physiologic and functional parameters that affect chemical fate, yielding predictions of internal dose under a variety of exposure scenarios. Such predictive models are especially needed in assessing children's risks from environmental toxicants because obtaining pharmacokinetic data in children for such toxicants is typically not feasible for ethical reasons. PBPK analyses have been useful in removing some of the uncertainty in extrapolation of exposure and risk between rodents and human adults (Andersen et al., 1987; Bois et al., 1990; Rao & Ginsberg 1997; Hattis et al., 1993). This approach should also be feasible for extrapolating dose from adults to children, with recent reviews pointing out how pharmacokinetic parameters may need adjustment for the development of PBPK models for children (Clewell et al., 2002; Ginsberg et al.,

in press; Price et al., 2002). However, to date there have been relatively few attempts at PBPK modeling in children (Price et al., 2003; Pelekis et al., 2001; Gentry et al., 2002). These efforts have adjusted adult PBPK models for known physiologic differences that occur in early life, but have made predictions for children's dosimetry of environmental toxicants without the benefit of testing the model against actual pharmacokinetic data in children. This situation arises because of the lack of pharmacokinetic data for environmental agents in children.

In contrast to the situation with environmental chemicals, there is an extensive pediatric pharmacokinetic database for therapeutic drugs (Ginsberg et al., 2002; Renwick et al., 2000). These data provide an opportunity for calibrating and validating physiologically based pharmacokinetic (PBPK) models in children that also have relevance for environmental toxicants. This is especially true when modeling drugs whose primary metabolic pathway is also of importance to the activation or clearance of environmental toxicants. Such is the case with the two drugs chosen for analysis in the current study: theophylline and caffeine (Aldridge et al., 1979; Bonati et al., 1981; Carrier et al., 1988; Kraus et al., 1993). The primary route for clearance of these methylxanthine drugs in adults is metabolism via CYP1A2, a CYP that is also central to the metabolic activation of aromatic amines that occur in cigarette smoke, the workplace, and diet (Eaton et al., 1995). Theophylline and caffeine are useful case-study chemicals because clearance in neonates and infants is considerably different (slower) than in adults. Further, although these two xanthines are structurally very similar to each other (Figure 1), theophylline half-life and clearance data in neonates indicate approximately three times faster removal than for caffeine (Dorne et al., 2001; Ginsberg et al., 2002). Given that adult clearance of caffeine is slightly faster than theophylline, the neonate/adult clearance differential is substantially greater for caffeine than for theophylline (Table 1). This observation suggests that the pharmacokinetic mechanisms that govern xanthine metabolism in neonates differ from those in adults. However, to date this has not been explained.

The goal of the current research is to develop adult and neonatal PBPK models for theophylline and caffeine that can reproduce the available pharmacokinetic data. Neonates were chosen for special focus for these drug substrates because pharmacokinetics in neonates are most unlike adults in comparison to other children's age groups (Ginsberg et al., 2002; Dorne et al., 2001). It is hoped that this approach will provide insights into the functional consequences of CYP1A2 immaturity in early life across structurally related chemicals. It is also hoped that the developed models will aid in deriving PBPK models for environmental toxicants that are also substrates for CYP1A2. The next section describes the datasets and approaches used to develop caffeine and theophylline models in neonates and adults. The section which follows after that describes modeling results in terms of matches to empirical data and mechanisms for clearance differences across age groups and across chemicals.

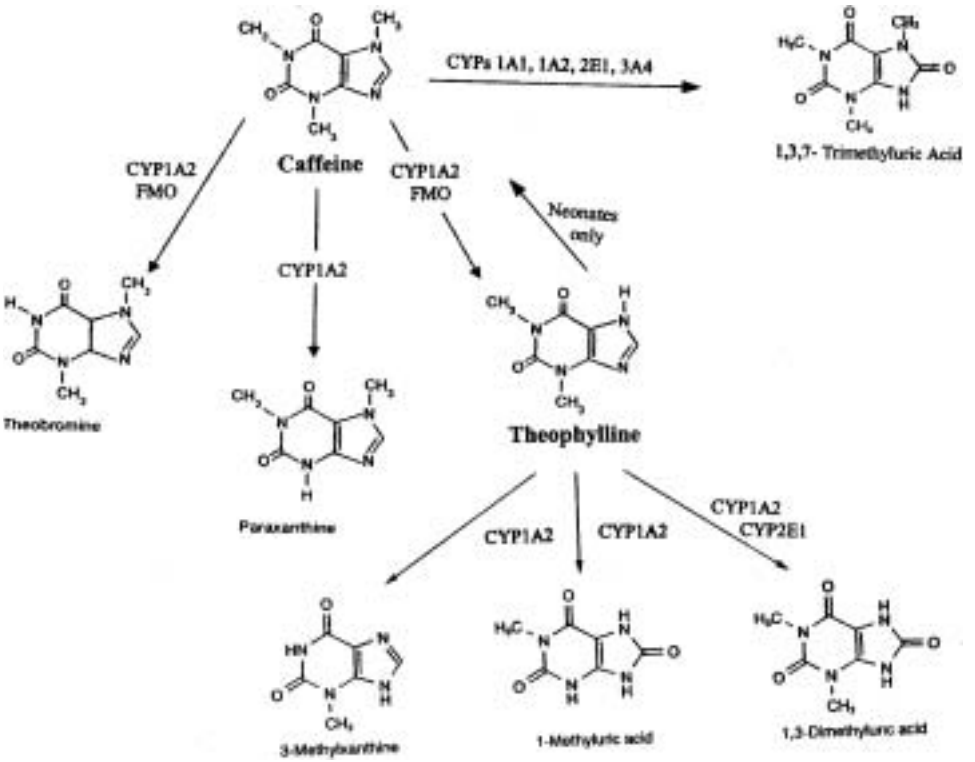

FIGURE 1. Major metabolic pathways for caffeine and theophylline.

MODELING OF THEOPHYLLINE AND CAFFEINE

Fate of Caffeine and Theophylline in Adults

As shown in Figure 1, theophylline and caffeine are stuctural analogues, differing only in that caffeine has one additional *N*-methyl group. Table 1 summarizes the fate of caffeine and theophylline in adults and neonates. The hepatic clearance of both caffeine and theophylline are saturable processes (Cheng et al., 1990; Denaro et al., 1990; Tang-Liu et al., 1982; Dahlqvist et al., 1984), with the primary metabolic pathway in adults being CYP1A2-mediated N-demethylation (Figure 1). Metabolic clearance far outweighs renal elimination in both cases. For caffeine (1,3,7-trimethylxanthine), the primary metabolic step results in an array of dimethylxanthines, one of which is theophylline itself (1,3-dimethylxanthine).

Theophylline's metabolism is also mediated to a large extent by CYP1A2, with N-demethylation (to monomethylxanthines) and 8-hydroxylation (to 1,3-dimethyluric acid) being of most significance (Ha et al., 1995; Bonati et al., 1981; Ogilvie, 1978). Theophylline is also excreted unchanged in urine, which occurs to a greater extent (approximately 10% of dose) than for caffeine (about 1%) in adults.

TABLE 1. Caffeine/Theophylline Fate Comparison in Adults and Neonates

Drug	Age group	$t_{1/2}$ (h)	Total body clearance (ml/kg/min)	Major clearance pathways	Minor clearance pathways	References
Caffeine	Adults	6.0	1.57	CYP1A2 N-demethylation	Urinary excretion; 8-hydroxylation	Parsons and Neims (1978)
Caffeine	Neonates	80–103	0.15	Urinary excretion	N-Demethylation; 8-hydroxylation	Gorodischer and Karplus (1982); Aranda et al. (1979); Parsons et al. (1976)
Theophylline	Adults	7.3	0.86	CYP1A2 N-demethylation; 8-hydroxylation	Urinary excretion	Powell et al. (1977); Miller et al. (1984); Mitenko and Ogilvie (1973); Jusko et al. (1978); Pfeifer et al. (1979); Ellis et al. (1976)
Theophylline	Neonates	20–34	0.3–0.4	Urinary excretion; 8-hydroxylation	N-Demethylation; back-methylation to caffeine	Hilligoss et al. (1980); Jones (1979); Gabriel et al. (1978); Boutroy et al. (1978)

The half-life and clearance kinetics of these two xanthines are similar in adults, with caffeine somewhat more rapidly cleared (Table 1). While CYP1A2 had traditionally been thought of as the primary enzyme responsible for oxidative metabolism of these xanthines, several studies in microsomes and recombinant systems have shown that other CYPs and flavin-containing monooxygenases (FMO) can also participate (Ha et al., 1995, 1996; Chung & Cha, 1997; Tassaneeyakul et al., 1994; Tjia et al., 1996) (Figure 1). This becomes important when considering neonatal metabolism of caffeine and theophylline. The deficiency in CYP1A2 at this developmental stage creates the possibility that other clearance pathways can take on added importance. The involvement of these additional pathways is further evaluated next.

