Incorporating Children's Toxicokinetics into a Risk Framework

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Children's responses to environmental toxicants will be affected by the way in which their systems absorb, distribute, metabolize, and excrete chemicals. These toxicokinetic factors vary during development, from *in utero* where maternal and placental processes play a large role, to the neonate in which emerging metabolism and clearance pathways are key determinants. Toxicokinetic differences between neonates and adults lead to the potential for internal dosimetry differences and increased or decreased risk, depending on the mechanisms for toxicity and clearance of a given chemical. This article raises a number of questions that need to be addressed when conducting a toxicokinetic analysis of *in utero* or childhood exposures. These questions are organized into a proposed framework for conducting the assessment that involves problem formulation (identification of early life stage toxicokinetic factors and chemical-specific factors that may raise questions/concerns for children); data analysis (development of analytic approach, construction of child/adult or child/animal dosimetry comparisons); and risk characterization (evaluation of how children's toxicokinetic analysis can be used to decrease uncertainties in the risk assessment). The proposed approach provides a range of analytical options, from qualitative to quantitative, for assessing children's dosimetry. Further, it provides background information on a variety of toxicokinetic factors that can vary as a function of developmental stage. For example, the ontology of metabolizing systems is described via reference to pediatric studies involving therapeutic drugs and evidence from *in vitro* enzyme studies. This type of resource information is intended to help the assessor begin to address the issues raised in this paper. *Key words:* children, dosimetry, risk assessment, toxicokinetics. *Environ Health Perspect* 112:272–283 (2003). doi:10.1289/ehp.6013 available via *http://dx.doi.org/* [Online 25 November 2003]

Children Can Be a Tough Group to Figure Out

Perhaps the risk assessment community has difficulty embracing the arena of children's risk because of the imposing task. Children are difficult to study for a number of reasons, not the least of which is a practical one: it is not ethically feasible to introduce chemicals of environmental concern, even at trace amounts, into infants and children. Thus, there is very little toxicokinetic data (i.e., pharmacokinetic data for environmental toxicants) in this age group. One can gather data from "natural experiments" in which children are exposed inadvertently to a pesticide or airborne chemical. The resulting biomarker data may be useful for investigators in assessing the extent of exposure, but these are not the well-controlled toxicokinetic data one needs for building a predictive children's model. Another equally daunting problem is that children are highly diverse, stretching in definition (for the purposes of this framework) from fetal through adolescent stages and beyond. Generalizations and defaults are not possible for such a sweeping range of development. Even within a narrow age range, there can be considerable variability, given the rapid and variable rate of development in early life. Thus, improving children's risk assessments through toxicokinetics is a worthwhile endeavor but one that will be hampered by data gaps and uncertainties.

Our goal in writing this article on toxicokinetic issues is to outline the toxicokinetic questions that need to be addressed if this area of study is to contribute to a children's risk assessment. We also hope to provide the reader with some background and resources that can help shed light on these questions by showing where this information can fit into a framework for children's risk assessment. We recognize that such risk assessments also need to address critical exposure and toxicodynamic issues, and that in some ways these areas overlap with toxicokinetics (Adams et al. 2000). We point out those interfaces, but for this discussion we will keep toxicokinetics as a separate consideration in the children's risk assessment process.

Toxicokinetics as a key element in children's risk assessments. The importance of toxicokinetics in risk assessment has been increasing as we have learned more about how toxicokinetic processes (especially chemical metabolism) are involved in mechanisms of toxicity and how these processes can differ markedly across species. In numerous cases, toxicokinetic analyses have allowed replacement of the traditional dose metric—applied dose per body weight per day—by a more relevant internal dose metric that facilitates extrapolation of animal dose–response data to humans (Andersen et al. 1987; Bois et al. 1990; Hattis et al. 1993; Rao and Ginsberg 1997). Most commonly this is accomplished with physiologically based pharmacokinetic (PBPK) models, which simulate chemical uptake, distribution, metabolism, and excretion in both animals and humans.

Although progress in toxicokinetics has removed some of the uncertainty in cross-species extrapolations in risk assessment for adults, these principles have yet to be applied in a systematic manner to the risk assessment of children. This is a critical need, given that children's toxicokinetics differ from adults in a number of ways: smaller body size; different ratios of fat, muscle, and water; higher breathing and metabolic rates per body weight; and immaturity of clearance systems and enzymatic reactions (Anderson et al. 1997; Besunder et al. 1988; Ginsberg et al. 2002; Kearns and Reed 1989; Renwick 1998). Another obvious difference is that children are more diverse, undergoing a developmental program of growth and maturation that continuously alters how chemicals are processed and cleared. Thus, incorporating children's toxicokinetics into risk assessment is complicated by the need to consider many developmental stages, ranging from *in utero* to adolescence, and by the extensive variability that can occur within each age group.

Pharmacokinetic differences between children and adults with respect to the clearance of therapeutic drugs have been recognized for years. These differences have spawned numerous clinical pharmacokinetic studies for the purpose of better titrating drug dosage to a particular age or body size (Anderson et al. 1997; Ginsberg et al. 2002; Morselli 1989; Renwick et al. 2000). The focus on children as a pharmacological receptor has not been matched by a similar focus on children as a toxicant receptor. In large part this is because of the lack of pediatric toxicokinetic studies, as mentioned above. This leaves us with a relatively rich pharmacokinetic database for

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children but a nearly empty toxicokinetic database. Challenges facing the children's risk assessment framework include how to combine basic information on children's physiologic development with what can be learned from children's pharmacokinetic data sets and how to develop an analytic process that allows us to compare internal doses of environmental toxicants between adults and children.

Proposed building blocks for a children's toxicokinetic assessment. In crafting a general framework for conducting children's risk assessments, a 3-phase approach has been recommended that involves problem formulation, data analysis, and risk characterization. [See companion article by Daston et al. (2004)]. An overview of this approach is provided in Figure 1, which serves to organize the information on children's toxicokinetics into a stepwise analytical approach.

Phase I: Toxicokinetic Questions in Problem Formulation

A risk assessment of children will likely begin with a number of questions regarding age groups and exposures, toxicokinetic handling of the chemical(s), and whether there are susceptible periods or unique toxic effects (toxicodynamic issues). These questions and the preliminary answers developed in problem formulation help to scope the remainder of the analysis. A major toxicokinetic question likely to arise in problem formulation is "Can early life stages be considered as part of the overall human variability distribution and thus be within the 3.2-fold toxicokinetic uncertainty factor"? This broad question can be broken into the following more-defined areas that should help the risk assessor better evaluate the issues of children's toxicokinetics:

- What types of data, resources, and analytic approaches are needed to determine whether *a*) children of certain ages experience higher exposures because of higher ventilation rates, cardiac outputs, or greater absorption of chemical(s); *b*) children of certain ages experience a higher internal dose of parent compound or active metabolite (per unit of administered dose) because of distributional, metabolism, or elimination factors; and *c*) children of certain ages process the chemical(s) by novel toxicokinetic pathways in order to generate metabolite profiles that are qualitatively different from those of adults?

In this article we discuss these broad, scoping toxicokinetic questions to provide background information and to help us consider what kinds of resources and analyses will be needed for their resolution.

- Does children's toxicokinetics warrant an uncertainty factor that differs from the current practice of allowing an approximately 3.2-fold factor for intersubject variability in toxicokinetics?

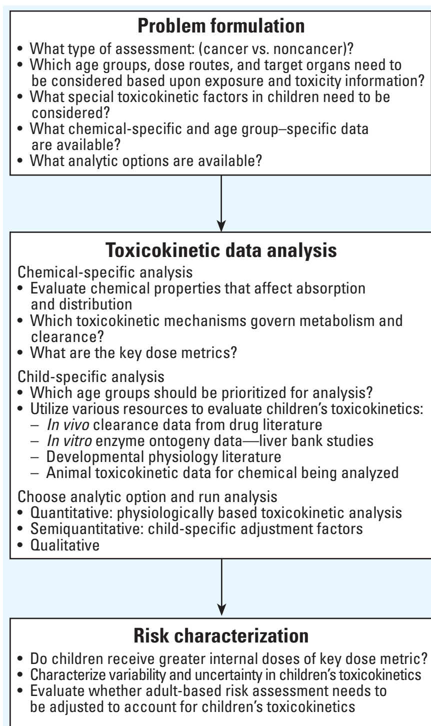

Figure 1. Outline of toxicokinetic assessment process for children.

Noncancer risk assessments are concerned with threshold effects, with the public health exposure level set well below the observed threshold seen in animal or high-exposure human studies [U.S. EPA (U.S. Environmental Protection Agency) 1989]. A series of uncertainty factors are used to attempt to conservatively cover what we do not know about risks; the uncertainty factors lower the allowable exposure level from the animal or human no observable adverse effect level (NOAEL) or benchmark dose. Prominent among these is the interindividual variability factor, typically a 10-fold factor. This factor results from a general recognition that the animal species or worker cohort from which the NOAEL is determined rarely, if ever, represents the range of diversity present in the human population at large. This uncertainty factor should encompass variability from a wide array of sources: inherited traits and genetic polymorphisms, gender and hormonal differences, lifestyle factors such as amount of stress, exercise, tobacco and alcohol use, obesity, nutritional status, and finally, disease conditions, some of which can alter the handling of xenobiotics. Related to this last factor is the ingestion of prescription and over-the-counter medications, many of which can alter the pharmacokinetics of other chemicals. Another circumstance that this intersubject uncertainty factor is asked to cover is age, encompassing a range from prenatal to elderly.

