

# Review and Evaluation of the Potential Impact of Age- and Gender-Specific Pharmacokinetic Differences on Tissue Dosimetry

Harvey J. Clewell, Justin Teeguarden, Tracy McDonald, Ramesh Sarangapani, Greg Lawrence, Tammie Covington, Robinan Gentry, and Annette Shipp

The K.S. Crump Group, Inc., ICF Consulting, 602 East Georgia Avenue, Ruston, LA 71270, 318-255-2277

**ABSTRACT:** In standard risk assessment methods for carcinogenic or noncarcinogenic chemicals, quantitative methods for evaluating interindividual variability are not explicitly considered. These differences are currently considered by the use of statistical confidence limits or default uncertainty factors. This investigation consisted of multiple tasks aimed at making quantitative predictions of interindividual differences in susceptibility by using physiologically based pharmacokinetic (PBPK) models. Initially, a systematic, comprehensive review of the literature was conducted to identify any quantitative information related to gender- or age-specific physiological and biochemical factors that could influence susceptibility to chemical exposure. These data were then organized from a pharmacokinetic perspective by process and by chemical class to identify key factors likely to have a significant impact on susceptibility as it relates to internal target tissue dose. Overall, a large number of age- and gender-specific quantitative differences in pharmacokinetic parameters were identified. The majority of these differences were identified between neonates/children and adults, with fewer differences identified between young adults and the elderly. The next phase of this work consists of using PBPK models to develop examples of approaches through the development of case studies. The goal of the case studies is to continue to develop a methodology that incorporates PBPK modeling to assess the likelihood that a chemical or class of chemicals may present an age- or gender-specific risk. The case studies should also demonstrate practical methods for quantitatively incorporating information on age- and gender-specific pharmacokinetic differences in risk assessments for chemicals.

## I. INTRODUCTION

### A. The Role of Tissue Dosimetry in Risk Assessment

The biological basis of interindividual variability is not explicitly considered in standard carcinogen risk assessments. Rather, statistical confidence limits are typically applied to account for this and other uncertainties inherent in the use of animal bioassay data or high-dose occupational data. Non-cancer hazard assessments usually apply a default uncertainty factor of 10 to account for interindividual variability. While it may be possible to distinguish specific groups of individuals, such as infants or the elderly, who appear to be more susceptible to a chemical's potential toxicity, numerous factors contribute to this variability within a defined population (interindividual) and among subgroups within a larger

population (intraindividual). These factors may be placed into two broad categories: those that influence the target tissue dose for a given external exposure (pharmacokinetic factors) and those that influence the target tissue response at a given target tissue dose (pharmacodynamic factors).

In recent years, there has been an increasing use of pharmacokinetic data for a chemical or class of chemicals in risk assessment. In particular, physiologically based pharmacokinetic (PBPK) models have been applied in chemical risk assessments to help make key pharmacokinetic factors more explicit and provide a means for estimating the significance of these factors in the final risk estimates. Use of these data provides a stronger biological basis for extrapolation across species or dosing patterns (from high to low dose, across temporal dosing patterns or routes of exposure) and provides estimates of the relevant tissue

dose for the target population. However, because of variability in populations and subgroups within populations, tissue doses may vary even at the same exposure level. PBPK modeling has the capability to quantitatively describe the potential impact of the pharmacokinetic aspects of this variability in individual susceptibility. Specifically, a PBPK model can provide a quantitative structure for determining the effect of various age- and gender-specific factors in the relationship between the external (environmental) exposure and the internal (biologically effective) target tissue exposure. For example, PBPK models can be used to determine the impact of differences in key metabolic enzymes not only due to normal variation in enzyme activities within the general population, but also due to differences in metabolism between males and females and across age groups. The focus of this investigation is to identify those pharmacokinetic factors that may be used quantitatively to assess intraindividual variability across lifestages (infant, child, adult, elderly) and between genders.

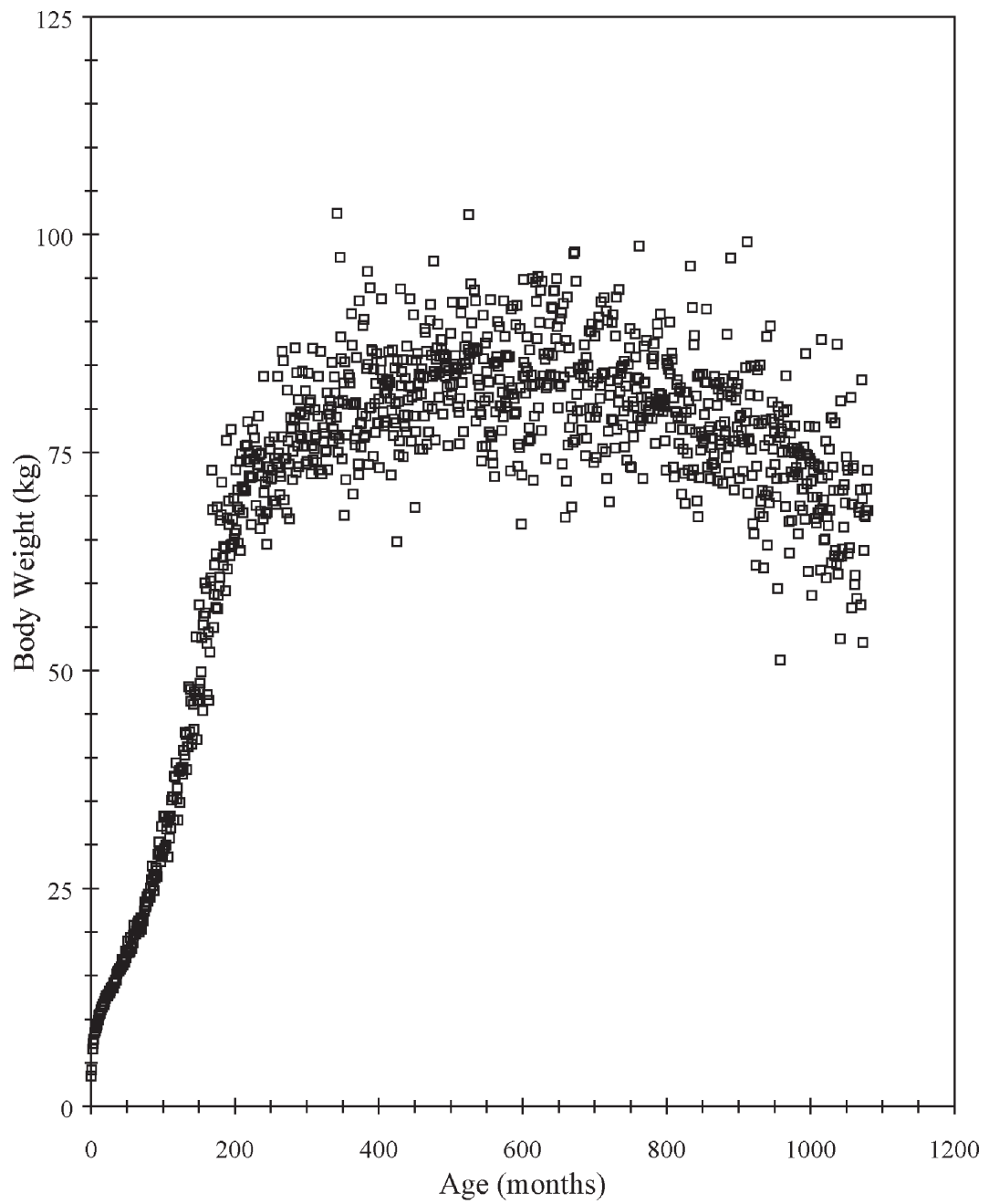
## **B. General Description of Age- and Gender-Specific Differences in Physiology and Metabolism with Potential to Impact Tissue Dosimetry**