Fate of Caffeine and Theophylline in Early Life

Due to the immaturity of CYP1A2 at birth, compensatory pathways such as renal elimination are more predominant for both caffeine and theophylline. While only a little more than 1% of caffeine is excreted unchanged in urine in adults, 80–90% is cleared in this manner in neonates (Aldridge et al., 1979). This neonate/adult differential is less dramatic with theophylline, where nearly 50% is excreted unchanged in neonates compared to approximately 10% in adults (Bonati et al., 1981; Lowry et al., 2001). However, renal elimination is not as efficient as CYP1A2-mediated metabolism and so the overall clearance rate in neonates is reduced relative to adults; for caffeine this decrease in total body clearance is 10-fold, while for theophylline it is 2- to 3-fold (Table 1).

Metabolite recovery in neonate urine is low after caffeine administration (10–15% of dose) and consists of small amounts of a wide variety of demethylated metabolites (Aldridge et al., 1979; Al-Alaiyan et al., 2001; Carrier et al., 1988). Excretion of theophylline metabolites in the urine of newborns differs from the pattern seen for caffeine in a number of ways: (1) Up to 40% of the theophylline dose may be processed via 8-hydroxylation in neonates. While substantial, this is still below the percentage of this metabolite found in adult urine. (2) A unique metabolite is found in neonatal urine, caffeine. This back-methylation reaction in which a methyl group is added to theophylline has been reported to account for 5–10% of the overall urinary excretion found in short-term experiments (Bonati et al., 1981; Kraus et al., 1993; Grygiel & Birkett, 1980; Tserng et al., 1983; Skopnik et al., 1992).

Data Used to Model Caffeine and Theophylline Metabolism

Data Describing Xanthine Metabolism by Individual CYPs Given that a variety of enzymes in addition to CYP1A2 can potentially act on caffeine and theophylline, it was essential to have detailed information on these enzymes from in vitro studies. Fortunately, recombinant systems in which CYP genes have been expressed in human B-lymphoblastoid cell lines have been used to study the activity of various CYPs toward these xanthine substrates. Ha et al. (1995, 1996) used this system to evaluate the biotransformation of caffeine and theophylline by seven different CYPs (1A1, 1A2, 2A6, 2B6, 2D6, 2E1, and

3A4). The Michaelis–Menten constants for the metabolism of caffeine or theophylline to specific metabolites are shown in Table 2 for those CYPs that had demonstrable activity.

For caffeine, formation of the major metabolite, paraxanthine, is catalyzed almost exclusively by CYP1A2 (V_{max}/K_m of 161 L/h-mmol CYP). The formation of other demethylated metabolites, theobromine and theophylline, occurs less efficiently, with the V_{max}/K_m ratio for these reactions one to two orders of magnitude lower than for paraxanthine formation. This is consistent with in vivo evidence that these are minor demethylation products in adult subjects (Carrier et al., 1988). Formation of theobromine and theophylline is most efficient with CYP1A2 but can also occur via other CYPs (2E1, 1A1). Caffeine conversion to 1,3,7-trimethyluric acid (1,3,7-TMU) was most efficiently performed by CYPs 1A2 and 1A1, but low-affininty, high-capacity pathways involving CYPs 3A4 and 2E1 were also detected.

Metabolism of theophylline to demethylated products was catalyzed almost exclusively by CYP1A2 (Table 2), with CYP1A1 also exhibiting some activity in the formation of 1-methylxanthine. Unlike caffeine, the 8-hydroxylation metabolism of theophylline occurred at a rapid rate, with both CYP1A2 and CYP2E1 having substantial activity. The CYP1A2-mediated formation of this hydroxylation product (1,3-dimethyluric acid) occurs with higher affinity and so will be predominant at lower doses but is more readily saturated than the CYP2E1-mediated reaction. The back-conversion of theophylline to caffeine

TABLE 2. Michaelis–Menten Constants for Theophylline and Caffeine From In Vitro Single CYP Systems in Mammalian Cells Transfected With CYP c-DNAs (Data of Ha et al., 1995, 1996)

A. Theophylline Metabolism to 1-MX, 3-MX, and 1,3-U

	1-Methylxanthine (1MX)			3-Methylxanthine (3MX)			Dimethyluric acid (13U)		
CYP isoform	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
1A1	13.1	0.31	42.26	nd	nd	nd	nd	nd	nd
1A2	360	0.38	947	148	1.09	135.8	439	0.23	1909
2E1	nd	nd	nd	nd	nd	nd	4120	15.3	269

B. Caffeine Metabolism to 1,7-x, 3,7-x, 1,3-x, and 1,3,7-U

	Paraxanthine (1,7X)			Theobromine (3,7X)			Theophylline (1,3X)			Trimethyluric acid (1,3,7U)		
CYP isoform	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
1A1	2.69	0.59	4.56	0.82	0.41	2.0	nd	nd	nd	3.78	0.26	14.5
1A2	30.5	0.19	161	3.0	0.16	18.8	1.12	0.25	4.5	1.95	0.27	7.2
2E1	nd	nd	nd	0.48	1.44	0.33	0.36	0.84	0.43	2.95	1.04	2.84
3A4	nd	nd	nd	nd	nd	nd	Nd	nd	nd	27.5	46	0.60

Note. V_{max} in mol metabolite formed/h/mol CYP. K_m in mmol/L. nd, Activity not detected.

was not assessed in this in vitro system but, as already mentioned, has been detected in vivo in neonates.

These data provide an initial starting point for simulating the conversion of caffeine and theophylline to specific metabolites by specific CYPs. They document that in addition to CYP1A2, CYPs such as 2E1 and 3A4 may be involved in caffeine and theophylline metabolism, depending on the amount of these CYPs present in a given tissue and on substrate concentration. While CYP1A1 is also able to metabolize both substrates, the normally low levels of this CYP in liver precludes it from making a substantial contribution to their metabolism. This assumption is based on the common modeling convention that places all metabolism in the liver, an assumption for which there is no empirical data in the case of the two xanthines modeled presently, and in fact has rarely been tested for other chemicals.

Data Describing Amount of Individual CYPs Present in Liver An essential input for scaling xanthine metabolism from in vitro recombinant systems to in vivo is to adjust the CYP V_{max} values shown in Table 2, expressed per mole CYP, to account for the amount of CYP protein per whole liver (Lipscomb et al., 1998). For adults, this in vitro to in vivo scaling relied on the following data: (1) CYP content per milligram microsomal protein (Shimada et al., 1994): CYP1A2, 0.042 nmol, CYP2E1, 0.022 nmol; CYP3A4, 0.096 nmol; (2) microsomal protein per gram liver: 52.5 mg (Alcorn & McNamara, 2002; Lipscomb et al., 2003); and (3) size of adult liver: 1508 g (Diem & Lentner, 1970). For neonates, liver bank studies have shown that a number of CYPs are deficient at birth (Cresteil, 1998; Tateishi et al., 1997). However, this is partially offset by the fact that liver mass per body weight is larger in early life than in adults and tends to scale according to surface area (Gibbs et al., 1997). These factors were combined by Alcorn and McNamara (2002) to develop equations describing the postnatal ontogeny of CYP content in liver as compared to adults, termed the infant scaling factor. Table 3 shows these scaling factors for the CYPs involved in xanthine metabolism for the neonatal period (first week of life).

Since these infant scaling factors are relative to CYP levels in adult liver, they must be multiplied by the CYP composition of adult liver as shown in Table 3 (Shimada et al., 1994). The table shows that the CYP2E1 content of neonatal liver microsomes is closer to the adult level (31.1% of adult level) than is the case for the other two CYPs. The absolute amount of CYP2E1 in neonatal liver is less than CYP3A4, but substantially greater than CYP1A2. Modeling CYP-specific rates for caffeine and theophylline metabolism involved using the reported Michaelis–Menten constants for each metabolite pathway and CYP (Table 2) and adjusting the V_{max} for each pathway for the amount of CYP present per total adult or neonatal liver. Table 3 also shows the relative amounts of the three CYPs primarily involved in metabolism of these xanthines. These proportions indicate that relative to adult liver, the contribution to xanthine metabolism of CYP1A2 in neonates will be greatly diminished while the relative contribution of CYP2E1 will be increased.

TABLE 3. Scaling of V_{max} From In Vitro (Ha, et al., 1995, 1996) to In Vivo

	CYP1A2	CYP2E1	CYP3A4
Adult hepatic CYP content (mol CYP per whole liver)[a]	3.32E-06	1.74E-06	7.60E-06
Ontogeny scaling factor[b]	0.0185	0.311	0.127
Relative hepatic scaling factor[c]	1.87	1.87	1.87
Neonate scaling factor[d]	0.035	0.582	0.310
Neonate content of CYP (mol CYP per whole liver)[e]	6.1E-09	5.3E-08	9.4E-08
Relative proportion of each CYP active toward these xanthines—adult liver	26.2%	13.7%	60.0%
Relative proportion of each CYP active toward these xanthines—neonatal liver	4.0%	34.6%	61.4%

[a] Derived by multiplication of CYP content of adult microsomes (Shimada et al., 1994) by mg microsomal protein per gram liver (Alcorn & McNamara, 2002) and size of adult liver (Diem & Lentner, 1970).