As mentioned above, the noncancer uncertainty factor is 10, which can be understood to consist of a half-log factor (3.16-fold) for toxicokinetic variability and a similar half-log factor for pharmacodynamic variability (Renwick 1998). Thus, the interindividual variability in toxicokinetics created by the various genetic, lifestyle, physiologic state, and age factors must fit within a 3.2-fold factor for the default to be adequate. In other words, one must determine whether the upper or lower ends of the children's distribution (e.g., low-end metabolizers) are captured within the bounds created by the adult central tendency value plus or minus a 3.2-fold uncertainty factor. If not, then a special children's uncertainty factor or other more quantitative approaches to characterize variability might be needed.

It is important to point out that a similar uncertainty factor does not exist for cancer risk assessment. These assessments are generally considered conservative because of the nature of the linear low-dose modeling often used and because interspecies scaling of the unit risk factor employs a generic body weight function that increases human potency relative to that of rodents (Andersen et al. 1995; U.S. EPA 1992). However, the lack of any special consideration of children's risk in cancer assessments leaves open the question of whether the derived unit risks are appropriate for all age groups. In fact, one recent cancer risk assessment did adjust the unit risk for child-specific factors, but this adjustment was not based on toxicokinetics (U.S. EPA 2000).

Thus, in the absence of precedents for using a children's toxicokinetic analysis to adjust cancer or noncancer assessments, a framework is needed for doing this in a rational and consistent manner. The hope is that such a process will foster an understanding of the size of adult/child differences in key toxicokinetic pathways relative to the amount of interindividual variability we have come to expect in noncancer assessments.

Phase II: Data Analysis and Development of Risk Assessment Approach

In this phase, chemical-specific data regarding the fate of the chemical(s) being analyzed are combined with children's data relating to the developmental profile of toxicokinetic systems (from *in utero* periods through adolescence). This will foster an understanding of how children of various ages are likely to handle the chemical(s). Obviously, the best case is to have metabolism and disposition data for the chemical(s) in children. However, it will be extremely rare to find such toxicokinetic data. Following are some approaches to consider for filling these data gaps:

Use of toxicokinetic data for the chemical(s) under analysis to characterize the major fate and transport mechanisms. Typically, animal data, and in some cases, adult human data also, will be available for

characterizing the chemical's fate and disposition. Once the major toxicokinetic factors governing chemical fate are known, then the approaches that follow can be evaluated for utility and feasibility.

Use of surrogate chemicals (typically therapeutic agents) for which pharmacokinetic data in children exist. Even if the target chemical(s) has not been evaluated in children, a chemical that is similarly processed may have been tested. Information for surrogate chemicals can help delineate the maturation of key pathways and how the target chemical will be handled at certain developmental stages. This section describes the kinds of data sets and resources available for this part of the phase II assessment.

Over the past several years children's pharmacokinetic databases have been developed in which therapeutic drugs tested in both children and adults are identified and key pharmacokinetic parameters are compared across ages (Ginsberg et al. 2002; Renwick 1998; Renwick et al. 2000). The metabolism and clearance pathways of many of the drugs are known, making them useful indicators for particular pathways. For example, dextromethorphan and debrisoquine are known substrates for a particular cytochrome P450 (CYP) enzyme, CYP2D6; trimethadione, chlorzoxazone, and halothane are markers for CYP2E1 activity; morphine is predominantly processed by glucuronidation; and a host of antibiotics are not extensively metabolized but are mostly excreted unchanged by the kidneys (Bertz and Granneman 1997; Dollery 2000; Kurata et al. 1998; Tanaka 1998).

A well-known example of child–adult differences in metabolic processing is caffeine, a process in which an initial *N*-demethylation reaction is catalyzed by CYP1A2, and a secondary *N*-acetylation step is catalyzed by *N*-acetyltransferase (Welfare et al. 2000). The CYP1A2 reaction is the primary factor governing the half-life of this drug. Caffeine's half-life in newborns is 14-fold higher than in adults, which is likely the result of the immaturity of CYP1A2 at this age (Dorne et al. 2001; Ginsberg et al. 2002; Parsons et al. 1976; Parsons and Neims 1978). The metabolic clearance of caffeine and another CYP1A2 substrate, theophylline, becomes more like the adult rate by 2–6 months of age, after which time the half-life becomes significantly shorter than in that in adults (Dorne et al. 2001; Ginsberg et al. 2002). This suggests that once CYP1A2 function approaches adult levels, the greater size and blood flow to the liver (per body weight) in young children can lead to greater enzymatic clearance of CYP substrates (Gibbs et al. 1997). However, this also means that chemicals activated to toxicants by this pathway [e.g., aromatic amines, polycyclic aromatic hydrocarbons (PAHs), aflatoxin B1] may produce more active metabolite in this age group compared with adults. Unfortunately, there are no data that can be used to directly evaluate this possibility.

This drug has also been used to show that the *N*-acetyltransferase step is also deficient in early life. Once caffeine is demethylated by CYP1A2, it can be *N*-acetylated to form 5-acetylamino-6-formylamino-3-methyluracil (AFMU) or excreted in urine in the nonacetylated form (1-methylxanthine). The ratio of AFMU to 1-methylxanthine has been used to phenotype the *N*-acetyltransferase trait in adults and children. In children the ratio of acetylated to nonacetylated metabolite is low in newborns through 2 months of age (12% of adult ratio), but then rises to 65% of the adult ratio during the 2- to 6-month interval (Pariente-Khayat et al. 1991). This agrees with data showing that most newborns are slow acetylators but that beyond 100 days, the underlying genetic polymorphism (fast vs. slow) becomes evident (Pons et al. 1989; Szorady et al. 1987). Such data with caffeine are examples of the information that can be obtained from pharmacokinetic data for therapeutic agents.

Table 1 is a compilation of information obtained from *in vivo* pharmacokinetic analyses of drugs with *in vitro* analyses of enzyme levels from blood or liver samples. The combination of the two types of information for a given clearance pathway can provide a strong indication of how the pathway's function develops in the postnatal period. *In vivo* pharmacokinetic data have been analyzed across chemicals that share a common mode of elimination in order to develop a more complete evaluation of the function of specific pathways (Ginsberg et al. 2002; Renwick et al. 2000). Table 1 shows data for six CYP enzymes, several phase II conjugation pathways, renal and biliary clearance, other metabolic functions (e.g., epoxide hydrolase, alcohol dehydrogenase), and two esterases involved in the detoxification of organophosphate (OP) pesticides. This compilation of pharmacokinetic data by pathway and age group indicates a fairly consistent pattern, that is, premature neonates, full-term neonates, and infants up to 6 months of age tend to have less metabolic and clearance capacity than adults. The two exceptions in the chart are for enzymes expressed primarily in the fetal and early postnatal period: CYP3A7 and glutathione *S*-transferase (GST) pi. These fetal forms are replaced during the first year of life by corresponding (but not enzymatically equivalent) adult forms. Beyond 6 months, many CYP enzymes are sufficiently active that clearance *in vivo* is actually greater than that in adults. This appears to be due to the greater liver size and blood flow in children compared with adults (Gibbs et al. 1997). Table 1 provides further evidence for this phenomenon in that the *in vivo* data indicate greater clearance capacity than suggested by the *in vitro* protein levels or enzyme activities for CYP1A2, CYP2E1, CYP2C9/19, CYP2D6, and CYP3A4, particularly at 6 months of age.

These overall patterns are illustrated for a specific CYP, CYP3A4, as evidenced by therapeutic drugs that depend upon this CYP for clearance (Figure 2) (Ginsberg et al. 2002). CYP3A4 is the major CYP in adult human liver, but its function is evidently deficient in early life. This pattern appears to be widely applicable; an analysis across 40 therapeutic drugs involving 11 different metabolic/excretory mechanisms shows a similar shift from immaturity in the earliest age categories to evidence of drug half-lives shorter than adults beyond 6 months of age (Figure 3).

The period of shorter half-lives in children relative to adults may represent a time in which there is faster removal of parent compound but greater formation of metabolites, which can be a concern if chemical metabolism leads to more toxic moieties. However, this may not lead to greater toxicity if the metabolites are also cleared more readily in children. Prediction of the health implications of shifts in xenobiotic metabolism must be chemical specific and must consider both parent compound and toxic metabolites.

The period of accelerated clearance is in contrast to the earlier periods in which hepatic metabolism is slower than that in adults, leading to the potential for prolonged retention and higher levels of parent compound. This situation may be compounded by the slower renal and biliary function at these times. In terms of detoxification systems, Table 1 shows that epoxide hydrolase is active at birth but apparently only at 50% of adult function. Although the data are very limited, it appears that two forms of GST may be deficient (40–60% of adult levels) in early life. These data may have risk implications, given that GSTmu is critical to epoxide conjugation with glutathione and that epoxide hydrolase also appears to be slow in this age group. Thus, the newborn and very young infant may have a relative deficit in detoxifying epoxides, although compensating mechanisms (e.g., other GSTs) may exist. It should be noted that further research into the developmental profile of these detoxification systems is an important research need.