The many physiological and biochemical determinants of the concentration time course (pharmacokinetic) of a chemical can be broadly organized into four secondary processes, absorption, distribution, metabolism, and elimination, collectively referred to as ADME. Each of these secondary processes results from one or more primary processes that correspond to actual physiological or biochemical elements. Elimination, for example, is a secondary process that is comprised of fecal excretion, metabolism, glomerular filtration, tubular secretion, and pulmonary exhalation. These processes are dynamic, in some cases changing significantly during development, maturation, and aging in humans. General changes in body weight (Figures 1 and 2) and corresponding changes in organ volumes, particularly the fat composition, occur throughout the human lifespan. For example, glomerular filtration rates (GFRs) are relatively low in human newborns, as is he-

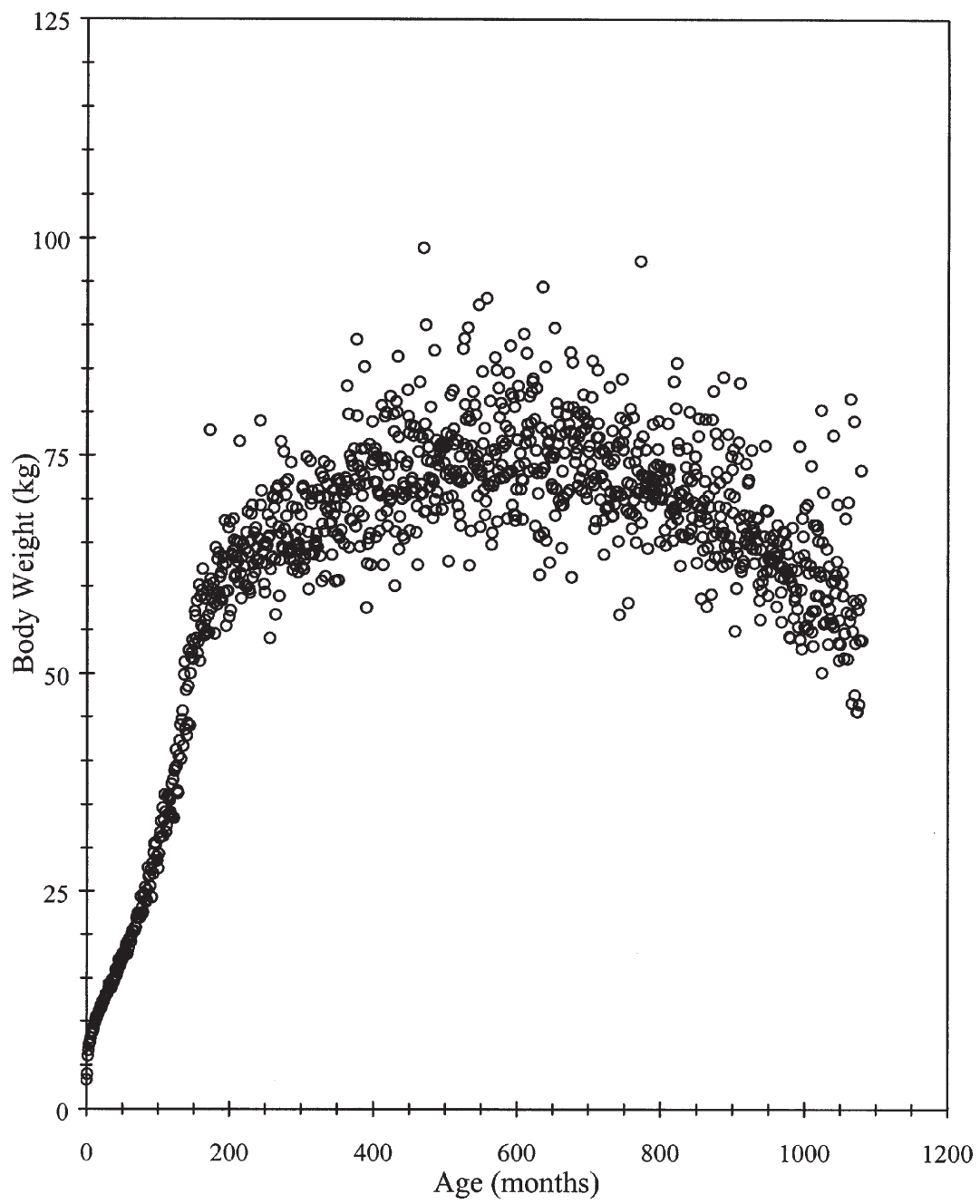
patic metabolism by the cytochrome P450, but both rise rapidly after birth, reaching adult levels after several months. The capacities of some enzyme systems involved in xenobiotic metabolism decline during old age, as does renal elimination. Changes in lung morphology also occur during development and maturation. The dynamic, developmentally related state of these processes implies that there is a uniqueness to each lifestage with respect to its exposure-tissue dose relationship. This applies equally to gender differences, where such differences can also be significant. While the pharmaceutical sciences have begun to develop chemical- and lifestage-specific data useful for characterizing and assigning significance to the effects of age and gender on pharmacokinetic processes, risk assessment for environmental xenobiotics has not. Because the development of PBPK models is strongly influenced by *a priori* knowledge regarding the significance of the various pharmacokinetic processes that determine the tissue concentrations of a chemical of interest, there is significant value in developing tools to broadly define, for chemical classes, the important processes at various lifestages.