[b] Relative amount of CYP in neonatal (1–7 d old) livers (nmol/mg microsomal protein) as compared to adult liver microsomal content of the CYP (Alcorn & McNamara, 2002).

[c] Scaling factor to account for different in liver mass per body weight between neonates and adults.

[d] This overall scaling factor is the ratio of neonatal CYP content per body weight relative to adults, and is the product of the preceding two lines in the table.

[e] Product of first and fourth lines of table, adjusted by body weight ratio between newborns and adults (3.5/67.2 kg).

CYP3A7 is an isozyme that is present in high amounts in fetal microsomes, with its levels declining postnatally as CYP3A4 levels increase (Cresteil, 1998). Due to its abundance, it is potentially important for caffeine and theophylline metabolism in neonates. However, there are no data describing caffeine or theophylline metabolism by this CYP, so its potential contribution to fetal disposition of these xanthines could not be included in the models.

Model Overview

Caffeine and theophylline are water soluble drugs of low volatility that are thought to be metabolized primarily in the liver and eliminated as parent compounds or metabolites in urine. A basic five-compartment flow-limited model was developed to simulate the fate of these xanthines as shown in Table 4: rapidly perfused tissues (vessel-rich group), slowly perfused tissues (muscle as prototype tissue), liver, kidney, and fat. In addition, the models included arterial and venous blood compartments to simulate the delivery of xanthine to tissues and its subsequent removal via partitioning into the venous circulation. It was not necessary to estimate an oral absorption rate constant because much of the data came from iv infusion studies. In these cases, doses of caffeine or theophylline were introduced into the venous blood compartment according to the experimental protocol being simulated. Where oral dosing was simulated, these were repeat-dose studies with blood levels reported at steady state so that there was no peak blood concentration against which to backfit the oral absorption coefficient. These experiments were also simulated

TABLE 4. Summary of Basic Inputs for Neonate and Adult PBPK Modeling

Model parameter	Neonate	Adult	Data source
Body weight (kg)	3.5	67.2	Diem and Lentner (1970)
Cardiac output (L/min)	0.678	5.835	Regression based on multiple data sources
Compartment volumes (L)			
Liver	0.147	1.508	Regression equation from Price et al. (2003), Diem and Lentner (1970)
Kidney	0.025	0.267	Regression equation from Price et al. (2003)
Vessel-rich group (except liver and kidney)	0.373	6.90	Regression equation from Price et al. (2003)
Vessel-poor group (muscle)	1.934	28.60	Regression equation from Price et al. (2003)
Fat	0.235	15.10	Regression equation from Price et al. (2003)
Venous pool blood	0.22	3.65	Jonsson and Johanson (2001)
Arterial pool and lung blood	0.093	1.57	Jonsson and Johanson (2001)
Blood flows (L/min)			
Liver	0.122	1.52	Neonates: % basal metabolic rate (Holliday, 1971) Adults: Fiserova-Bergerova (1995)
Kidney	0.025	1.14	Neonates: PAH clearance (West et al., 1948) Adults: Fiserova-Bergerova (1995)
VRG (vessel rich group)—except liver and kidney	0.454	1.43	Neonates: Cardiac output – Σ(other tissue flows) Adults: Fiserova-Bergerova (1995)
VPG (vessel poor group)—predominantly muscle	0.047	1.46	Neonates: % basal metabolic rate (Holliday, 1971) Adults: Fiserova-Bergerova (1995)
Fat	0.03	0.29	Neonates: set to adult fraction of cardiac output Adults: Fiserova-Bergerova (1995)

with pulse iv doses of xanthine to achieve the reported steady-state blood concentration, with model performance checked against postdosing xanthine clearance from blood and appearance of parent compound or metabolites in urine.

Metabolism of both xanthines was simulated to occur in the liver only, and to include the contribution of a number of different CYPs. As described later in this section, the Michaelis–Menten constants for each CYP's metabolism of caffeine or theophylline were derived by adapting in vitro kinetic constants to whole liver, with backfit optimization needed for certain pathways. The fate of xanthine metabolites was not simulated, except in the case of the back conversion (methylation) of theophylline to caffeine. Renal elimination of unchanged (parent) xanthine was modeled via a first-order process backfit to empirical data as described later in this section.

Physiological Parameters for Adults and Neonates Model inputs for adults and neonates are shown in Table 4 along with the source or derivation of the value. Body-weight values were taken from Diem and Lentner (1970), with data averaged across male and female subjects. Total cardiac output was estimated for adults and neonates from regression equations fitted to a variety of adult (Miles et al., 1981; Moore et al., 1992; Rowland et al., 1997; Turley & Wilmore, 1997; Vinet et al., 2001) and neonate datasets (Braden et al., 1990;

Costeloe et al., 1977; Tibby et al., 1997; Walther et al., 1985; Wyse et al., 1975). The regression equations for each age group are shown next.

Regression for child cardiac output:

$$\log Q \text{ (L/min)} = 0.8914[\log (\text{body weight in kg})] - 0.654$$

Regression for cardiac output in adults:

$$\text{Cardiac output } (Q, \text{ L/min}) = -2.86 + 5.85(\text{BSA})$$

where adult body surface area (BSA) was taken as $1.5\ m^2$.

Compartment volumes for neonates were from age regressions presented by Price et al. (2003), who used data from Altman and Ditmer (1962), ICRP (1975), and NRC (1993) regarding the ontogeny of organ growth and body fat content. Data for adult compartment volumes were from Diem and Lentner (1970) for liver and kidney, from Fiserova-Bergerova (1995) for vessel-rich and vessel-poor groups, and from age regresssions for body fat (Price et al., 2003).

Neonatal blood flows reflect the well-documented low flow to the kidney (Hook & Bailie, 1979) and disproportionately large size of and blood flow to the brain at birth (Renwick, 1998). Renal blood flow in neonates was derived from data showing the increase in *p*-aminohippuric acid (PAH) clearance with increasing age beginning at birth (West et al., 1948). PAH clearance provides an estimate of renal plasma flow, which was adjusted to renal blood flow by the hematocrit (Ht) in neonates [blood flow = plasma flow/(1 − Ht)] (Diem & Lentner, 1970). Other sources reporting neonatal renal blood flow data (Calcagno & Rubin, 1963; Hook & Bailie, 1979) support the value shown in Table 4. Blood flows for liver and muscle were derived on the basis of the percent of total basal metabolic rate (BMR) contributed by these tissues in neonates, as reported by Holliday (1971). Since specific data pertinent to adipose tissue blood flow in neonates were not available, the percentage of cardiac output directed toward fat in the adult model was also used for neonates. Blood flow to the vessel-rich group (VRG), minus liver and kidney, was assumed to constitute the remainder of the cardiac output. This yields a VRG flow that is approximately two-thirds of the cardiac output. This is reasonable given that the VRG compartment contains the brain, which is larger and contributes a considerably greater percentage of BMR in neonates than in adults. Tissue blood flows in adults were taken from Fiserova-Bergerova (1995).

The inputs for cardiac output and tissue blood flows shown in Table 4 are for a basal level of activity. An "activity model" was also developed to represent parts of the day with higher breathing rate and energy expenditure (Black et al., 1996). This model involved increased cardiac output with muscle receiving the majority of the extra blood flow (Fiserova-Bergerova, 1995). However, xanthine pharmacokinetics were not materially changed by activity-based adjustments to the model, so these data are not presented.

Partition Coefficients Basic methodology for the estimation of tissue/blood partition coefficients from information on octanol/water partition

coefficients has been described previously (Ginsberg et al., 1996, 1999), patterned after methodology and reasoning first described by Patterson and Mackay (1989).

From data on blood solubilities and octanol/water partition coefficients of relatively hydrophilic compounds, Patterson and Mackay (1989) derive the relationship:

$$S_B = C_W(1+0.0035K_{OW})$$

where S_B and C_W are the solubility of the chemical in blood and water under some standard conditions, K_{OW} is the octanol–water partition coefficient, and 0.0035 represents their estimate of the effective octanol-equivalent fat content of blood. Similarly, solubilities in various tissues are represented as:

$$S_T = aC_W + bS_O$$

From these two, it follows that tissue/blood partition coefficients, S_T/S_B, should be given by:

$$\frac{aC_W + bS_O}{[C_W(1+0.0035K_{OW})]}$$

or, taking the denominator's C_W into the numerator, and noting that $S_O/C_W = K_{OW}$,

$$\frac{a + bK_{OW}}{1 + 0.0035K_{OW}}$$

or, more generally,

$$\frac{a + bK_{OW}}{1 + cK_{OW}}$$

where a is related to the effective water content of the tissue, b is related to the effective lipid content of the tissue, and c depends on the effective lipid content of blood.