Another potentially important deficit in children is glucuronidation during the first 2 months of life. Because of the relative deficiency of glucuronide and high bilirubin levels (an endogenous glucuronidation substrate), it is possible that there will be greater internal exposure early in life to xenobiotics (or their metabolites) that require glucuronide conjugation. This is the case with the antibacterial drug chloramphenicol, in which a relative lack of glucuronidation contributed to its accumulation and toxicity (anemia) in infants (Mulhall et al. 1983; Vest 1965). In

Table 1. Postnatal developmental profile of pharmacokinetic functions.

Pharmacokinetics pathway	Premature neonates	Full-term neonates	1 Week–2 months	2–6 Months	6 Months–1 year	1–2 Years	> 2 Years
Renal clearance		35% - *in vivo* $t_{1/2}$ for 7 drugs[a] GFR ~20% adult[b]	28% - *in vivo* $t_{1/2}$ for 7 drugs[a]	88% - *in vivo* $t_{1/2}$ for 7 drugs[a] GFR ~100% adult[b]	137% - *in vivo* $t_{1/2}$ for 7 drugs[a]	137% - *in vivo* $t_{1/2}$ for 7 drugs[a]	185% - *in vivo* $t_{1/2}$ for 7 drugs[a]
CYP1A1	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
CYP1A2		2% - PL[e] 2% - *in vitro* EA[f] 11% - *in vivo* $t_{1/2}$ for 2 drugs[d]	4% - PL[e] 3% - *in vitro* EA[f] 4% - *in vitro* EA[g] 23% - *in vivo* $t_{1/2}$ for 2 drugs[d]	16% - PL[e] 9% - *in vitro* EA[f] 13% - *in vitro* EA[g] 81% - *in vivo* $t_{1/2}$ for 2 drugs[d]	25% - PL[e] 15% - *in vitro* EA[f] 13% - *in vitro* EA[g] 175% - *in vivo* $t_{1/2}$ for 2 drugs[d]	175% - *in vivo* $t_{1/2}$ for 2 drugs[a]	54% - PL[e] 35% - *in vitro* EA[f] 185% - *in vivo* $t_{1/2}$ for 2 drugs[d]
CYP2A6		20% - Nicotine $t_{1/2}$[ff]					
CYP2E1		13% - PL[h] 27% - *in vitro* EA[i] 27% - Serum DMO/TMO[j]	22% - PL[h] 39% - *in vitro* EA[i] 27% - *in vivo* serum DMO/TMO[j]	30% - PL[h] 47% - *in vitro* EA[i]	36% - PL[h] 41% - *in vitro* EA[i] 97% - Serum DMO/TMO[j]	92% - *in vivo* serum DMO/TMO[c]	82% - PL[h] 83% - *in vitro* EA[b] 92% - Serum DMO/TMO[c]
CYP2C9/19	21% - PL[k] 33% - *in vitro* EA[l]	30% - *in vivo* $t_{1/2}$ for 1 drug[m]	29% - PL[k] 30% - *in vitro* EA[l]	38% - PL[k] 45% - *in vitro* EA[l]	36% - PL[k] 83% - *in vitro* EAI 182% - *in vivo* $t_{1/2}$ for 1 drug[m]	36% - PL[k] 83% - *in vitro* EA[l] 182% - *in vivo* $t_{1/2}$ for 1 drug[m]	130% - *in vivo* $t_{1/2}$ for 1 drug[m]
CYP2D6		13% - PL[n]	22% - PL[n]	34% - PL[n]	45% - PL[n]		88% - PL[n]
CYP3A4	19% - *in vivo* $t_{1/2}$ for 8 drugs[o]	17% - *in vitro* EA[p] 50% - *in vivo* $t_{1/2}$ for 8 drugs[o]	29% - *in vitro* EA[p] 55% - *in vivo* $t_{1/2}$ for 8 drugs[o]	37% - *in vitro* EA[p]	46% - *in vitro* EA[p] 200% - *in vivo* $t_{1/2}$ for 8 drugs[o]	110% - *in vitro* EA[p] 200% - *in vivo* $t_{1/2}$ for 8 drugs[o]	189% - *in vivo* $t_{1/2}$ for 8 drugs[o]
CYP3A7		1100% - *in vitro* EA[q]	600% - *in vitro* EA[q]	300% - *in vitro* EA[q]	200% - *in vitro* EA[q]		
Epoxide hydrolase		42% - PL[r] 50% - CBZ-E/CBZ[s]	50% - CBZ-E/CBZ[s]	50% - CBZ-E/CBZ[s]	50% - CBZ-E/CBZ[s]	50% - CBZ-E/CBZ[s]	65% - CBZ-E/CBZ[s]
Glucuronidation	23% - *in vivo* $t_{1/2}$ for 6 drugs[t]	34% - *in vivo* $t_{1/2}$ for 6 drugs[t]	47% - *in vivo* $t_{1/2}$ for 6 drugs[t]	102% - *in vivo* $t_{1/2}$ for 6 drugs[t]	84% - *in vivo* $t_{1/2}$ for 6 drugs[t]	84% - *in vivo* $t_{1/2}$ for 6 drugs[t]	74% - *in vivo* $t_{1/2}$ for 6 drugs[t]
Sulfation/glucuronidation		84% - APAP clearance[u]		149% - APAP clearance[u]		70% - APAP clearance[u]	
Acetylation		83% slow phenotype[v]	12% - *in vivo* caffeine *N*-acetylation[w]	65% - *in vivo* caffeine *N*-acetylation[w]			48% slow phenotype[v]
GSH transferase		$GST\alpha_{B1}$ ~ 100% - PL[x] $GST\alpha_{B2}$ ~ 40% - PL[x] GSTmu ~ 60% - PL[x] GSTpi >2100% - PL[x]	$GST\alpha_{B1}$ ~ 100% - PL[x] $GST\alpha_{B2}$ ~ 40% - PL[x] GSTmu ~ 60% - PL[x] GSTpi >2100% - PL[x]	$GST\alpha_{B1}$ ~ 100% - PL[x] $GST\alpha_{B2}$ ~ 40% - PL[x] GSTmu ~ 60% - PL[x] GSTpi >2100% - PL[x]	$GST\alpha_{B1}$ 112% -PL[x] $GST\alpha_{B2}$ 62% - PL[x] GSTmu 93% - PL[x] GSTpi 2100% - PL[x]		121% - *in vivo* $t_{1/2}$ of busulfan[y]
Biliary excretion	57% - BSP clear[z]	70% - BSP clear[z]	90% - BSP clear[z]				
Serum protein	Few binding sites[aa]	Few binding sites[aa]	Few binding sites[aa]	Increasing # sites[aa]	Increasing # sites[aa]	Adult level[aa]	Adult level[aa]
Serum cholinesterase	50% - EA[bb]	57% - EA[bb]	101% - EA[bb]	91% - EA[bb]	104% - EA[bb]		
Serum arylesterase		28% - EA[cc]	34% - EA[cc]	52% - EA[cc]	84% - EA[cc]	80% - EA[cc]	
Alcohol dehydrogenase		55% - *in vivo* alcohol clearance[dd]	15% - *in vitro* EA[ee]		32% - *in vitro* EA[ee]		91% - *in vitro* EA[ee]

Abbreviations: APAP, acetaminophen; BSP, bromosulfophthalein; CBZ, carbamazepine; CBZ-E, carbamazepine-epoxide; DMO, dimethadione; EA, enzyme activity; EH, epoxide hydrolase; GFR, glomerular filtration rate; PL, protein level; TMO, trimethadione.