## **C. Purpose and Objectives**

The purpose of the analyses described in this article is to perform a comprehensive review of the literature to determine the information available in humans to support the use of PBPK modeling to address the impact of age- and gender-specific physiological, biochemical, and pharmacokinetic differences in individual risk. One objective is to organize, from a pharmacokinetic perspective, the key factors that are likely to have a significant impact on susceptibility, as it relates to estimates of target tissue concentrations. A second objective is to develop a predictive pharmacokinetic framework that can be used to characterize the effect of age and gender differences on tissue dosimetry for a chemical or class of chemicals. A major objective would be to use the available data in combination with a PBPK model to develop examples of approaches that could provide data-derived uncertainty factors to be used in place of default values in risk assessment.



**FIGURE 1.** Male body weight data from NHANES data.



**FIGURE 2.** Female body weight data from NHANES data.

## D. Technical Approach

The data needs for this analysis were extensive, comprising two levels of organization: summary descriptions of age and gender differences and primary literature providing specific quantitative differences in relevant physiological parameters and processes. A systematic, comprehensive literature survey was conducted using available electronic databases for journals and reference books. On occasion, principle investigators were contacted directly.

The retrieved literature was reviewed to determine: (1) which pharmacokinetic or primary process or physiological parameters give rise to lifestage or gender-specific differences in tissue dosimetry; (2) chemical-specific examples of these effects; and (3) quantitative data sufficient to support additional analyses. The literature was grouped for review into one of two major categories, physiological parameters that can impact pharmacokinetics (i.e., body weight, ventilation rate) or data on pharmacokinetic processes. The pharmacokinetic processes category was divided into the four classic subcategories of absorption (A), distribution (D), metabolism (M), and elimination (E). These four subcategories were further divided by chemical class, chemical name, enzyme system, or a specific process (e.g., glomerular filtration). The relevant quantitative and qualitative information on age- and gender-specific differences in pharmacokinetics, as well as the age- and gender-related information on physiological parameters, was then placed in a tabular matrix using the relevant key words for rapid organization. Table 1 presents the classifications used to characterize the physicochemical characteristics of the compounds that are a part of this analysis.

These data were organized from a pharmacokinetic perspective, and the key factors that are likely to have significant impact on susceptibility as it relates to estimates of dose metrics or target tissue concentrations were identified and evaluated. A predictive pharmacokinetic framework was then developed, based on the available data, which can be used to characterize the effect of age and gender differences on tissue dosimetry for a chemical or class of chemicals. In addition, this manner of organizing the data resulted in the

identification of physicochemical or biochemical data that would be needed to place a chemical within the predictive framework. It also allowed for identification of chemicals for which adequate data were available to conduct further quantitative analysis of the impact of age- and gender-specific differences in pharmacokinetics on risk assessment.

As described in this report, the effect of age/gender on pharmacokinetics is relatively well characterized for pharmaceuticals, but less well characterized for xenobiotics. Pharmaceutical pharmacokinetics generally focus on aggregate properties, such as volume of distribution and whole body clearance, while PBPK modeling is typically more concerned with primary processes, such as tissue partitioning and metabolism. The results of the literature searches reflect this difference. Much of the data focus on secondary processes: clearance (elimination), bioavailability, and measures, such as area under the blood concentration time curve (AUC). There are fewer cases where data are available on primary processes, for example, absorption, metabolism, and excretion. While it is possible to use aggregate properties in a PBPK model, it is preferable to relate these secondary processes to the physiological and biochemical differences that give rise to them within the quantitative framework developed here. The latter approach is more informative and makes it possible to use the predictive power of the PBPK modeling to extrapolate across chemicals. These considerations are reflected in the choice of data sets used throughout the manuscript.

## II. SUMMARY AND DEVELOPMENT OF A PREDICTIVE PHARMACOKINETIC FRAMEWORK

Based on our review of the literature, a large number of age- and gender-specific quantitative differences in absorption, distribution, metabolism, and elimination were identified. The quantitative differences are summarized in a matrix (Table 2) that can be used to predict age- and gender-related pharmacokinetic differences for a particular class of chemicals (lipophilic, water soluble, etc.). The majority of the age-related differences identified were between neonates/children and adults, with

**TABLE 1**  
**Classification of Chemicals by Their Physicochemical Properties**

Class	Number of Chemicals in Matrix	Typical Example
<b>A. Volatile: v.p. &gt; 1 mm-Hg</b>		
1. Reactive (lung only)	0	Formaldehyde
2. Water soluble: Kow <1	2	Ethanol
3. Lipophilic: Kow >1	6	Chloroform
<b>B. Nonvolatile: v.p. &lt;1 mm-Hg</b>		
1. Water soluble		
a. Hydrophilic: Kow <1	19	Methotrexate
b. Acidic: pKa <5	6	Benzoic acid
c. Basic: pKa >9	3	Caffeine
2. Lipophilic: Kow >1		
a. Moderately: Kow <4	32	Phenobarbital
b. Highly: Kow >4	4	Estradiol
<b>C. Insoluble (lung only)</b>	0	Carbon dust

fewer differences reported between young adults and the elderly. With the exception of differences in distribution and P450 metabolism, no gender-specific differences were identified.