The central estimates of the fitted constants from this modeling for 29 chemicals are shown in Table 5A. Table 5B shows the number of data–chemical comparisons available for each tissue and the standard deviation of the log (observed/model predicted partition coefficients). The model parameters shown in Table 5A result from minimizing the sum of squares of the log ratios (observed/predicted) across all observations. The fit was evaluated based on the deviation of individual data points from the sum of squares best fit relationship, which yields an overall standard deviation of 0.148 for all tissues. This means that 95% of the partition coefficients in the data set are within $10^{(0.148\times1.96)} = 1.95$, or about two fold of the best fit line.

When combined with the octanol/water partition coefficient for caffeine, 0.84 (average of values given by Leo et al., 1971; Guy et al., 1985; and Schiffman et al., (1994), and for theophylline of 0.955 (Dollery, 1999), the model coefficients in Table 5A resulted in the estimated tissue/blood partition coefficients shown in Table 5C. The same set of partition coefficients was used for neonates and adults.

Calculation of Volume of Distribution PBPK modeling for caffeine and theophylline yields estimates of blood concentration that take into account various distributional phenomena. The volume of distribution of each compound was estimated from the model output total milligrams of drug remaining in the system at a particular time point divided by the blood concentration (mg/L drug) to yield the volume over which the drug was distributed in liters. For this purpose, we used a time point late in the simulation—well after any short-term changes in drug distribution due to the initiation of dosing.

TABLE 5. Derivation of Partition Coefficients for Modeled Xanthines in Human Tissues

A. Fitted Tissue Constants for Partition Coefficient Equation[a]

Equation input	Parameter value
Common "*c*"	0.00180
Liver *a*	0.77333
Liver *b*	0.01475
Kidney *a*	0.78986
Kidney *b*	0.00771
Fat *a*	0.06339
Fat *b*	0.38840
Muscle *a*	0.67896
Muscle *b*	0.00963

B. Fit of the Data to the Model for 29 Chemicals

	Liver	Kidney	Fat	Muscle	All tissues
Number of observed vs. predicted comparisons	22	25	29	29	105
Standared deviation log (observed/model "predicted")	0.149	0.143	0.170	0.132	0.148

C. Model-Derived Partition Coefficients

Tissue	Theophylline	Caffeine
Liver	0.79	0.78
Kidney	0.80	0.80
Fat	0.43	0.39
Muscle	0.69	0.69
VRG (average of kidney and liver)	0.79	0.79

[a] Parameter values are shown to more significant figures than are warranted by the statistical uncertainty of

Running the Models

Simulations were run in Excel spreadsheets in which the columns represented the amounts of caffeine or theophylline (or metabolites) in various body compartments, and the rows represented successive time steps. The length of the time step was set to be short enough to ensure that there were no appreciable departures from appropriate mass balance of theophylline or caffeine. With the exception of metabolism in the liver, all transfers in the model were linear. Thus, for example, the caffeine (mg) in muscle tissue at time = 100.1 min would be:

(Muscle caffeine$_{100\,\mathrm{min}}$ + (0.1 min × muscle blood flow) (L/min) ×
[arterial blood concentration (mg/L) − (muscle caffeine$_{100\,\mathrm{min}}$/muscle
to blood partition coefficient)]

In this equation, absolute cell references were used for a bank of constants, including the blood flows and tissue volumes needed to calculate tissue concentrations, and relative cell references were used for the contents of various compartments.

The equation for the kidney had an additional linear loss term representing the fraction of incoming arterial kidney blood flow that was cleared to the urine per minute. This fraction was an adjustable parameter in the model fitting, tuned to reproduce the urinary output of unchanged caffeine or theophylline from the system observed in the various calibrating data sets. The backfit percent renal blood flow eliminated varied across xanthines and age groups as follows: caffeine adult, 0.08%; caffeine neonate, 1.7%; theophylline adult, 0.6%; theophylline neonate, 2.9%.

Metabolism in the liver was represented as a series of Michaelis–Menten loss terms corrresponding to the various CYP enzymes as exemplified for CYP1A2:

mg/min metabolized by CYP1A2 =
[liver xanthine conc. (mg/L) × microsomal V_{max} [mol/(h-mol CYP) ×
conversion factor/[microsomal K_m (mg/L) + liver xanthine conc. (mg/L)]

The starting point for V_{max} and K_m inputs were the Michaelis–Menten parameters derived for xanthine metabolism by recombinant CYPs (Ha et al., 1995, 1996; see also Table 2). For the a priori models, scaling of the V_{max} from in vitro to in vivo utilized a conversion factor as shown in the preceding equation. This scaling factor was derived from data on the size of the liver, the amount of microsomal protein per gram of liver, the overall concentration of all CYP enzymes per microgram of microsomal protein isolated from the liver, and the relative abundance of that CYP among all liver microsomal CYPs for a particular age group. For the backfit version of the models, a V_{max} multiplier parameter was used to optimize hepatic extraction in place of the in vitro-to-in vivo scaling

factors. Initial model runs were conducted in backfit mode for both renal and hepatic clearance mechanisms. The V_{max} multiplier was backfit to match the reported theophylline or caffeine $t_{1/2}$ in adults and these calibrated models were used to estimate overall hepatic clearance. Then the models were rerun except that in the second set of runs, the V_{max} values reported in vitro (Table 2) were scaled to in vivo directly from the scaling factors described earlier and in Table 3 (mol CYP/mg microsomal protein × mg microsomal protein per gram liver × g liver per whole liver). Comparison of overall hepatic clearance in the scaled-up version of the model to the fully backfit version indicates how well the in vitro-based Michaelis–Menten parameters and the assumption that all metabolic clearance takes place in the liver predict in vivo metabolism.

The Excel spreadsheets encoding the models are available on request to one of the authors (dhattis@aol.com).

RESULTS

Comparison to Empirical Data

While PBPK models for these xanthines were not previously published in animals or humans, the current effort was facilitated by the availability of in vitro metabolic constants for human CYPs together with human adult and neonate pharmacokinetic data with which to calibrate the models.

Calibration of the models with respect to hepatic metabolism involved backfit of the V_{max} multiplier. This multiplier converts the in vitro-derived V_{max} (mol metabolized/h/mol CYP) to total hepatic metabolism (mol/h/liver) and was either derived by using the in vitro-to-in vivo scaling factors described earlier (a priori metabolic rate) or was simply backfit to the in vivo clearance data. Backfit of the V_{max} multiplier yielded a model that could reproduce theophylline pharmacokinetic data with the difference between the backfit and the a priori V_{max} multiplier two-to three-fold as described later.

In the case of caffeine, adjustment of the V_{max} alone would not match in vivo clearance and half-life data. To further explore this disparity, the ability of the in vitro-derived K_m for caffeine to simulate the saturation in caffeine clearance was evaluated. A number of studies have reported caffeine $t_{1/2}$ in adults following single dose administration (Cheng et al., 1990; Bonati et al., 1982; Murphy et al., 1988; Lelo et al., 1986; Parsons & Neims, 1978). These studies are compiled in Figure 2, which shows the relationship between caffeine $t_{1/2}$ and dose (filled black squares, unconnected by lines). Saturation is evident over this dose range as the $t_{1/2}$ becomes longer with increasing dose. The figure also shows a series of adult caffeine model expectations. The flattest line, labeled K_m mult = 1, reflects the use of the Ha et al. (1996) K_m values without further modification. This model input leads to a prediction of no apparent saturation over this dose range, a result that clearly diverges from the actual data. This suggests that the in vitro-based K_m is too high relative to the in vivo CYP metabolism of caffeine. Decreasing the K_m multiplier below unity, which decreases all

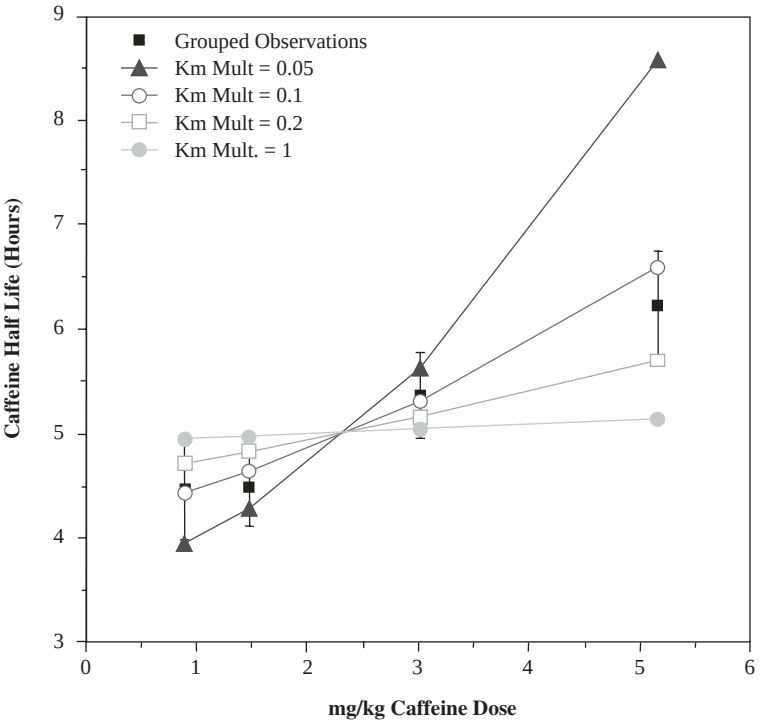

FIGURE 2. Backfit of caffeine K_m to the observed nonlinearity in caffeine half-life.