Pathway function is shown as % adult. [a]Ginsberg et al. (2002) analyzed renally cleared drugs: ampicillin, cimetadine, furosemide, piperacillin, ticarcillin, tobramycin, vancomycin. [b]GFR in mL/min/kg (Besunder et al. 1988; Morselli 1989). [c]CYP1A1 protein or enzyme activity was not detectable in microsomes from liver bank samples at any age, indicating very low constitutive levels (Sonnier and Cresteil 1998). [d]Ginsberg et al. (2002) analysis of drugs cleared primarily via CYP1A2: caffeine, theophylline. [e]Sonnier and Cresteil (1998) measured CYP1A2 protein levels via immunochemical methods in microsomes from liver bank samples (n = 6–23 per age group). [f]Sonnier and Cresteil (1998) measured CYP1A2 activity in microsomes from liver bank samples using methoxyresorufin as substrate (n = 6–23 per age group). [g]Cazeneuve et al. (1994) measured CYP1A2 activity in microsomes from liver bank samples via caffeine *N*-demethylation. [h]Vieira et al. (1996) measured CYP2E1 protein levels in liver bank microsomes by immunochemical means (n = 4–23 per age group). [i]Vieira et al. (1996) measured CYP2E1 activity levels in liver bank microsomes using chlorzoxazone as substrate (n = 2 for group 1–10 years of age; otherwise, n = 9–21 per age group). [j]Tanaka (1998) and Nakamura et al. (1998) report the ratio of oxidized metabolite DMO to parent drug TMO in serum of children dosed at the following ages: < 4 weeks (n = 5); 1 month–1 year (n = 5); 1–10 years (n = 21); adult (n = 20). CYP2E1 converts TMO to DMO. [k]Treluyer et al. (1996) measured CYP2C protein levels in microsomal preparations from liver bank samples with immunochemical techniques. [l]Treluyer et al. (1996) measured CYP2C19 enzyme activity in microsomal preparations from liver bank samples using diazepam as substrate. [m]Ginsberg et al. (2002) analyzed tolbutamide, a drug cleared primarily via CYP2C. [n]Treluyer et al. (1991) measured CYP2D6 protein levels in microsomal preparations from liver bank samples with immunochemical techniques. [o]Ginsberg et al. (2002) analyzed drugs cleared primarily by CYP3A: alfentanil, carbamazepine, fentanyl, lidocaine, midazolam, nifedipine, quinidine, triazolam. [p]LaCroix et al. (1997) measured CYP3A4 EA in microsomes from liver bank samples using testosterone as substrate (n = 12 newborns; 9 at 1 week–1 month; 13 at 1–3 months; 11 at 3–12 months; 2 at > 1 year; 11 adults). [q]LaCroix et al. (1997) measured CYP3A7 EA in microsomes from liver bank samples using dehydroepiandrosterone as substrate (n = 12 newborns; 8 at 1 week–1 month; 20 at 1–3 months; 14 at 3–12 months, 0 at > 1 year; 12 adults). [r]Ratanasavanh et al. (1991) measured epoxide hydrolase protein levels by Western blot on liver bank microsomal preparations (n = 5 for 1 day–1 week of age; n = 5 for adults). [s]EH levels were estimated by CBZ-E to CBZ ratio in blood in different age groups at steady state after repeat drug administration in epileptic patients. Data were pooled across 2 studies (Korinthenberg et al. 1994; Pyonnonen et al. 1977). Higher CBZ-E/CBZ ratios in children are indicative of slower EH activity, as CBZ-E formation and CBZ clearance is slower in young children (Eichelbaum et al. 1975, 1985; Kuhnz et al. 1983; Ohmori et al. 1998). [t]Ginsberg et al. (2002) analysis of drugs cleared directly via glucuronidation: lorazepam, morphine, oxazepam, trichloroethanol, valproic acid, zidovudine. [u]Ginsberg et al. (2002) compilation of kinetic data for APAP across 5 five studies; n = 7–24 per age group. APAP is a substrate for various conjugation reactions, with sulfation predominating in early life (Besunder et al. 1988; Levy et al. 1975). [v]Pariente-Khayat et al. (1991) measured the ratio of acetylated to nonacetylated metabolite in urine after caffeine administration in children (n = 10–26). Younger age groups were compared against an older age group (6 months–2 years), as adult data were not available and onset of rapid phenotype occurs by this age. [w]Szorady et al. (1987) phenotyped 100 newborns 2–3 days old as well as 100 or more subjects in older age groups, using acetylation of sulfadimidine (as appears in urine). [x]Strange et al. (1989) measured GST protein levels by radioimmunoassay in liver bank tissue cytosols. $GST\alpha_{B1}$ levels were 70% of adult *in utero* and 112% of adult at 5–10 months postnatal. Therefore, the estimate is that newborn through 6-month levels would be approximately equal to adult. $GST\alpha_{B2}$ protein levels were 25% of adult during the *in utero* period and 62% of adult at 5–10 months postnatal. Therefore, the estimate is that newborn through 6-month levels are 40% of adult. GSTmu is 22% of adult during *in utero* period and 93% of adult at 5–10 months. Therefore, the estimate is that newborn through 6-month levels are 60% of adults. GSTpi is 5300% of adult during *in utero* period and 2100% of adult at 5–10 months. [y]Gibbs et al. (1997) measured busulfan half-life in a group of 14 children (average age = 3 years) relative to adults. Busulfan is metabolized predominately by $GST\alpha_{B1}$. [z]Jusko (1972) measured clearance of BSP in groups of 5 or more children; values in young children were compared against an older child group (3 months–14 years of age), as adult data were not available. [aa]Serum protein binding sites (albumin, α_1-acid glycoprotein) are low in newborns but increase to adult levels by 1 year (Besunder et al. 1988). [bb]Data were pooled across 4 studies (Augustinsson and Barr 1963; Ecobichon and Stephens 1972; Lehmann et al. 1957; Zsigmond and Downs 1971) in which serum cholinesterase levels were measured with acetylcholine, benzoylcholine, butyrylcholine, or procaine as substrate. [cc]Augustinsson and Barr (1963) and Ecobichon and Stephens (1972) measured serum arylesterase activity with phenyl acetate as substrate. [dd]Idanpaan-Heikkila et al. (1972) followed the removal of ethanol from mother and newborn blood (n = 6 for each age); newborns were exposed transplacentally and born with equal blood alcohol concentration as mother. [ee]Pikkarainen et al. (1967) measured alcohol dehydrogenase activity in liver samples from newborns 1 week–2 months (n = 2); infants 6 months–2 years (n = 2); older children 5–15 years (n = 3); and adults (n = 3). Data from 9 fetuses were approximately 25% of the newborn levels. [ff]Dempsey et al. (2000) meas-ured nicotine elimination from newborns (n = 5) exposed transplacentally.

contrast, some xenobiotics can undergo conjugation with alternative cofactors, which allows a shift in metabolic profile if the primary pathway is compromised or immature. This occurs with acetaminophen, where sulfation predominates early in life until glucuronidation takes over (Levy et al. 1975).

The findings described above are consistent with other pediatric pharmacokinetic summaries (Dorne et al. 2001; Renwick 1998, 2000) in which clearance in childhood is slower than in adults for a variety of drugs and age groups. There are also cases in which clearance is more rapid, particularly when the pediatric group was at least several months of age (Renwick 1998). This type of age-specific and pathway-specific pharmacokinetics information in children should prove useful in predicting how children (particularly neonates) may differ from adults in internal dosimetry and ultimate risk.

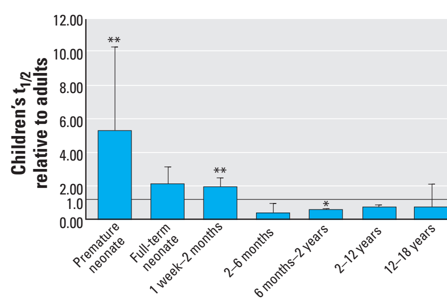

Figure 2. Analysis of children's pharmacokinetic database half-life results for CYP3A substrates—alfentanil, carbamazepine, fentanyl, lignocaine, midazolam, nifedipine, quinidine, and triazolam. Child/adult half-life ratios were derived by a composite analysis of the eight CYP3A drug substrates in the children's pharmacokinetic database. Data were organized into age categories shown. Figure reproduced from Ginsberg et al. (2002) with permission from Oxford University Press.
*$p < 0.1$; **$p < 0.05$.

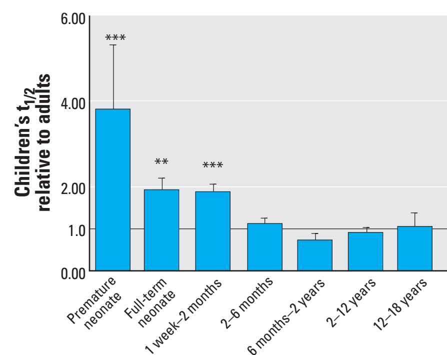

Figure 3. Analysis of children's pharmacokinetic database: half-life results for full database—40 substrates. Child/adult half-life ratios were derived by a composite analysis of the 40 drug substrates in the children's pharmacokinetic database containing half-life results. Data were organized into age categories shown. Figure reproduced from Ginsberg et al. (2002) with permission from Oxford University Press.
**$p < 0.01$; ***$p < 0.001$.

Simply knowing the function of particular pathways may not be sufficient to predict *in vivo* handling of a xenobiotic at a particular age. Numerous toxicokinetic factors are involved in chemical processing and clearance, including partitioning into body compartments, protein binding, respiration, and organ flows. Therefore, a more comprehensive analysis, such as PBPK modeling, may be needed to integrate the various factors at work and predict xenobiotic fate in children. The information provided in Table 1, combined with basic physiologic information, may make PBPK modeling of children more feasible. Where this is pursued, the uncertainty and data gaps surrounding input parameters for children must be recognized and made transparent.

Although characterization of pathway function on the basis of indicator drugs is a useful approach, it carries the uncertainty that a drug may be processed by several clearance pathways. If one pathway is deficient at a certain age, but another is more functional, then the overall half-life or clearance rate may not be affected. Instead, parent compound may be shunted from the less active to the more active pathway, leading to a shift in metabolite profile. The assignment of chemicals to particular pathways is based on the fate of the majority (generally 60% or more) of the administered dose in adult humans, as ascertained from the literature. Shifts in metabolic processing in early life would tend to obscure child versus adult clearance differences based on overall half-life or blood clearance. Thus, in some cases, the data in Table 1 and Figures 2 and 3 may be an underestimate of the degree of child–adult difference that might actually exist for a given pathway.

Another caveat for some of the *in vivo* data sets cited in Table 1 and Figures 2 and 3 is that they represent clinical pharmacokinetic trials in children who are not in full health. The data sets summarized in Ginsberg et al. (2002) were screened not only for sufficient numbers of subjects per age group but also for the health of the subjects on test. Data for children who were critically ill or severely compromised, especially with hepatic or renal conditions that would affect xenobiotic handling, were excluded from the database. However, it is possible that the clinical state of the children being tested may have affected factors such as volume of distribution or other pharmacokinetic factors in certain data sets. The overall concordance between *in vitro* and *in vivo* data shown in Table 1 suggests that this factor is not a systematic issue here.