### **A. Absorption**

The observed differences in absorption are the net result of the interplay between multiple physiological processes and the physicochemical characteristics of the compound. The size, in particular the surface area, flow rates of blood, air and gastric contents, status of the tissue forming the barrier as well as the biochemistry, such as

gastric pH and lung surfactant, can influence rates of absorption.<sup>5-9</sup> For instance, changes in absorption would be expected to parallel increases or decreases in surface area. In addition, the effect of each of these physiological properties on absorption depends on the physicochemical properties of the ingested compound.<sup>8</sup> Lipophilicity, water solubility, and reactivity of the exogenous chemical have a particularly large impact on absorption.

#### **1. Oral Absorption**

Sufficient data on the absorption of lipophilic and water-soluble pharmaceuticals were collected

**TABLE 2**  
**Summary of Age- and Gender-Dependent Changes**

<b>Absorption</b>			
Route of Exposure	Neonate/Child	Elderly	Gender
Oral			
Lipophilic	↑↓	↑↓	M=F
Water Soluble	↑↓	↑↓	M=F
Inhalation			
Lipophilic	↑	I	M=F <sup>1</sup>
Water Soluble	↑	I	I
Particulates	↑	I	M=F
Dermal			
Lipophilic	I	↓	I
Water Soluble	I	↓	I
<b>Distribution</b>			
Compound Characteristics	Neonate/Child	Elderly	Gender
Lipophilic	↑	↑	F↑
Water Soluble	↑	↓	F↓
Protein Binding	↓	↓	↑↓
<b>Metabolism</b>			
Metabolic Pathway	Neonate/Child	Elderly	Gender
Glutathione-S-Transferase	↓	I	I
Sulfotransferase	↑	I	I
Glucuronyl Transferase	↓	I	F≤M
P450	↓	I	M>F
Carboxylesterase	↓	I	I
Alcohol Dehydrogenase	↓	I	I
<b>Elimination</b>			
Compound Characteristics	Neonate/Child	Elderly	Gender
Lipophilic			
Protein binding	I	I	I
Glomerular filtration	↓	↓	M=F
Tubular secretion	↓	↓	I
Tubular re-absorption	I	I	I
Water Soluble			
Protein binding	I	I	I
Glomerular filtration	↓	↓	M=F
Tubular secretion	↓	↓	I
Tubular re-absorption	I	I	I

<sup>1</sup> Data for one compound, methylene chloride

↑ Higher than adult; ↓ lower than adult; ↑↓ increases and decreases demonstrated

I Insufficient data for conclusion

F Female; M=Male

to generalize absorption following oral exposure to these classes of compounds in the neonate and elderly populations. To the extent that  $T_{\max}$  values reflect differences in rates of absorption, the rate effect of age on absorption was not predictable by chemical class. Compared with adults, increases and decreases in the rate of absorption were reported for both lipophilic and water-soluble compounds in neonates and the elderly. While the data generally suggest a lower rate of absorption in elderly vs. adult populations, the extent of absorption was the same. This was similarly true for the neonate. A recent review of gender-related differences in pharmacokinetics provides no convincing data that gender differences in gastrointestinal absorption rate constants unrelated to differences in first pass intestinal metabolism exist.<sup>10</sup>

## **2. Dermal Absorption**

Data on the dermal absorption of xenobiotics in neonates and children were not available. The epidermis of the fetus is unkeratinized, becoming fully keratinized 3 to 5 days after birth. Because the keratinized epidermal layer represents a protective barrier, an increase in dermal absorption during this time frame would be expected.<sup>11</sup> However, Cunico et al.<sup>12</sup> reported no differences in penetration for infants and adults. The hydration state of the epidermis is also elevated in neonates,<sup>13</sup> suggesting the potential for increased absorption of some compounds.

Data on the dermal absorption of xenobiotics in the elderly, which indicated lower, but not statistically different absorption of lipophilic compounds, conformed with expectations based on reports of lower hydration of the epidermis.<sup>14</sup> In general, the available data provide mostly qualitative suggestions of age-dependent differences, but are not sufficient to guide a more quantitative PBPK analysis.

## **3. Respiratory Tract Absorption and Deposition**

The available literature provided excellent quantitative characterizations of age-dependent changes in pulmonary parameters affecting up-

take of volatile compounds and deposition of particulates. Lung surface area, total lung capacity, vital capacity, functional residual capacity, and residual volume all increase in proportion to lung growth. The number of conducting airways is complete at birth and increase only in size with age.<sup>15</sup> However, the size of the pulmonary airways increases from birth to adolescence, primarily by addition of new alveoli.<sup>11,16</sup> The resulting surface area for respiratory absorption also increases.<sup>17</sup> Age-related differences in ventilation parameters have been reported, with the greatest differences occurring between neonates and adults.<sup>18</sup> Morphometric studies have consistently found a decrease in surface area of airspace wall per unit of lung volume beginning in the third decade of life, which continues throughout life.<sup>19</sup> Total lung capacity does not change significantly with age.<sup>20</sup> These age-specific differences in characteristics of the respiratory tract lead to age-specific differences in uptake and deposition.

The characterization of age-dependent differences in the pulmonary uptake of anesthetics is indicative of expected behaviors for similar volatile organic xenobiotics. The uptake rate of volatile anesthetics is higher in both infants and children than in adults.<sup>21</sup> A larger alveolar ventilation rate, relative to FRC and body weight, as well as greater perfusion rates and lower fat content, are believed to be responsible for this age-specific difference in pulmonary absorption.<sup>21</sup> To the extent that these data reflect the behavior of water-soluble and lipophilic volatile organics, it appears the uptake of both classes of compounds is higher in infants and children. Age-dependent changes in the physiological parameters affecting these lifestage-specific effects can be integrated into a predictive PBPK model to determine the effect of these differences on uptake and on steady-state blood concentrations for water-soluble and lipophilic compounds.