K_m values in the Ha et al. (1996) data set by the same factor, allows the saturation phenomenon to become more evident with the 0.1 multiplier (10-fold reduction in K_m), producing results that approximately match the actual data (Figure 2). This adjustment also increased metabolic clearance, improving the fit of the adult caffeine model in this regard. For these reasons, all subsequent caffeine model runs utilized a 10-fold reduction in the Ha et al. (1996) K_m values relative to the in vitro-derived values.

One other point of calibration in the scaled up models was for the back-methylation of theophylline to caffeine in neonates. In vitro data on this conversion were not reported by Ha et al. (1995) or in other data sets. Thus, this reaction was modeled as a first-order process via backfit against data describing the urinary excretion of caffeine post theophyllline dosing in neonates (Bonati et al., 1981).

Figure 3 presents the fit of the scaled-up models (without V_{max} multiplier adjustment but with caffeine K_m adjustment and backfit of renal clearance rate) to hepatic clearance data as derived from the fully backfit model. The following data sets were used for this purpose: Bonati et al. (1981) for theophylline clearance in adults (2.82 mg/kg iv dose, $n = 3$) and in neonates (5.5 mg/kg iv bolus followed by 1.1 mg/kg maintenance doses every 12 hours, $n = 12$); Lelo

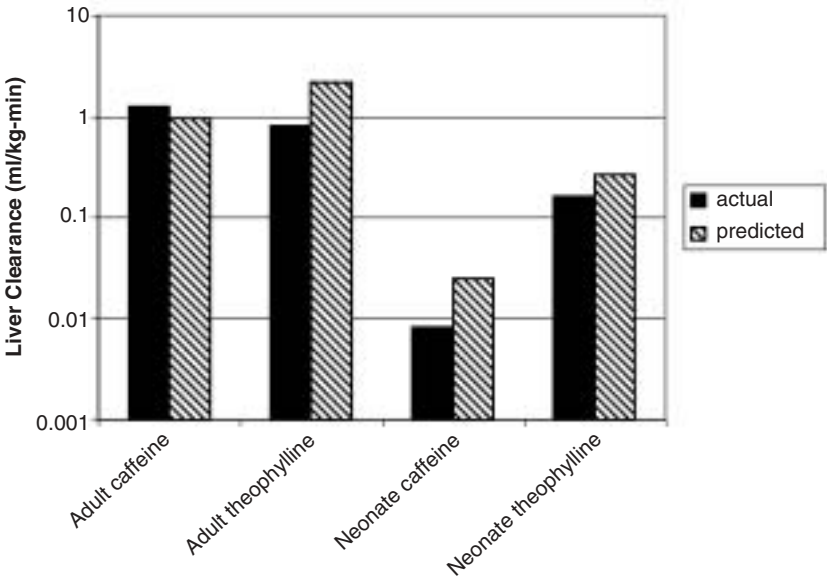

FIGURE 3. Metabolic clearance of caffeine and theophylline in adults and neonates: actual vs. predicted.

et al. (1986) for caffeine clearance in adults (oral administration of 2.41 mg/kg in 6 adults every 8 h for 3 d); Aranda et al. (1979) for caffeine clearance in neonates (10 mg/kg iv dose in 12 neonates). It should be noted that the neonatal data sets consist of a range of subject ages and dosing regimens, with some neonates premature at birth while others were full term, and with dosing beginning as early as postnatal d 1 in some cases but not until d 32 for other neonates (Bonati et al., 1981; Aranda et al., 1979). Further, the Bonati et al. data set involved follow-up maintenance dosing over a 4- to 19-day period Thus, the infants for which pharmacokinetic data were available represent a broad age range spanning the first month of life. PBPK modeling of these experiments assumed enzyme function and physiologic parameters suitable for full-term neonates in the first week of life. This was considered appropriate even though infant age went beyond 1 wk, since many of the infants would have particularly immature pharmacokinetics due to pre-term birth. Since neonatal data were reported in pooled fashion, it was not possible to simulate more discrete age groupings. Figure 3 indicates reasonable agreement (less than twofold difference) between actual hepatic clearance and that predicted based on the scaled up model for caffeine in adults. Part of the reason for this fit is that the in vitro K_m values for caffeine metabolism were adjusted from that reported in vitro (Ha et al., 1996) in order to match caffeine saturation kinetics in adults as described earlier. When the caffeine model was adapted to neonates, the fit was also reasonable although in this case there was a model overshoot of liver metabolism by threefold. Regarding the theophylline model,

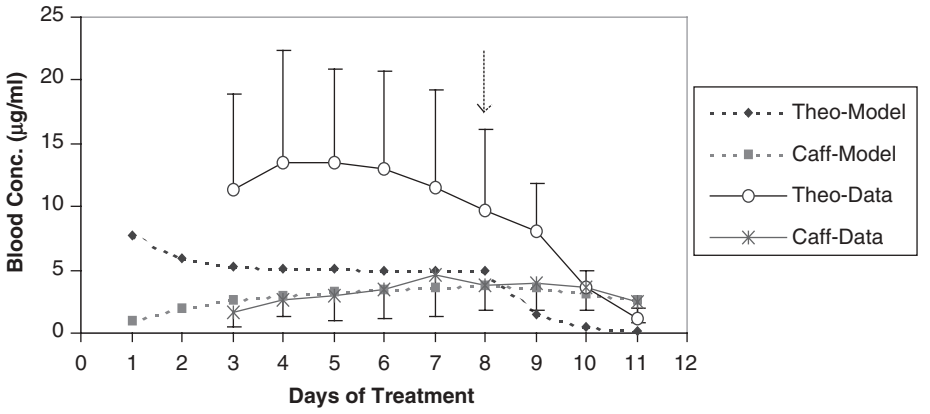

FIGURE 4. Modeled vs. actual theophylline and caffeine blood concentrations (data from Bonati et al., 1981). Arrow indicates time when theophylline maintenance dosing ceased. Error bars represent SD.

the scaled up model overpredicted liver clearance by two- to threefold in adults and neonates. The neonatal fit of the theophylline data was assisted by the fact that a portion of the liver clearance (back-methylation to caffeine) was not scaled up from in vitro but was necessarily derived by backfit as described earlier.

The potential reasons for some overprediction of caffeine and theophylline metabolism in neonatal liver are discussed in the next section. Overall, the results in Figure 3 suggest that the various scaling procedures needed for this modeling (in vitro to in vivo; adult to neonate) yielded a working model of xanthine metabolism. The modeling system was able to describe the orders of magnitude difference in liver clearance between adults and neonates while at the same time simulating the more rapid rate of neonatal metabolism of theophylline relative to caffeine. These models were then used to assess other aspects of caffeine and theophylline metabolism.

Back-methylation of theophylline to caffeine was calibrated against urinary excretion data in Bonati et al. (1981) as described earlier. That paper also describes theophylline and caffeine blood concentrations in neonates during and after a theophylline repeat-day dosing regimen that lasted for 8 d. These data were simulated with a form of the model that included both caffeine and theophylline disposition so that clearance of caffeine formed from theophylline could also be modeled. The reported blood concentrations together with model estimates for this dose regimen using the a priori model are presented in Figure 4. While actual data are not available until d 3, model results are shown beginning on simulated d 1. The observations and model results both show low levels of caffeine relative to theophylline at early time points, with caffeine levels gradually increasing over the course of the 8-d regimen. Model predictions were generally two- to threefold lower than actual theophylline blood concentrations. In contrast, the model closely matched the reported

caffeine blood concentrations. The models were also able to simulate the post-dosing decline in xanthine blood concentrations. These results suggest that the theophylline neonatal model provides reasonable estimates of the back-methylation of theophylline to caffeine.