Use of* in vitro *data to ascertain how toxicokinetic pathways change during development. The liver bank data describing CYP protein levels and enzyme activities, as well as the serum esterase data shown in Table 1, can be used as indicators of pathway function at particular ages. These data are made more reliable for risk assessment when there are corroborating *in vivo* half-life or clearance data for indicator chemicals. An example is CYP2E1 function, in which *in vitro* liver bank studies (Figure 4A,B) generally agree with *in vivo* drug metabolism data for the CYP2E1 substrate trimethadione (Figure 5) (Nakamura et al. 1998; Tanaka 1998; Vieira et al. 1996). These developmental profiles can be essential for estimating the degree to which CYP2E1-mediated metabolic activation may occur for a wide variety of environmental toxicants (e.g., benzene, chlorinated solvents, ethanol).

Use of developmental information to evaluate how toxicokinetics can affect susceptibility during the* in utero *period. The maternal–fetal environment presents unique toxicokinetic considerations. The developing organism is exposed as part of the maternal system via placental transport. Although maternal factors (distribution, metabolism, excretion) govern fetal exposure, exposure to the fetus can be quantitatively or qualitatively different from that in the mother. This may result from chemical accumulation in the fetal compartment (NRC (National Research Council) 2000) or from *in situ* metabolism that causes fetal exposure to metabolites different from that experienced in the maternal system. The following sections are a summary of the key toxicokinetic factors during *in utero* development.

Placental transport. Between the maternal system and conceptus is a specialized set of membranes that chemicals must cross. The placenta has its origins in the conceptus, with its unique anatomical and functional features influencing chemical transfer to the developing conceptus. This set of membranes undergoes considerable change with development and also exhibits substantial species differences. As pregnancy progresses, there is closer contact between the fetal blood and the syncytiotrophoblast—the placental cell layer most important to placental function and maternal–embryo/fetal exchange (Garbis-Berkvens and Peters 1987). This leads to greater exchange of nutrients and chemicals across the placenta as gestation proceeds. On the macroscopic level, the effects of gestational age can be exemplified by comparing the tremendous change in the ratio of placental/fetal weight (ratio equals 4 at 10 weeks and 0.2 at 40 weeks) (Hytten and Leitch 1971).

Any substance in the maternal circulation can be transferred, to some extent, across the placenta unless it is metabolized/eliminated before or during its placental passage (Garbis-Berkvens and Peters 1987; Slikker 1987). A useful method for characterizing placental transfer across chemicals and species is the dually perfused placenta. This is an *in vitro* preparation in which placental transfer is defined as a clearance ratio. Studies of non-metabolized model compounds indicate

decreases in placental transfer as the molecular weight of the model compounds increases (Illsley et al. 1985). Under these very controlled conditions without maternal or fetal involvement, differences in placental transfer between species are evident, with sheep being more different than guinea pig from human. These types of functional assessments help to predict placental transfer and fetal exposure after maternal dosing.

Additional factors that influence the placental transfer of chemicals are uterine/placental blood flow, placental permeability, and placental metabolism (Juchau 1980a, 1980b; Mihaly and Morgan 1983; Miller et al. 1976; Mirkin and Singh 1976; Waddell and Marlowe 1981). These factors are not static during pregnancy but may change as gestation progresses.

Chemical delivery to the developing conceptus relies primarily on blood flow to the placenta. Although chemicals may transfer from mother to fetus via the amniotic fluid after crossing the amnion, the majority of agents gain access to the conceptus via placental passage (Nau and Liddiard 1978). In addition to the changes in placental blood flow that occur during gestation, changes in blood flow as a result of chemical exposure must also be considered. Although experimentally induced changes in placental blood flow can alter normal development of the conceptus (Barr and Brent 1978; Greiss and Gobble 1967; Millicovsky and DeSesso 1980), the effect of such perturbations on placental transport of xenobiotics needs to be evaluated.

Placental permeability to a chemical is influenced both by placental characteristics (e.g., thickness, surface area, carrier systems, and lipid/protein content of the membranes) and also by characteristics of the chemical agent (e.g., degree of ionization, lipid solubility, protein binding, and molecular weight) (Mihaly and Morgan 1983; Mirkin and Singh 1976; Welsch 1982). The rate of placental transfer is rapid for nonionized, lipid-soluble chemicals of low molecular weight (less than 1,000 daltons) and is largely controlled by placental blood flow (Mihaly and Morgan 1983; Mirkin and Singh 1976). However, charged molecules such as tubocurarine can also enter the fetus (Kivalo and Saarikoski 1972, 1976). Similarily, chemicals highly ionized at normal blood pH, such as the salicylates, readily cross the placenta (Wilson et al. 1977). The question is thus not whether a compound crosses the placenta but rather at what rate.

Maternal considerations. A chemical must pass through and may interact with several anatomical compartments on its journey from the site of maternal exposure to the organ systems of the developing conceptus. Maternal factors act to either enhance or diminish the concentration of an active chemical in the conceptus. Maternal detoxification decreases the amount of parent compound available for placental transport, whereas maternal bioactivation may make more active chemical available. The entire set of maternal toxicokinetic factors (e.g., absorption, distribution, serum binding, and elimination) also influences the concentration of active agent at the target site. Because of the physiologic changes that occur during pregnancy, the influence of these maternal factors on chemical delivery may also change during gestation (Bogaert and Thiery 1983; Cummings 1983; Juchau 1995; Juchau and Faustman-Watts 1983; Noschel et al. 1980). These issues have been reviewed (Brock-Utne et al. 1980; Juchau and Faustman-Watts 1983; Krauer et al. 1980; Levy 1981; Slikker and Miller 1994).

For chemicals that bind avidly to plasma proteins, this factor can retard placental transfer. In general, only the free drug crosses the membranes of the placenta (Krauer et al. 1980; Welsch 1982). Protein binding is usually reversible, and there are a finite number of binding sites. Thus, binding is saturable, and equilibrium may be described by the law of mass action (Krauer et al. 1980; Miller et al. 1976). As long as binding is reversible, it does not prevent the chemical from crossing membranes but only slows the rate at which the transfer occurs (Levine 1973).

Placental biotransformation. This may be the most critical factor in influencing the delivery of chemicals to the developing conceptus. Placental biotransformation of a chemical prior to fetal delivery may dramatically alter the chemical profile in the conceptus from that in the mother (Slikker et al. 1982). Equilibrium factors, which influence the rate of placental transfer, can result in quantitative differences of exposure. Placental metabolism, however, can qualitatively alter the exposure of the conceptus to potentially toxic chemicals. Placental metabolism is less well characterized than hepatic metabolism, but existing data suggest that the placenta has considerably less metabolic capacity than adult liver (Juchau 1980b; Mirkin and Singh 1976). A listing of some of the human placental xenobiotic and hormone-metabolizing enzymes or isoenzymes is presented in Table 2.

Embryo/fetal considerations. As with most organ systems, the various chemical-metabolizing systems undergo quantitative if not qualitative changes during development.

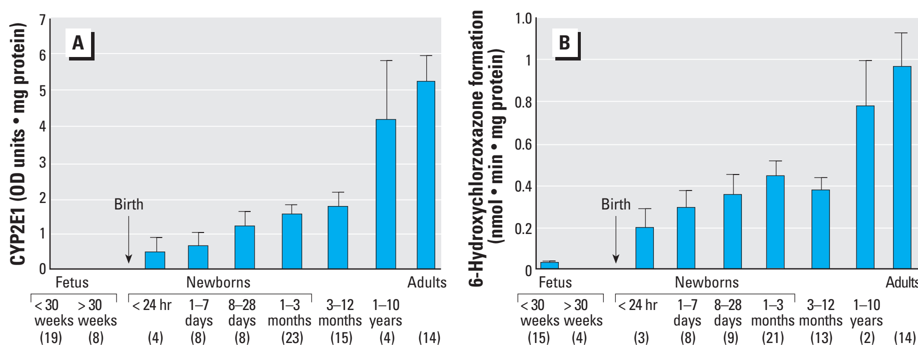

Figure 4. (*A*) Age-related variations of CYP2E1 protein in the human liver. Microsomal proteins (60 µg) were separated on a 9% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nylon membranes. CYP2E1 was immunochemically detected with a polyclonal antibody raised against rat ortholog CYP2E1; the antigen–antibody complex was visualized after addition of peroxidase-conjugated anti-(rabbit IgG) antibody using 4-chloro-1-naphthol as the dye and quantified by scanning with an image analysis system. Results are expressed as the mean ± SE of densitometric values of immunochemically detected CYP2E1 per milligram protein. Optical density (OD) units are an arbitrary measure of the density. Number per group is indicated between brackets. (*B*) Age-related variations of chlorzoxazone hydroxylation in human liver. Microsomal samples (0.3 nmol P450) were incubated with 500 µM chlorzoxazone and an NADPH-generating system. The formation of the 6-hydroxylated metabolite was monitored at 287 nm after separation by high performance liquid chromatography. Results are expressed as the mean ± SE of activity measured as rate of formation of 6-hydroxychlorzoxazone per mg microsomal protein. Number of samples in each group is indicated between parentheses. Figure reproduced from Vieira et al. (1996) with permission from Blackwell Publishing.