Children also receive a higher particle dose per unit surface area of the lung compared with adults. Direct measurement of vapor deposition has not been conducted to explore age-related effects. However, mathematical models that have been developed to quantitatively describe the effect of postnatal changes in the human lung on dosimetry predict a higher deposited fraction in children compared with adults, for both inhaled

reactive vapors and particulates. Reports of differences in pulmonary uptake between elderly populations were not available. Only a single report of gender-related differences in the uptake of volatile organics was identified and does not provide sufficient data to form expectations for gender differences in pulmonary uptake.

The available quantitative data on respiratory tract anatomy and physiology can be integrated into a predictive model structure to provide estimates of age-related differences in the uptake of volatile organic compounds and the deposition of particulates. Detailed morphometric measurements of lung volume, surface area, minute ventilation, breathing frequency, and airway dimensions are available in humans for different age groups (from neonates to adults). Case studies that provide specific quantitative characterization of age-related effects of particulate deposition from the neonatal through adult periods would provide useful insights into expectations for differences in risk in these age groups. In addition, developing a similar quantitative approach for organic vapors would be useful for predicting differences in tissue doses for two important classes of chemicals and for dispelling some currently held beliefs about the relationship between increases in uptake or ventilation rates and steady-state blood concentrations.

## B. Distribution

Physiological processes and constructs, as well as the physicochemical properties of a chemical, influence distribution of a chemical throughout the body. The extent to which a chemical distributes throughout the body is influenced by five main physiological properties, which include body composition (body water, fat, lean body mass), blood flow, composition and concentrations of plasma binding proteins, tissue-protein concentration, and fluid pH.<sup>2</sup> In addition, plasma lipoprotein concentrations have an impact on the distribution of lipophilic compounds.<sup>22-24</sup> From the pharmaceutical literature, sufficient data were available to characterize age- and gender-specific effects on the distribution of both water-soluble and lipophilic compounds.

The main determinant of differences in volume of distribution is the relative size of three body compartments, total body water, fat, and lean mass. In general, the largest age-related differences in distribution occur in the first 10 to 12 months of life, after which distribution is similar to adults.<sup>24</sup> Based on a database published by Hattis et al.<sup>25</sup> 84% of the compounds with differences in Vd in adults and children had volumes of distribution that were higher in the neonate compared with the adult. For the majority of these compounds, the volume of distribution was 1.3- to 2.8-fold higher in the neonate. There was no apparent relationship to chemical class. The consistent effect for both lipophilic and hydrophilic compounds is likely attributable to a larger body water compartment and reduced binding to plasma proteins, including lipoproteins. Generally, reduced plasma protein binding relative to adult levels is observed in newborns.<sup>26</sup> Consistent with the observed decreases in lean body mass in the elderly, changes in the volume of distribution correlated with lipophilicity, with the volumes of distribution of some lipophilic compounds increasing and that of the water-soluble compounds decreasing with age. Two modestly lipophilic compounds did not fit this pattern.

Comparing men and women, the observed differences in the Vd of both lipophilic and hydrophilic compounds are consistent with gender-specific differences in lean body mass. The Vd of lipophilic compounds is higher, and that of water-soluble compounds lower, in women compared with men. In fact, normalization of the Vd for lean body mass minimizes gender-specific differences in Vd for some chemicals.<sup>10</sup> The relationship between body composition, chemical class, and Vd was clearest when men and women were compared and weakest when neonates and adults were compared. The relationship in newborns seems confounded by issues of plasma protein binding, but as a function of gender and in the elderly the relationship is sufficiently clear to be suitable for use in a predictive PBPK framework.

While examples of reduced protein binding in neonates were documented, this effect is chemical rather than chemical-class-specific and is not suitable for integration into a general predictive framework. Such an approach requires specific knowledge of binding characteristics, protein con-

centrations, and the effect, if any, protein binding has on hepatic and renal clearance.

### C. Metabolism

For Phase I reactions, the cytochrome P450 family of enzymes is the most abundant and the most important system with regard to xenobiotic metabolism. The overall activity of P450 has been reported to be approximately 50% higher in adults when compared with the fetus or neonate.<sup>24</sup> In addition, there have been age-related differences in specific P450 isozymes reported. Specifically, the activity of the CYP3A family, the most abundant P450 isozyme in the human liver and a family of isozymes that are involved in the metabolism of numerous xenobiotics, has been reported to be approximately 25 to 50% lower in newborns.<sup>27,28</sup> In addition, the profile of the CYP3A family is different in the fetus and neonate, when compared with the adult, in that CYP3A7 is responsible for up to 85% of the total P450 activity in the fetal liver.<sup>29</sup> However, within 3 to 12 months of age, CYP3A7 levels decline to adult levels and CYP3A4, which is not present in the fetal liver, becomes the major P450 isoenzyme in the newborn and adult liver.<sup>29-31</sup>

There is conflicting information regarding the decline of CYP3A4 levels with age with one investigator reporting a decrease with age.<sup>32</sup> However, there is no decrease in the activity of CYP3A4 with age, but there is a significant decrease in liver mass (35%), liver blood flow (35%), and volume (24 to 44%) between adulthood and late old age that could account for the decline in total systemic clearance of CYP3A4 prototype compounds.<sup>33</sup> Consequently, these age-related differences in the metabolic capabilities of the fetus/neonate and children/adults should be considered in a predictive framework for xenobiotics that are metabolized by the CYP3A isozymes. Moreover, the changes in liver mass, liver blood flow, and volume that occur with advancing age between adulthood and late old age that could account for the decline in total systemic clearance should be considered in a general predictive framework.

There are quantitative age- and gender-specific differences in the activity of several different P450 isozymes and age-related differences

in the capacity to form certain conjugates. An increase P450 metabolic activity does not infer a decrease in susceptibility to the toxicity of that chemical. Many chemicals are minimally toxic; however, when acted on by metabolic enzymes, such as the cytochrome p450 enzymes, these chemicals are activated, that is, the result of metabolism is the formation of toxic metabolites. Consequently, the presence of increased P450 for that particular chemical would potentially make that particular age group or sex a sensitive subpopulation. Therefore, the toxicity of the chemical and/or metabolites should be considered.