Another check of theophylline model predictions is the comparison of metabolite ratios found in urine in relation to model predictions of metabolite formation. These comparisons are specific to the CYP-related metabolites (i.e., excluding back-methylation to caffeine, which was derived by backfit), with data shown for adults and neonates in Table 6. The model predicts that the majority of theophylline's CYP metabolism will result in 1,3-dimethyluric acid, with 1-methyluric acid being of secondary importance and 3-methylxanthine being only a trace (5% or less) metabolite. These predictons are similar to reported urinary metabolite data in all cases except the Bonati et al. (1981) adult data set, in which 32% of metabolite formation is to 3-methylxanthine. However, the Grygiel and Birkett (1980) data set provides a metabolite pattern more similar to the modeled data.

Table 7 provides renal clearance data for caffeine and theophylline in adults and neonates. When compared with liver clearance data in Figure 3, it

TABLE 6. Model Prediction vs. Actual Data for CYP-Related Metabolite Formation From Theophylline

	Percent formation of metabolite[a]		
	1-Methyluric acid (1MU)	3-Methylxanthine	1,3-Dimethyluric acid
Bonati et al. (1981); adults	30	32	38
Grygiel and Birkett (1980), adults	27	13	60
Model, adults	31	5	64
Bonati et al. (1981), neonates	24.9	Not detected (< 5%)	75.1
Model, neonates	17.4	2.6	80

[a] percentage of each metabolite relative to the total amount of CYP-related oxidation products found in urine following theophylline dosing in adults or neonates. Back methylation to caffeine not included in these percentages.

TABLE 7. Model-Predicted Pharmacokinetic Parameters for Caffeine and Theophylline

	Renal clearance (ml/kg-min)	Volume of distribution(L/kg)		Blood $t_{1/2}$ (h)	
		Model	Observed	Model	Observed
Caffeine—adult	0.015	0.56	0.53	6.47	5.02
Caffeine—neonate	0.073	0.74	0.92	48.2	97.8
Adult/neonate ratio	0.21	0.80	0.63	0.13	0.05
Theophylline—adult	0.106	0.54	0.44	2.92	7.3
Theophylline—neonate	0.121	0.75	0.83	16.0	26.8
Adult/neonate ratio	0.88	0.80	0.53	0.18	0.27

Note. Liver clearance results are presented in Figure 3.

is evident that in adults xanthine clearance by the liver is considerably more rapid than from the kidney. However, in neonates, renal clearance predominates in the case of caffeine elimination while renal and hepatic clearance are comparable for theophylline. The results also show that liver clearance in adults is approximately two orders of magnitude greater than in neonates for caffeine but only fivefold faster for theophylline. In contrast, renal clearance is estimated to be equal to or faster than adults in neonates for both xanthines (Table 7).

Table 7 also shows model results compared to actual data for two other parameters: volume of distribution (V_d) and blood $t_{1/2}$. Model projections for both xanthines indicate greater V_d in neonates compared to adults. This is consistent with reported V_d data, although the reported data suggest a somewhat larger V_d age differential than that shown in the model output. Model predictions of blood half-life are close to actual data for caffeine in adults and for theophylline in neonates. In the case of the other two simulations, caffeine in neonates and theophylline in adults, model predictions of $t_{1/2}$ were two- to threefold shorter than the actual observations (Table 7). These shorter $t_{1/2}$ values stem from the somewhat faster hepatic clearance predicted by the scaled-up models than what may actually occur, as described earlier and in the next section.

The preceding results describe model checks against in vivo datasets in neonates and adults. A dataset which describes the metabolism of caffeine in incubations with neonatal or adult microsomes is also available (Cazeneuve et al., 1994). The models were scaled to in vitro metabolism conditions to simulate these microsomal experiments, with model output compared to the observed data in Figure 5. The microsomal results are qualitatively similar to in vivo results in that demethylation of caffeine to paraxanthine, theobromine, and theophylline are one to two orders of magnitude slower in neonates than in adults. However, caffeine conversion via 8-hydroxylation to trimethyluric acid is not appreciably different in microsomal preparations derived from

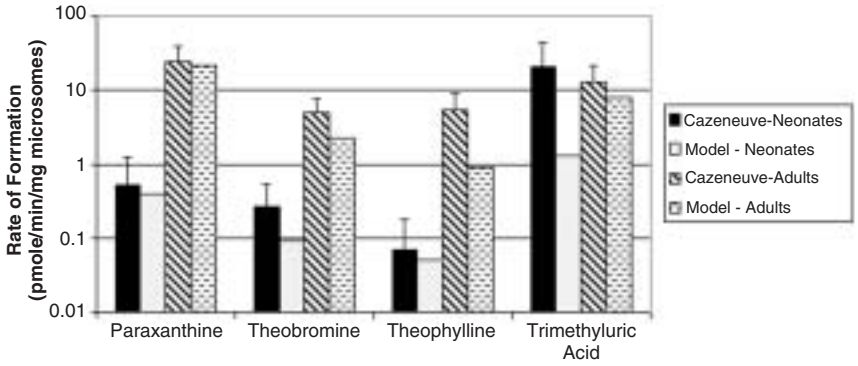

FIGURE 5. Prediction of caffeine microsomal metabolism compared to data from Cazeneuve et al. (1994). Error bars represent SD.

neonates and adults. Model simulations reproduce the pattern of demethylation results with good fit to the paraxanthine data, but model underprediction of the microsomal theobromine and theophylline production is apparent. The model provided a good fit to trimethyluric acid formation in adults but not in neonates, where there was a nearly 20-fold discrepancy between observations and model predictions. As later discussed further, discrepancies in model fit to microsomal data may be due to the presence of metabolizing systems (e.g., FMOs; CYP3A7) in addition to those present in the recombinant CYP expression systems (Ha et al., 1995, 1996) from which the scaled up model was derived.

Use of the Model to Explore Differences in Caffeine and Theophylline Metabolism in Neonates

The scaled-up xanthine models were used to examine the theophylline/caffeine differential in liver clearance in neonates. Figure 3 demonstrates that liver clearance of theophylline is approximately an order of magnitude greater than clearance of caffeine in neonates. Model results for throughput via individual metabolic pathways enabled the breakdown of hepatic clearance by pathway, expressed as a percentage of total hepatic clearance (Figure 6) or total body clearance (Figure 7). As expected, metabolic clearance of both xanthines is dominated by CYP1A2 in adults but not in neonates. For caffeine, CYP1A2 is still the major metabolic route in neonates, but CYP2E1 and CYP3A4 are also of some consequence. In contrast, the major route of theophylline metabolism in neonates is the back-methylation reaction to form caffeine, which comprises approximately 70% of total hepatic clearance. As with caffeine, CYP2E1 becomes of somewhat greater importance in neonates than in adults, with the contribution from CYP1A2 being substantially lower in early postnatal life than in adults.

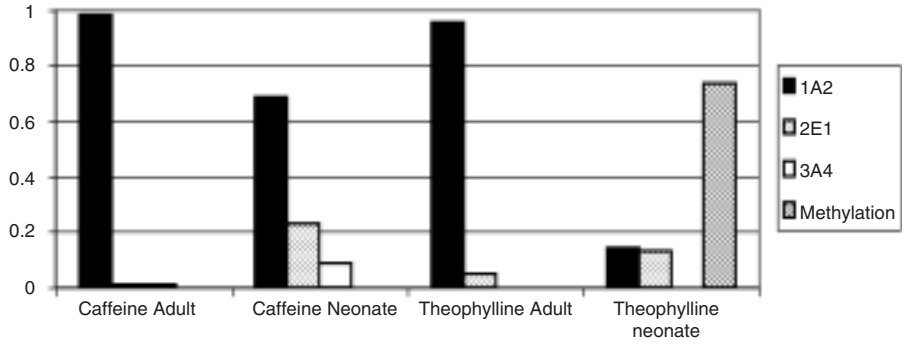

FIGURE 6. Percent contribution of individual CYPs to total hepatic clearance of xanthines.

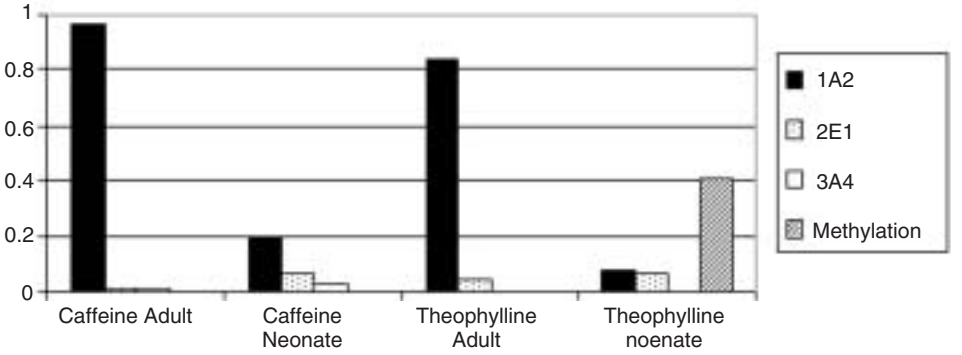

FIGURE 7. Percent contribution of individual CYPs to total body clearance of xanthines.