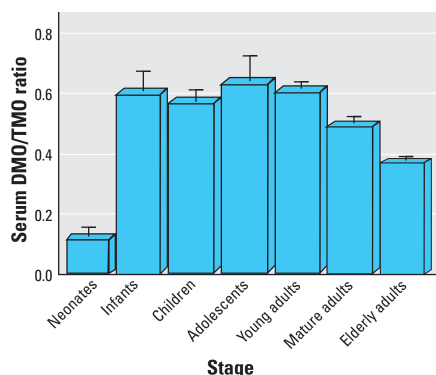

Figure 5. Age-related changes over seven stages of life in the serum dimethadione (DMO)/trimethadione (TMO) ratio 4 hr after oral administration of TMO (4 mg/kg). The seven stages were neonates (< 4 weeks, $n = 5$), infants (< 12 months, $n = 12$), children (< 10 years, $n = 21$), adolescents (< 20 years, $n = 3$), young adults (< 40 years, $n = 20$), mature adults (< 65 years, $n = 20$), and elderly adults (> 65 years, $n = 40$). Mean ± SE. Figure reproduced from Tanaka (1998) with permission from Blackwell Publishing.

Chemical biotransformation by the developing conceptus has been extensively reviewed (Dutton 1978; Dutton and Leakey 1981; Dvorchik 1981; Eltom et al. 1993; Juchau and Faustman-Watts 1983; Leakey 1983; Nau and Neubert 1978; Neims et al. 1976; Pelkonen 1977, 1980; Rane and Tomson 1980; Slikker 1987, 1994; Slikker and Miller 1994). Despite that data have been collected using a variety of techniques and some data gaps exist because of technical or ethical reasons, several general conclusions may be drawn from the literature.

- During prenatal development, the activities of most enzymes that catalyze phases I and II reactions are lower than those in adults.
- As in the adult, the conceptus exhibits substrate specificity in its ability to metabolize chemicals, suggesting the existence of several sets of enzymes or isozymes, which may or may not be the same as those in the adult.
- These enzyme systems may be inhibited or induced by maternal pretreatment with a variety of chemicals.
- Enzyme activity generally increases with gestational age.
- The ontogeny of each enzyme may be different, and the controlling mechanisms of maturation of enzyme activity are incompletely understood.
- Prenatal human and nonhuman primates exhibit higher levels of many metabolizing enzymes (especially P450s) than do commonly used laboratory species.
- As in the adult, the liver of the conceptus appears to have the greatest capacity for chemical metabolism. The fetal adrenal, kidney, lung and brain also exhibit metabolic capabilities.

Table 3 summarizes recent data concerning some CYP isozymes and their occurrence in human fetal tissues.

Fetal distribution and elimination. The majority of chemicals entering the fetal circulation do so via the umbilical vein after passage through the placenta. A portion of the blood flow entering the liver of the conceptus is shunted via the ductus venosus directly to the inferior vena cava and to the heart for total body distribution (Rudolph and Heymann 1967). The remaining umbilical flow enters hepatic tissue and exits to the vena cava via the portal vein (Dawes 1968). There is potential for a significant first-pass effect from chemical passing through the fetal liver prior to other sites; however, a substantial fraction passes directly to the remaining tissues (Power and Longo 1975). As in the adult, approximately 16% of the fetal cardiac output is directed toward the fetal brain (Behrman et al. 1970).

Just as placental transfer of chemicals is the predominate pathway from the maternal system to the conceptus, placental transfer is also the predominate route for embryo/fetal elimination of chemicals. The same toxicokinetic rules apply for fetal-to-maternal transport across the placenta: chemicals that are nonionized and lipid soluble will diffuse across the placenta according to the concentration gradient from conceptus to mother. However, if a chemical has been conjugated by the fetus (e.g., glucuronidation, sulfation) or otherwise metabolized to a more polar form, the rate of return to the maternal circulation will be slower than that for the parent compound (Dancis et al. 1958; Goebelsmann et al. 1968, 1972; Levitz et al. 1960). PBPK models to predict the fate of toxicants in fetal tissues as a function of development are emerging (Kim et al. 1996; Sandberg et al. 1996).

Use of animal toxicokinetic information to evaluate how development can affect internal dosimetry. Laboratory animals can be useful in a number of respects in providing toxicokinetic data and related information for children's risk assessments of environmental pollutants (Harroff et al. 1997). The discussion here will be limited primarily to mice and rats, as most toxicokinetic and toxicology studies employ these two rodent species.

Diversity is one of the more daunting problems anticipated when studying children because of the numerous variability factors described earlier. Intersubject variability is substantially lower in homogeneous rodent populations provided by major animal suppliers. Uniform groups of animals of the same established genetic and husbandry backgrounds can be maintained under defined and carefully controlled conditions. Therefore, it is possible with animal studies to control more variables and to better focus on age-dependent differences in chemical metabolism and disposition.

Animal toxicokinetics and susceptibility to xenobiotics. Series of studies of OPs in rats have clearly shown that the relatively low detoxification capacity of weanlings places them at increased risk of acute, high-dose OP poisoning. Benke and Murphy (1975) concluded that the lower acute toxicity of parathion and methyl parathion in adult rats was due to the adults' greater detoxification capacity. More recent studies have confirmed and extended these findings. The maturational profiles of P450-catalyzed desulfuration (activation) and dearylation (inactivation) as well as carboxylesterase and arylesterase (inactivation) activities have been characterized in the liver and/or blood of rats (Atterberry et al. 1997; Moser et al. 1998). Despite very low carboxylesterase and arylesterase activities, rat fetuses and sucklings appear to be protected from chlorpyrifos because of limited transfer of the compound from the mother (Mattsson et al. 2000). However, this protective effect would not be expected if neonates were exposed directly (i.e., not via the mother) to OPs. On the basis of somewhat anecdotal information of groups of people poisoned by these pesticides, the suggestion is that young

Table 2. Partial list of human placental xenobiotic- and hormone-metabolizing enzymes or isoenzymes.[a]

Phase	Type	Reaction (gene)	Substrate	Constitutive	Inducer	Inhibitor
I	MFO	*O*-Deethylase (*CYP1A1*)	7-Ethoxycoumarin	(+)	Cigarette smoke	Aminoglutethimide
I	MFO	Aryl hydrocarbon hydroxylase (*CYP1A1*)	PAH	(?)	Cigarette smoke	Alpha-naphthoflavone
I	MFO	Hydroxylase (*CYP3A7*)	Cortisol	+	–	–
I	MFO	Aromatase (*CYP19*)	Androgens	(+)	–	Aminoglutethimide
I	MFO	Cholesterol side-chain cleavage (*CYP11A*)	Cholesterol	(+)	–	Aminoglutethimide
I	MFO	Estrogen catechol formation, 2-Hydroxylation (*CYP1A1*) 4-Hydroxylation (*CYP1B1*)	Estrogens	(+)	Cigarette smoke	–
I	MFO	25-Hydroxycholecalciferol hydroxylase	25-Hydroxycholecalciferol		–	–
I	Oxidoreductase	17β-Hydroxydehydrogenase		(+)	–	16-Methylene estradiol
		Type 1	Estrone to estradiol			
		Type 2	Estradiol to estrone			
I	Oxidoreductase	11β-Hydroxydehydrogenase	Cortisol/cortisone	(+)	–	–
I	Oxidation	Dehydrogenase	Alcohol/acetaldehyde	(+)	–	–
I	Oxidation	Monoamine	Norepinephrine	(+)	–	Monoamine oxidase inhibitors
II	Sulfatase	Sulfate cleavage	Steroid sulfates	(+)	–	–
II	Conjugation	GST	Epoxides	(+)	–	–
II	Conjugation	Catechol-*O*-methyltransferase	Catecholamines, catechol estrogens	(+)	–	–

Abbreviations: MFO, mixed-function oxidase; (+), the enzyme is constitutively expressed in human placenta; (?), it is unclear whether the enzyme is constitutively expressed; –, no known inducer or inhibitor for the placental enzyme. [a]Data modified from Slikker and Miller (1994), with additional data from Arcuri et al. (1999); Juchau (1995); Moghrabi et al. (1997); Rasheed et al. (1997); and Zusterzeel et al. (1999).

children (0–4 years of age) are at increased risk (Diggory et al. 1977; Zwiener and Ginsburg 1988). However, the exposure information is not detailed suffciently in these cases to confirm the sensitivity of any particular person or age group. Ecobichon and Stephens (1972) reported that blood arylesterase activity is relatively low in young children (1–2 years of age), with this group having pronounced intersubject variability. No more recent information could be located on the time course of maturation of arylesterase or related enzymes in human blood or liver.

Comparative studies show that neonatal rodents are frequently more susceptible to toxicants than adult animals, but such findings should be interpreted with caution when extrapolating to humans. Done (1964) and Goldenthal (1971) compiled the results of LD_{50} (median lethal dose) studies of several hundred chemicals in neonatal and mature rodents. The neonatal animals were more sensitive to many but not all the compounds. Almost all the age-dependent differences in LD_{50} values were less than an order of magnitude; indeed, most varied no more than 2- to 3-fold. More pronounced interage differences were seen for a few drugs, some of which (e.g., chloramphenicol, diazepam) are poorly metabolized and accumulate to toxic levels in human newborns. As full-term human newborns are more mature than their rodent counterparts with respect to liver metabolism, interage differences might be less pronounced in humans. However, maturation is much more rapid in rodents, such that even a few days of growth can result in marked disparity in chemical metabolism, disposition, and effects. Results from the Done (1964) acute toxicity compilation (Figure 6) suggest dramatic differences in sensitivity in early postnatal life. These types of examples from the animal literature may be excellent research opportunities for exploring the relative importance of toxicokinetic versus toxicodynamic mechanisms in determining susceptibility in early life. Although the animal research may be able to point out mechanisms of toxicokinetic susceptibility, extrapolation of temporal trends across species is difficult because organs and their associated functions mature at different rates in different species. This makes the choice of an appropriate animal model for children complex and cross-species extrapolations more uncertain than what we are accustomed to in adult risk assessments [Bruckner 2000; NAS (National Academy of Sciences) 1993].