Quantitative differences in the levels of esterases in the neonate and in adults have been reported.<sup>34</sup> For example, the levels of plasma pseudocholinesterase have been reported to be 50 to 60% that of adults.<sup>34</sup> This difference would be an important factor in sensitivity to chemicals that are detoxified by these enzymes, such as organophosphorous pesticides.

Gender-related differences in activity have also been reported for the P450 enzymes, CYP1A2,<sup>10,32,35,36</sup> CYP3A4,<sup>32</sup> CYP2D6,<sup>37</sup> and CYP2E1.<sup>28,37</sup> CYP1A2 activity has been reported to be greater in males than in females.<sup>10,32,35,36</sup> One study reported an approximate 1.5-fold decrease in half-life for theophylline, a CYP1A2 substrate, in males when compared with females.<sup>37</sup> CYP1A2 is involved in the metabolism of polycyclic hydrocarbons, heterocyclic amines, and aromatic amines. Consequently, the half-lives of these types of chemicals would be expected to be longer in females than in males. The activities of CYP3A4, CYP2D6, and CYP2E1 have also been reported to be higher in males.

One of the most important Phase II pathways, glucuronidation, was reported to be at most 20% of the adult levels in the fetus during the first 4.5 months of gestation.<sup>38</sup> Thus, at least during early gestation, the ability of the fetus to detoxify xenobiotics by glucuronidation is limited. The fetal liver has been reported to up to 20-fold less glutathione activity than adult livers.<sup>39-41</sup> Based on studies with acetaminophen, the ability to form glucuronide conjugates is also less in the neonate and young children, when compared with adults. In infants and children 12 years of age or

less, approximately 50% of the administered dose was eliminated as a sulfate conjugate.<sup>42</sup> In adults, approximately 50% of the dose was eliminated as glucuronide conjugates. Thus, the sulfation pathway was more important in children, and the glucuronide pathway more prominent in adults. These quantitative differences should be considered when either of these pathways is important in the detoxification of chemicals. The data regarding gender differences in Phase II capacity are mixed with no evident differences in the ability to form conjugates by any of the Phase II pathways identified.

#### D. Elimination

The effects of development and aging on renal function and elimination have been well characterized for lipophilic and water-soluble pharmaceuticals. The renal clearance rates of compounds that are GFR-dependent and tubular secretion-dependent are both reduced in the neonate. Renal clearance was lower for all chemical classes, lipophilic and water soluble, including organic ions. The magnitude of reduction was significant, being on the order of 30 to 50%. These reductions parallel the immaturity of glomerular filtration and tubular secretion systems in the neonate. Reductions in renal clearance that are similar in magnitude are also observed in the elderly. In the elderly, reductions in renal clearance parallel age-dependent deficits in renal function. Very complete quantitative data are available that detail the postpartum maturation of GFR and tubular secretion systems, and the rate of decline after age 30. These data, which include rates, are both necessary and sufficient to develop quantitative, age-dependent predictions of the effect of clearance on steady-state blood concentrations across the entire lifespan. While gender effects in renal clearance generally suggest a lower renal clearance in women compared with men, the data on differences in GFR between men and women are not consistent. Quantitative data on gender differences in tubular secretion were not available. The case for supporting gender differences in renal clearance is not as strong as for age-dependent changes.

### III. AGE AND GENDER DIFFERENCES IN ADSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION

#### A. Absorption

In the context of pharmacokinetics, absorption is the movement of external material across biological membranes at one of four principle portals of entry, the respiratory tract, skin, gastrointestinal tract, or, in the case of fetal exposure, through the placenta<sup>11</sup> into the systemic circulation. Absorption is the net result of the interplay between multiple physiological processes and the physicochemical characteristics of the compound. Physiological characteristics, such as the size, surface area, blood flow rates, gastric contents, status of the tissue forming the barrier, as well as biochemical parameters, such as gastric pH and lung surfactant, can influence rates of absorption.<sup>5-8,43</sup> For instance, changes in absorption would be expected to parallel increases or decreases in surface area exposed. In addition, the effect of each of these physiological properties on absorption depends on the physicochemical properties of the compound.<sup>8</sup> Lipophilicity, water solubility, and reactivity have a particularly large impact on absorption; therefore, these properties were the basis for the categories within which compounds were organized.

The following sections contain a description of information concerning age- and gender-specific differences in the physiological processes that influence absorption and their impact on absorption of chemicals following oral, dermal, inhalation or fetal (placental) exposure. This information is presented at two levels of organization, grouped first by route of exposure and second by physiological construct or process (organ size, flow rates). Because the preponderance of data is on pharmaceuticals, compounds will be classified by physicochemical properties (i.e., lipophilicity) to highlight the relationship to less well studied, environmentally relevant compounds with different physicochemical properties.

#### 1. Oral Absorption

The anatomical structures comprising the gastrointestinal tract—stomach, small and large intes-

tines—are optimized to support its primary function, absorption of nutrients, and water. The wall of the stomach is covered by folds or rugae, while the walls of the small intestine are lined with villi, both of which serve to maximize surface area. The external surface of each villus is covered by microvilli. Lipophilic compounds are primarily absorbed by passive diffusion, although water-soluble compounds may also be absorbed to a limited extent by passive diffusion.<sup>44</sup> However, the absorption of most water-soluble compounds occurs via active transport systems that are also involved in the absorption of nutrients.<sup>44</sup> For example, several divalent ions, including lead, may be absorbed via the calcium transport system.<sup>45</sup> Active transport systems have been identified for several nutrients, including sugars, amino acids, gamma globulins (newborns), pyrimidines, triglycerides, fatty acids, bile salts, vitamins, electrolytes ( $\text{Na}^+$ ,  $\text{Cl}^-$ ) and iron. For xenobiotics to be absorbed via these systems they must be structurally similar to the nutrients allowing them to be transported by these specialized transport systems. For this portal of entry, net absorption may be influenced by the available surface area in the upper and lower gastrointestinal tract, blood flow rates, gut transit times and pH, as well as the status of the intestinal mucosa (villi and microvilli).<sup>5-8,43</sup>