When the pathway-specific results are expressed as a function of total body clearance (Figure 7), the quantitative importance of individual metabolic pathways to overall xanthine disposition is depicted. While again CYP1A2 is the predominant mode of clearance in adults, now none of the CYP pathways are of major importance in neonates. This is due to the low rate of these pathways relative to renal excretion of parent xanthine. The metabolic pathway of greatest signficance in neonates is the back-methylation of theophylline to caffeine, which accounts for 40% of total body clearance for this xanthine. There is no corresponding pathway in neonatal metabolism of caffeine, and thus this back-methylation reaction provides the primary explanation for theophylline's greater hepatic clearance rate.

DISCUSSION

Theophylline and caffeine offer an excellent opportunity for modeling neonatal and adult metabolism and clearance. Three tiers of metabolism data are available, which enables building a model from the individual CYP level up to whole liver. These data sets include a first tier describing the metabolic capacity of individual CYPs in recombinant expression systems (Ha et al., 1995, 1996), a second tier describing the ability of neonatal and adult liver microsomes to metabolize caffeine (Cazeneuve et al., 1994), and a third tier describing the in vivo metabolism of both xanthines (Bonati et al., 1981; Lelo et al., 1986; Carrier et al., 1988; Aldridge et al., 1979; Aranda et al., 1979; Parsons & Niems, 1978). The availability of data on these three levels allowed for the development and refinement of scaled-up metabolism models, that is, models constructed from Michaelis–Menten parameters for individual CYPs, which were then scaled up to whole liver, taking into account differences in hepatic content of individual CYPs between neonates and adults (Alcorn & McNamara, 2002). The fact that these models could approximate metabolism of caffeine by neonatal microsomes (Figure 5) as well as in vivo metabolic

clearance of theophylline and caffeine (Figure 3, Table 7) indicates that scaling up from in vitro results is feasible for estimating neonatal metabolism in vivo.

While the current data support the utility of using scaled up in vitro data for predicting neonatal metabolism, there are a number of caveats that should be kept in mind. The in vitro K_m values for caffeine metabolism by individual CYPs were too high, with a 10-fold decrease in K_m needed to match in vivo caffeine saturation data (Figure 2). The reason for this need to adjust the K_m for caffeine but not theophylline is unclear. Difficulties in scaling up CYP expression system results to human microsomes have been noted, particularly for CYP1A2 (Venkatakrishnan et al., 2000). However, it is important to note that the current K_m adjustment, which was made to fit in vivo caffeine data in adults, also brought caffeine neonatal metabolism into the proper range (Figure 3). This suggests that once a working adult model is in place, there is a reasonable expectation that scaling metabolic activity to neonatal enzyme levels will provide useful simulations of neonatal metabolism.

The scaled-up, a priori model overpredicts the rate of hepatic extraction of both xanthines in neonates, but particularly in the case of caffeine (Figure 3). This translates to a model prediction of caffeine $t_{1/2}$ in neonates that is approximately twofold shorter than observed (Table 7). One factor that may have caused this model overprediction of caffeine metabolism is that the developmental age assumed in the model may be an overestimate. The caffeine neonatal data come from a study of premature infants that were 28.5 wk average gestational age at birth and were put on study at an average postnatal age of 11.5 d (Aranda et al., 1979). The model assumption for this group was that they were developmentally similar to full-term neonates aged 1–7 d, which translates to approximately 1 wk of delayed development of metabolism due to premature birth. However, it is possible that the effect of prematurity in this group was greater. In fact, if the model assumed a d 0 postnatal age, liver metabolism would have been 70–80% lower for the major CYPs involved in caffeine metabolism; this assumption would have produced a better fit to the caffeine half-life data. Model fit to neonatal theophylline hepatic clearance and blood half-life data was closer than that seen for caffeine (Figure 3, Table 7). In that case, 9 of the 12 infants on study were premature (average gestational age for entire group = 30.8 wk) and the average postnatal age when placed on study was 8.3 d. The 2-wk greater gestational age for the theophylline group as compared to the caffeine group is a plausible explanation for the better fit of the neonatal model to the theophylline data. This comparison shows the importance of taking prematurity into consideration when modeling metabolism during the first weeks of life. This adjustment would need to be a function of both gestational and postnatal age. However, since many premature infants remain in the hospital during the early postnatal period, pharmacokinetic modeling of this level of immaturity may have limited applications in environmental risk assessment.

Another caveat is that models based on results with purified CYPs may not account for other enzyme systems that can act on the substrate but were not

included in the recombinant test system. For example, Ha et al. (1995, 1996) tested the activity of seven different CYPs against theophylline and caffeine (1A1, 1A2, 2A6, 2B6, 2E1, 2D6, 3A4). However, other enzymes of potential importance to xanthine metabolism were not examined in this system. The activity of CYP3A7 is of interest when considering fetal or neonatal metabolism because this is the predominant CYP in utero with expression levels still high in the early postnatal period (Cresteil, 1998; Hakkola et al., 1998). This CYP has 88% homology and overlapping substrate specificity with CYP3A4 (Wrighton et al., 2000). This raises the possibility that CYP3A4 substrates such as caffeine are also substrates for CYP3A7. In agreement with this concept are the microsomal results of Cazeneuve et al. (1994), which indicate that neonatal metabolism of caffeine to timethyluric acid occurs at a rate greater than in adult microsomes, a result that far exceeds the expectations of the model (Figure 5). The metabolic product of CYP3A4 incubation with caffeine is trimethyluric acid (Table 2), a finding that suggests that CYP3A7 present in neonatal liver microsomes might also produce trimethyluric acid. In fact, fetal microsome data reported by Cazenueve et al. (1994) indicate high levels of trimethyluric acid formation in these prenatal samples, again suggesting that a prenatal form of CYP3A4 (3A7) is responsible for this metabolic pathway. Given that the scaled-up model does not account for CYP3A7 metabolism of caffeine, it is not surprising that the model underpredicted trimethyluric acid production in fetal microsomes (Figure 5).

An important question is how this might affect projections of in vivo metabolism of caffeine in neonates. Figure 2 shows that in spite of the scaled-up model not accounting for the contribution of CYP3A7 to caffeine metabolism, there was still some model overshoot of caffeine hepatic clearance data in neonates (Figure 3). This would be consistent with a relatively high K_m for the CYP3A7 reaction such that at the single caffeine concentration tested in the microsomal system (1 m*M*; Cazeneuve et al., 1994) there could be significant substrate turnover by this CYP, while at the much lower liver concentrations that are associated with the in vivo neonatal experiments, very little metabolism would occur via this route. Supporting the concept of a high K_m for the putative interaction between CYP3A7 and caffeine is the high K_m reported for CYP3A4 (46K_m, Table 2b).

Another enzyme system lacking in the scaled-up model due to insufficient data is FMO. This system is of interest to the current case study because it can make a substantial contribution to the microsomal metabolism of caffeine to theobromine and theophylline, while contributing much less to paraxanthine formation (Chung & Cha, 1997). This is consistent with the model versus actual data comparison shown in Figure 5, wherein the model accurately predicted paraxanthine metabolism, a CYP1A2 product, but underpredicted theobromine and theophylline formation in both adults and neonates. This would suggest that better information regarding the role of purified CYP3A7 and FMO in metabolizing xanthines would improve modeling estimates in neonates and adults. However, even without these model refinements, it

appears that the scaled-up version does fairly well in projecting in vivo metabolism of caffeine and theophylline as indicated in Figure 3 (hepatic clearance data), Table 6 (percent metabolite data), and Table 7 ($t_{1/2}$ data).

This modeling effort has led to an explanation for the disparity of caffeine and theophylline clearance in neonates, which occurs in spite of the fact that these are close structural analogues that both rely principally on CYP1A2 for clearance in adults. Model output of total body clearance in Figure 7 shows that a major clearance pathway for theophylline in neonates is the back methylation of theophylline to caffeine. Since there is no analogous reaction available to caffeine, theophylline's metabolic clearance is considerably faster in neonates than is caffeine's. This leads to the neonate/adult metabolic clearance ratio to be much lower for caffeine than for theophylline. The back-methylation pathway can be thought of as theophylline clearance because very little of the formed caffeine would become demethylated back to theophylline, particularly in this early age group. Not only does this pathway not exist for caffeine, it is also not detectable for theophylline in adults, making it a novel reaction in neonates. Incubation of adult human hepatocytes has provided suggestive evidence for the ability of adult liver to also back methylate theophylline (Berthou et al., 1988). However, this metabolite is not detected in adults in vivo due to the predominance of other metabolic routes for theophylline and the rapid metabolism of any caffeine that would be formed.