Table 3. Some CYP isozymes and their occurrence in human tissues.[a]

CYP	Adult liver[b]	Adult brain[b]	Fetal liver[b]	Fetal brain[b]
1A1	+	+	+	
1A2	+++	–	–	
1B1	+		+	+
2A6	++		–	
2A7	+		–	
2B1/2B2	+	+		
2B6/2B7	+		–	
2C	+++		±	
2C8-19	+		+	+
2D6	+	+	±	
2E1	+	+	±	
2F1	–		–	
3A4	+++	+	±	
3A5	++		+	
3A7	+		+++	
4B1	–		–	

[a]Data modified from Carpenter et al. (1997); Chen et al. (1999); Farin and Omiecinski (1993); Hakkola et al. (1994, 1997, 1998a, 1995b); LaCroix et al. (1997); Maenpaa et al. (1993); Murray et al. (1992); Ravindranath and Boyd (1995); Slikker (1997); and Yang et al. (1995). [b]±, possibly present in small quantities, or mRNA observed but no protein confirmation; –, not detected; +, present in low levels; ++, present in moderate levels; +++, present in high levels.

Despite the dissimilarities in maturation mentioned previously, rodent studies can provide valuable information on mechanisms and specific immaturities that may be broadly applicable to infants and children. Heavy metals are a good example. It is widely recognized that dietary antigens, maternal antibodies, and other macromolecules may be absorbed through the immature gastrointestinal mucosa. Increased levels of relatively polar molecules in the cerebrospinal fluid of infants suggest that such compounds penetrate the blood–brain or blood–cerebral spinal fluid barrier more readily in infants than in children and adults (Adinolfi 1985; Dziegielewska et al. 2000). Therefore, it has been hypothesized that equivalent exposures of infants and adults to heavy metals such as mercury and lead will result in greater oral absorption and greater central nervous system deposition (and toxicity) in the infants. Kostial et al. (1978) demonstrated substantially greater gastrointestinal absorption and whole-body retention of lead, mercury, and cadmium by suckling rats than by adults. A similar phenomenon has been reported for lead in juvenile monkeys (Pounds et al. 1978) and human infants (Ziegler et al. 1978). Retention of intravenously injected lead was 8 times higher in the brains of suckling rats than in the brains of adults (Kostial et al. 1978). Brain retention of injected mercury was 19 times higher during the perinatal period. Rodents have been very useful in delineation of the toxicokinetic basis of age-dependent differences in heavy-metal toxicity, but subhuman primates are generally the animals of choice to examine toxicodynamic factors (Davis et al. 1990).

Relatively few *in vivo* data are available from kinetics studies of drugs or other chemicals in preadolescent animals because of technical difficulties in working with such small subjects and prior lack of interest in immature populations. Similarly, there is a paucity of information on the maturation of many physiologic processes in small animals. The maturation of hepatic xenobiotic metabolism in rats, in contrast, has been relatively well characterized (Imaoka et al. 1991; Renwick 1998; Watanabe et al. 1993). Although rat liver is immature at birth with respect to many metabolic functions, certain CYP functions, epoxide hydrolase, and glucuronidation function reach adult levels within the first week to 10 days. Other functions such as glutathione transferase and aryl hydrocarbon hydroxylase take longer to develop (Renwick 1998).

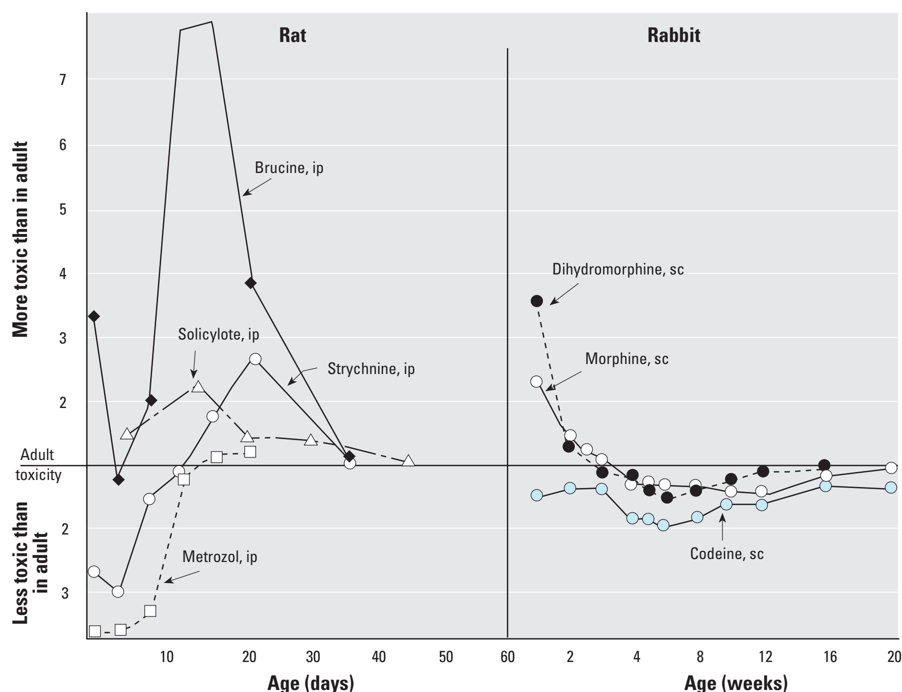

Figure 6. Changes in chemical susceptibility with age. Figure adapted from Done (1964).

Despite these known metabolic differences during development, findings of increased susceptibility in juvenile rodents have not been followed up to determine whether toxicokinetic mechanisms underlie these susceptibility differences. For example, Yoo et al. (1987) observed that CYP2E1-catalyzed metabolic activation of *N*-nitrosodimethylamine to a mutagen was substantially greater by hepatic microsomes of weanlings (25 days of age) than those of adult rats. Carbon tetrachloride, a chemical that also undergoes CYP2E1-catalyzed metabolic activation, was more hepatotoxic in 15-day-old than in 60-day-old male rats (Jahn et al. 1993). However, no accounts were located of animal studies relating susceptibility to injury by these CYP2E1 substrates to the time course of maturational changes in the chemical's metabolism.

Data gaps and challenges in applying animal data to children's toxicokinetic assessments. It is possible, under certain well-defined conditions, to make reasonable predictions of the disposition of drugs in adult humans on the basis of animal studies (Lin 1995). For example, the oral absorption of lipophilic compounds is relatively species independent, as is the volume of distribution of unbound drug (Fichtl et al. 1991; Lin 1995). Methods to extrapolate other parameters such as renal excretion of filtered drugs across species provide reasonable estimates for human pharmacokinetic function (Lin 1995). However, across species, differences in protein binding and drug metabolism can be substantial, leading to greater uncertainty in relying on pharmacokinetic data generated in animals (Nedelcheva and Gut 1994; Stevens et al. 1993). Nevertheless, metabolic clearance of low doses of flow-limited drugs should be less dependent on species-inherent rates of metabolism than on relative liver blood flow rates (Lin 1995). Thus, data from animal studies often can be useful in forecasting toxicokinetics in humans when their limitations are considered. However, this conclusion is based on comparison of adult animals with human adults. The development of toxicokinetic functions in early life is sufficiently different in rodents compared with humans to make direct extrapolation from juvenile animal studies difficult.

As mentioned above, one of the most promising approaches to quantitative estimation of internal doses of chemicals in children is PBPK modeling. Models have been developed to describe the disposition of a number of chemicals in pregnant rodents and their fetuses as well as in the lactating rat and nursing pup (Krishnan and Andersen 1998). Luecke et al. (1994) and Welsch et al. (1995) have adapted such models to human pregnancy to forecast potential teratogenic events. O'Flaherty developed PBPK models that accurately predicted time courses of lead in the blood and its deposition in bones of developing rats (O'Flaherty 1991) and children (O'Flaherty 1995). These models incorporated age-dependent changes in body weight, tissue volumes and blood flows, and bone formation and resorption rates. Pelekis et al. (2001) have made assumptions about distributions of physiologic and biochemical parameters in children and have estimated their effects on the disposition of volatile organic chemicals. Gentry et al. (2002) have modeled a variety of different chemicals across a wide range of ages by building functions into their model that adjust for metabolism and renal function changes during development.

Knowledge gained from PBPK modeling efforts with animals can be of significant benefit in the understanding of the kinetic behavior of chemicals in humans and in developing PBPK models for humans. Toxicokinetic studies in animals can yield a number of important parameters, including *in vivo* and *in vitro* partition coefficients, metabolic rate constants, and identity and stoichiometric yield of metabolites. Such chemical- and species-specific data are needed to construct an appropriate model. The model may be used to generate simulations of the time course of parent compound and/or metabolite(s) in blood, tissues, and urine. This information is often useful in design of the *in vivo* kinetics experiments needed to assess the accuracy of the model's predictions. When physiologic parameters are scaled allometrically to humans, the animal-based model becomes a starting point for making predictions regarding human pharmacokinetics. However, development of PBPK models for children would require extra effort to scale the model to the physiology and functional immaturity that occurs at specific developmental stages. Although it is tempting to use juvenile animal data to inform such children's PBPK modeling efforts, the across-species differences in physiologic and biochemical maturation add substantial uncertainty to this approach. Data in juvenile subhuman primates may be more promising because of their similarity to growing children (Conrad et al. 1995), but this would involve more expense.