No quantitative data regarding changes to surface area of the gastrointestinal tract were found, but clearly as the surface area increases with intestinal length, surface area increases with body weight<sup>4</sup> (Figure 3). The length, and presumably surface area of the small intestine, increases as a function of body weight at a higher rate than the large intestine, which is consistent with the role of the small intestine in nutrient uptake. However, atrophy of gastric mucosa and intestinal microvilli with a decrease in mucosal surface area has been reported in the elderly.<sup>46</sup>

In response to the nutrient demands of early development, the absorptive capacity of the intestine in children for some nutrients is higher when compared with adults. For example, calcium absorption from intraluminal contents is higher in children when compared with adults.<sup>11</sup> This could have important consequences for the absorption of other divalent metals such as  $\text{Pb}^{2+}$ . Pancreatic enzyme function and bile acid secretion are also lower in neonates.<sup>13</sup> Consequently, absorption

from the gastrointestinal tract of elderly individuals may be decreased, while it is increased for some compounds in the neonate.

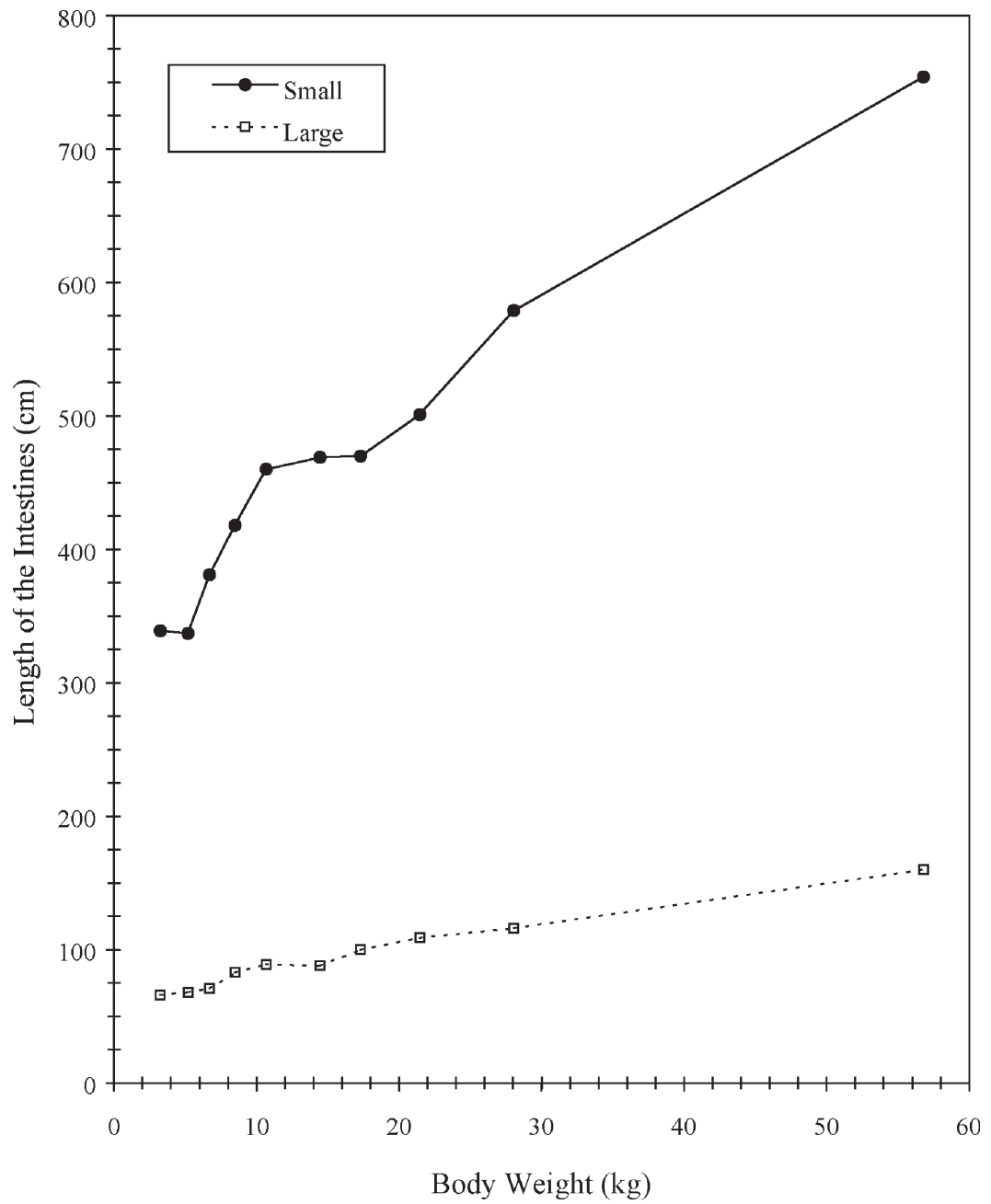
Changes in blood flow patterns with age may also impact the absorption of chemicals from the gastrointestinal tract. For example, splanchnic blood flow decreases with age at a greater rate than cardiac output, leading some investigators to hypothesize a reduced capacity for absorption from the gastrointestinal tract in the elderly.<sup>7</sup> It has been reported that splanchnic blood flow decreases approximately 40% by age 70.<sup>8</sup>

Gastric pH varies significantly with age; gender differences have also been reported.<sup>10</sup> Gastric pH is elevated in newborns relative to adult levels.<sup>11</sup> Adult values are typically 1 to 2.5,<sup>8</sup> while newborns have a gastric pH near neutrality.<sup>26</sup> Full acidification of the stomach does not occur until several months of age.<sup>11</sup> Women secrete less gastric acid and have a slower gastric emptying time, which is affected by increases in sex hormones, particularly in pregnancy and following the use of exogenous hormones. A decrease in gastric acid production causes an increase in gastric pH in the elderly.<sup>46</sup> The elevated pH in neonates or the elderly can influence the ionization state of ingested weak acids and bases, increasing or decreasing the neutral fraction, which is the species available for absorption by diffusion.