While back-methylation of theophylline to caffeine has been observed in numerous studies of neonates, it may not have been considered as a plausible explanation for the difference in neonatal metabolism of theophylline versus caffeine due to its relatively small percentage in urine (Bonati et al., 1981; Grygiel & Birkett, 1980; Kraus et al., 1993; Tserng et al., 1983). However, caffeine's long $t_{1/2}$ in neonates (Table 1, Figure 4) means that extended (multiday) dosing and urine collection periods are needed to see the full contribution of this reaction. By calibrating the model to the available urinary metabolite data, it has been possible to simulate total throughput via each metabolic route and thus demonstrate the importance of theophylline back methylation. While the enzyme responsible for N-methylation of theophylline has not been investigated, this activity is consistent with the action of soluble *N*-methyltransferases present in erythrocytes and a variety of tissues and that depends upon the methyl donor, *S*-adenonsylmethionine (Klaassen et al., 1996).

This case study points out that a novel metabolic pathway in neonates appears to be of sufficient consequence to be able to explain a large portion of the disparity in caffeine versus theophylline clearance in neonates. The fact that this pathway could not be anticipated from adult metabolism data highlights the importance of obtaining neonatal data when developing PBPK models for this age group. This is especially important for cases in which the predominant adult pathway is immature in neonates, leading to the opportunity for secondary metabolic pathways to take on greater signficance. While the current case study points this out most clearly for theophylline N-methylation, it is also true to a lesser degree for CYP2E1 metabolism. This CYP is more functionally

mature in neonates than is CYP1A2 (Alcorn & McNamara, 2002) and thus can make a more substantial contribution to hepatic and total body clearance in neonates than in adults (Figures 6 and 7).

Another finding of interest is the evidence that while hepatic clearance is slow in neonates, especially for caffeine (two orders of magnitude below adult hepatic clearance), renal clearance is not similarly deficient (Table 7). This results in the overall 10-fold slower clearance of caffeine in neonates as compared to adults (Table 1). Regarding renal clearance, this is expected to be immature in neonates, given the evidence of reduced renal blood flow and function at birth (West et al., 1948); Calcagno & Rubin, 1963; Hook & Bailie, 1979). However, tubular reabsorption and secretory mechanisms are also immature at birth (Besunder et al., 1988; Morselli, 1989) leading to the possibility that xanthine urinary excretion is promoted in neonates due to a lower capacity to reabsorb filtered solutes. Another possibility relates to the fact that the urinary excretion of caffeine and theophylline is enhanced by diuresis produced by their own action on the kidney (Mazkereth et al., 1997; Gillot et al., 1990). It is possible that this diuretic effect may be greater in neonates than in adults, leading to a more efficient excretion of these xanthines in neonates. Whatever the mechanism, it appears evident that renal excretion of xenobiotics in neonates, while slow for some pharmaceuticals (e.g., a number of antibiotics; Ginsberg et al., 2002), cannot be assumed to be slow in all cases.

Implications for Risk Assessments Involving Children's Exposures to Environmental Toxicants

While the ideal is to have children's as well as adult pharmacokinetic data for PBPK modeling and risk assessment, data in children are rarely available for environmental agents. Therefore, alternative approaches are needed. The current case study documents an approach that was suitable for modeling neonatal metabolism of two drugs. This approach could be applicable to environmental agents as follows: (1) Obtain enzyme-specific metabolic constants from purified recombinant systems for various CYPs (including CYP3A7) and other enzymes (e.g., FMOs) that might be involved in chemical metabolism. (2) Build a predictive model of metabolism via each active enzyme, adjusted for the microsomal content of these enzymes at specific ages. (3) Test predictions of metabolic rate against microsomal hepatocyte incubations from liver samples representative of different age groups (and/or studies in whole isolated hepatocytes). (4) Adjust the model to account for the microsomal or hepatocyte results, checking for novel metabolic pathways that may be of importance in neonates or other age groups. (5) Incorporate this core metabolism model into a complete PBPK modeling framework to predict the internal dosimetry of environmental agents in children and adults. (6) Check model results against any available in vivo pharmacokinetic data, which are much more likely to exist for adults than neonates. (7) Make final adjustments to the model to account for in vivo observations as available.

A simpler but less well defined approach might be considered for screening level risk assessments. The ontogeny of hepatic CYPs has been fairly well characterized in recent years; the present case study shows that use of this information to scale metabolism from adults to neonates is feasible. This information might be used to adapt an adult PBPK model for an environmental agent to neonates, even in the absence of confirmatory in vitro data from children's microsomes or isolated hepatocytes. For example, Gentry et al. (2002) have made internal dosimetry projections for a wide range of toxicants across a sprectrum of children's and adult age groups on the basis of such scaling information. The current modeling points out that this would project substantially less CYP1A2 metabolism in neonates than in adults, which can be a protective factor for chemicals such as aromatic amines, which are activated to mutagenic and carcinogenic metabolites by CYP1A2 (Eaton et al., 1995). A caveat to this approach is its potential to omit secondary or novel metabolic pathways that may not be important in adults but may be of primary importance in neonates. This would have occurred in the current case study for theophylline if not for the extensive in vitro and in vivo database available for xanthine metabolism in neonates. These data helped establish the somewhat increased importance of CYP2E1 metabolism and the novel back-methylation reaction for theophylline in neonates as compared to adults. Another consideration with aromatic amine substrates of CYP1A2 is that neonates and infants have delayed expression of *N*-acetyltransferases (Pariente-Khayat et al., 1991; Pons et al., 1989), a phase II conjugation system that appears to be protective against certain types of carcinogenic action by these agents (Grant et al., 1997; Marcus et al., 2000).

Thus, models that project internal dosimetry and neonatal risk for toxicants metabolized by CYP1A2 need to take into account at least four factors: (1) slower metabolism of parent compound, which may be protective if metabolism leads to more toxic/carcinogenic metabolites; (2) the potential for novel metabolic pathways to exist in neonates, which may lead to formation of the same toxicants as found in adults or to altogether different metabolites; (3) the greater importance of secondary metabolic routes in neonates due to the functional immaturity of CYP1A2; and (4) the potential for detoxification reactions to also be immature. These conclusions are based on the current drug case studies and in vitro liver bank data on CYP ontogeny as described earlier (Alcorn & McKenna, 2002; Cresteil, 1998; Hines & McCarver, 2002). However, caution must be used in extrapolating these conclusions to environmental toxicants in general. The array of CYPs involved in activation/detoxification will likely vary across toxicants as well as the relative importance of liver versus other sites of metabolism. The current case studies provide an approach for bringing this information into a PBPK modeling framework for evaluating child/adult dosimetry differences.

The current analysis does not attempt to capture inter-individual variability in the function of individual CYPs across adult or neonatal populations. Distributions of CYP function exist because of environmental factors that can affect

the expression of CYP genes, and also because of genetic polymorphisms that influence the function and expression of CYPs (Wormhoudt et al., 1999; Testai, 2002). For example, hepatic levels of CYP1A2 varied more than 10-fold across human livers based upon CYP1A2 mRNA and associated protein levels in microsomes (Sesardic et al., 1988; Schweikl et al., 1993). CYP2E1 protein content of human microsomes can vary 4- to 20-fold (Carriere et al., 1996; Lipscomb et al., 2003), while CYP3A4 microsomal content varied 3-fold across 20 human liver samples (Lipscomb et al., 2003). It should be noted that the CYP2E1 content of adult liver used in the current modeling based on Shimada et al. (1994) was at the low end of a population distribution seen in a recent paper (Lipscomb et al., 2003). This would suggest that the CYP2E1 contribution to hepatic xanthine metabolism could be appreciably higher in some individuals than that shown presently. A comprehensive risk assessment for an environmental toxicant can build on the current approach by incorporating interindividual variability to allow comparison of the full range of internal doses possible in children to that possible for adults.

Summary and Conclusions

The current analysis takes advantage of pharmacokinetic datasets in neonates and adults for two xanthine drugs to develop predictive PBPK models. This modeling effort has led to a greater understanding of the ways in which neonatal metabolism may differ from adults, and how to incorporate these differences into PBPK models. Specifically, neonatal metabolism can be approached both by scaling to whole liver from in vitro recombinant and microsomal systems, and also by scaling from adult metabolism data to neonates based upon the ontogeny of CYP expression. However, the latter approach may miss important metabolism pathways in neonates that are minor or nonexistent in adults. This is exemplified in the current case study by the finding that neonatal clearance of theophylline is faster than caffeine, due primarily to a novel metabolic pathway that is neither available to caffeine nor detectable in adults exposed to theophylline. These findings have implications for PBPK modeling of environmental toxicants in neonates, both for CYP1A2 substrates, which will have particularly slow metabolism in neonates, and for other types of agents for which the approaches described currently may also be applicable.

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