Use of basic physiologic differences across age groups to ascertain how factors such as renal clearance, protein binding, and lipid partitioning may vary with age. Renal clearance and protein binding capacity are immature at early postnatal stages and develop over the course of the first 6 months to year of life (Table 1). Glomerular filtration rate as well as transporter (secretory) systems in the proximal convoluted tubule are deficient at birth (Kearns and Reed 1989; Morselli 1989), leading to relatively slow clearance of a wide array of antibiotics and other renally cleared drugs and metabolites. Compounding the chances for greater chemical effect in the first months of life is a deficient protein binding status due to lower levels of albumin (which binds weakly acidic chemicals) and alpha-1-glycoprotein (which binds basic chemicals). Another issue is the relative deficiency to conjugate and excrete bilirubin early in life, which leads to greater occupation of serum binding sites by bilirubin. These factors may contribute to a greater ratio of free compared with bound chemical, leading to a higher potential for uptake into tissues and toxic action. Diminished protein binding can also lead to shorter duration of action because of greater availability of chemical for metabolism and elimination. These factors would likely be important only for chemicals that exhibit a high degree of plasma protein binding (e.g., trichloroacetic acid).

Summary of phase II toxicokinetic analysis. As information in the areas described above is gathered and processed, we can evaluate from an internal dose perspective which age group(s) appears to be most exposed. Age groups that appear to differ most from adults can be prioritized for more detailed analysis as the process continues. The following questions may be helpful:

Does the pharmacokinetic profile shift at natural age breakpoints? An early step in analyzing children's toxicokinetic data is deciding whether and how to create bins across ages. The rapid development of children requires that the population be broken into relatively small age groups, particularly in the early postnatal period. If larger bins are used, the data may become highly variable, with age specificity becoming a causality of the desire to simplify the assessment. Thus, bins must be constrained to reflect key developmental stages. Nevertheless, it is useful to make them as large as possible, as this maximizes the number of subjects per group and enhances the power of cross-age comparisons. It should be noted that similar age binning may occur in the exposure and toxicodynamic areas to reflect critical changes in behavior or windows of heightened vulnerability. At some point the children's kineticist may be asked to adjust his bins to match those created in these other areas for the sake of harmony and to allow risk calculations for neatly defined age groups. Age bins were developed to organize the data shown in Table 1 and Figures 2 and 3. These bins are somewhat arbitrary and were based on the overall availability of data for a variety of pathways and chemicals. For specific chemicals and pathways, alternative bins can certainly be considered.

Which pharmacokinetic parameters are most important in determining internal dose? Although PBPK models aim for the simplest approach that yields predictive results, there are inevitably many parameters that must be estimated to run the model. These parameters include compartment size, partition coefficients, cardiac output, organ blood flows, metabolic rates, lung ventilation rates, and

urinary elimination rates. Because these models contain multiple tissue compartments and we may be dealing with several different age groups, there is a potentially large number of parameter values that would require elucidation. However, the model may not be equally sensitive to all the inputs, making those parameters that have the greatest influence on model performance the most important to calibrate properly. Efforts to simplify pharmacokinetic modeling in both adults and children have relied on a series of algebraic equations that highlight key pharmacokinetic parameters while not including others that may be model insensitive (Pelekis et al. 2001). Such approaches point out the importance of identifying those pharmacokinetic parameters that drive internal dose. Which pharmacokinetic factors are most influential will depend on the properties of the chemical under investigation. The clearance of chemicals with high hepatic extraction (rapid liver metabolism) will depend primarily on hepatic blood flow, whereas chemicals with low hepatic extraction depend most on the rate of liver metabolism relative to other competing rates (urinary elimination or exhalation of parent compound). To determine the focus of a children's toxicokinetic analysis, the most important toxicokinetic parameters in a validated adult model must be determined. Additional effort can then be put into identifying how these parameters change as different child age groups are considered. Ultimately, the fate of any chemical will be determined by the interaction of all pharmacokinetic factors acting simultaneously. This kind of sensitivity analysis will ensure that those parameters with the greatest influence are given the attention they warrant.

Regardless of whether one is conducting a full PBPK assessment across ages or a less rigorous approach, it is important to know how key parameters change with age. As discussed above, various data resources and analytic approaches may be used to determine how key parameter values differ between children and adults. Similar to Table 1, it may be helpful to develop a table of key physiologic and metabolic parameters that affect the fate of the chemical in question and list what is known about those pathways for the *in utero* and postnatal periods. If the parameter values are not materially different between children and adults or if it appears that certain factors negate one another, one might conclude that it is not important to conduct further toxicokinetic assessment for that age groups(s). However, for those chemicals and age groups where children appear to receive a greater internal dose of active toxicant, further analytic steps may be considered as follows:

Semiquantitative approaches. Investigators should use information on the predominant disposition pathways to evaluate child/adult dosimetry differences. It may be possible to base conclusions on one or more particularly useful surrogates from the drug literature. In cases where pathways that govern chemical activation or clearance appear to be substantially different across early life and adults, an age group–specific adjustment factor may be considered. This may be based on the size of the child/adult ratio in that parameter value, using comparisons based on central tendency values or on bounding percentile in children versus central tendency in adults (e.g., 5th percentile of child metabolic function vs. adult median percentile). Such adjustment factors provide the opportunity for the risk assessment to consider child-specific toxicokinetic information readily available. However, it is a simplistic approach that does not consider the various ways in which changes in one pathway's function may be modified by other physiologic factors, leading to an overall influence greater or less than expected. A more complete analysis involving PBPK modeling is needed to integrate child/adult differences in a variety of parameters into across age-group comparisons of internal dosimetry.

Quantitative approaches—PBPK analysis. This involves constructing a child age group–specific model that incorporates the base physiologic parameter values specific to the age group(s) being analyzed as well as modifications in metabolism and renal clearance to reflect the major functional immaturities in early life. This model would then incorporate chemical-specific parameter values to simulate how children at specific developmental stages are likely to handle the chemical. It is essential to carefully consider data needs for such an analysis and whether sufficient data exist or can be generated/estimated.

Ideally, phase II of the children's toxicokinetic risk assessment would result in the following:

- An understanding of the toxicokinetic mechanisms that govern the fate of the target chemical
- Data resources that describe how these mechanisms change during development
- An initial prioritization of age groups for more detailed analysis based on likelihood for internal dosimetry differences relative to adults
- An analytic strategy (semiquantitative or quantitative techniques) for incorporating these data into the risk assessment process.

Phase III: Risk Characterization

The analytic approaches developed in phase II would be followed through so that phase III can attempt to answer the following questions:

- How similar/dissimilar are *in utero* and childhood stages to adults in terms of internal dose received per unit of administered dose?
- How variable are young age groups versus adults?
- Do all age groups fall within the default interindividual variability factor of 3.2?
- Do child:adult differences warrant the development of chemical- and age-specific uncertainty factors?
- Can internal dosimetry estimates for different age groups be developed and used in quantitative risk assessment?

To place the analytical results in perspective, the risk characterization will usually try to describe the degree of uncertainty and variability in the analysis. The following questions may be particularly pertinent to such a discussion.

Are children more variable in their pharmacokinetic responses than adults? How do we assess and apply this information? The role of variability in the risk assessment process can range from purely qualitatitive (e.g., people are different from one another, so we are not as certain as we would like to be about the degree of risk) to semiquantitative (e.g., people vary considerably in metabolizing chemical X, so it seems prudent to use a half-log toxicokinetic uncertainty factor) to fully quantitative (e.g., we have sufficient individual data to plot the population distribution of effective internal doses per unit of external dose, so let us show the risk manager the cleanup options that protect the 50th, 75th, 90th, and 95th percentiles). The fully quantitative mode of variability analysis is used in lead uptake/biokinetic modeling to enable risk assessors to calculate the amount of lead reduction in water, soil, air, or diet needed to bring 95% of the population of exposed children below the blood lead target of 10 μg/dL (U.S. EPA 1994). It is logical that children are more toxicokinetically variable than adults because of their variable growth and maturation rates, as well as the genetic, nutritional, disease, body composition, and prescription (and other) drug factors that create toxicokinetic variability in adults. Children also present the problem of variable growth rates, which can make even small age bins relatively heterogeneous. Greater variability can affect risk conclusions, especially if one is concerned about protecting the tails (e.g., 90th percentile) of the distribution. With greater variability, it is also more likely that a substantial fraction of a certain age group will lie outside the half-log toxicokinetic variability range we normally allocate to the adult defaults. Thus, it is critical that a children's toxicokinetic assessment characterizes in some way the degree of variability present in each age group's data set and determines whether that variability is greater than in the adult case.

By addressing these questions, the risk characterization will endeavor to determine whether toxicokinetic differences are likely between children and adults and whether these differences will be important to the overall risk assessment conclusions for this chemical/scenario. If so, the risk characterization can

describe the advantages and disadvantages of applying qualitative (e.g., professional judgment), semiquantitative (e.g., modified uncertainty factors), or quantitative approaches (PBPK modeling with distributional analyses). The major caveats and data gaps should be elaborated so that the limitations of the assessment are clear and critical research needs are identified. Finally, the characterization should describe the toxicokinetic factors that appear most influential in creating child/adult differences so that the key risk drivers for children are explained.

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