There is an increased incidence of intestinal stasis in the elderly compared with adults,<sup>46</sup> slowing the transit time of gut contents. In contrast, the gastric emptying time may increase with the increase in gastric pH in the elderly.<sup>8,46</sup> Two studies reported an increase in gastric emptying time, and two reported no change.<sup>2</sup> The impact of increases in gastric residence times would have a variable effect on absorption. If the stomach is the primary site of absorption, as might be expected for weak acids, absorption might be higher.<sup>8</sup> The contrary would be true for weak bases, where the site of absorption is the intestine. Any delay in movement from the stomach to the intestine would delay the absorption of these compounds.<sup>2</sup>

## **2. Dermal Absorption**

A primary function of the skin is to form a selectively permeable barrier to chemical sub-



**FIGURE 3.** The relationship between intestinal length and body weight. (Adapted from ICRP<sup>1</sup> and Ogiu *et al.*<sup>4</sup>)

stances. Materials passing through the skin into the systemic blood flow must first cross the nonperfused keratinized epidermal layer into the dermis, where they may be cleared by blood flow. Absorption through the epidermis occurs by passive diffusion via different pathways for nonpolar and polar compounds. Nonpolar compounds diffuse through the lipid matrix in the stratum corneum, while polar compounds may diffuse through the outer surface of protein filaments within the stratum corneum.<sup>43</sup> The status of the stratum corneum, thickness and hydration state, has a large impact on absorption.<sup>43</sup> The permeability of skin to hydrophilic compounds increases, and that of lipophilic compounds decreases with increasing hydration state. In general, the mass of a compound absorbed by this route is a function of surface area (exposed), the hydration state of the epidermis, blood flow to the dermal layer, and the physicochemical characteristics of the compound.

In adults, the keratinized epidermal layer (stratum corneum) of the skin is a protective barrier against transdermal absorption, particularly for hydrophilic compounds. The epidermis of the fetus is unkeratinized, becoming fully keratinized 3 to 5 days after birth.<sup>11</sup> This developmental change, unique to the fetus/newborn, could potentially result in an increase in dermal absorption during the first few days of life.<sup>11</sup> However, Cunico et al.<sup>12</sup> reported no differences in two parameters, indicative of skin penetration rates when 22 infants 1 to 3 days of age were compared with 30 adults. Based on these findings, Cunico et al.<sup>12</sup> concluded that penetration rates in adults and infants should be similar for polar compounds.

Aging affects changes in the physiology of skin in the elderly as well. Reduced hydration state of the stratum corneum and atrophy of the capillary network as well as a reduction in skin surface lipids have been reported.<sup>14</sup> Depending on chemical class, these changes in the elderly would be expected to reduce absorption, but predicting outcomes are difficult because one change (blood flow) is not chemical specific, while the others (reduced hydration, decrease in lipids) would have opposing effects on the absorption of a class of chemicals.

The skin surface area of newborns is three-fold larger per kilogram body weight than in adults,<sup>11,47</sup> suggesting the potential for higher per

kilogram doses for equivalent surface area exposures. Based on higher surface area to body weight ratios for children, exposure to a chemical that is capable of penetrating the skin is expected to result in a dose that is 40 to 50% higher in children on a body weight basis compared with adults.<sup>6</sup>

### **3. Respiratory Tract Absorption**

The respiratory tract consists of the nose, nasal cavity, trachea, bronchi, and bronchioles, with the bronchioles terminating in alveolar sacs that contain the alveoli, the primary region where gas exchange occurs. Chemicals may be absorbed across any of these structures. Respiratory tract absorption of volatile compounds is a function of the flow rate of mass through the lung, the available surface area, pulmonary blood flow, thickness of the airway epithelium. Absorption is also a function of tissue permeability that is determined by physicochemical parameters, such as molecular weight, lipid solubility, water solubility, and partition coefficients. The respiratory tract absorption of substances deposited in the airways additionally depends on airway clearance kinetics at the site of deposition. Airway clearance parameters do not show any significant variation with gender or age.<sup>48</sup>

The relative impact of mass flows and tissue blood flows depends on the physicochemical characteristics of the compound, as well as the nature of the absorptive surface. For instance, pulmonary absorption of highly water soluble, volatile organic compounds is lower than expected based on simple blood partitioning.<sup>49</sup> Drawing conclusions regarding the relationship between systemic tissue doses and ventilation rates without consideration of all the processes involved can be misleading. For highly water-soluble compounds, such as alcohols, the steady state arterial blood concentration is not, as commonly believed, dependent on ventilation rate, or fraction (as a percent of pulmonary ventilation) of inhaled material absorbed.<sup>50,51</sup> Differences in ventilation rates, however, may impact upper respiratory or pulmonary tissue doses of some compounds, such as particulates and reactive chemicals.

The human lung can be broadly divided into three regions based on structure and function: the

nasal cavity, the conducting airways, and the gas-exchange region or the pulmonary airways. The nasal cavity has a complex and convoluted structure and forms the first line of defense against inhaled toxicants. Its main function is to condition the inhaled air before entering the gas exchange region and filter the air of inhaled dust particles. The respiratory and olfactory tissue that line the nasal cavity are also metabolically active and well perfused by blood, resulting in an efficient scrubbing of inhaled vapors in this region. Certain metabolized vapors, such as vinyl acetate, are extracted in the nasal cavity by up to 90%, thus limiting the inhaled dose available for systemic exchange. Quantitative information on the human nasal cavity was characterized only available for adult males;<sup>52,53</sup> no information was available on age- and gender-related variations.

Lung surface area, total lung capacity, vital capacity, functional residual capacity, and residual volume all increase in proportion to lung growth. In infancy and childhood, the overall growth of the lung parallels that of the body as a whole and these changes are best correlated with body length or stature.<sup>54</sup> The rate of development is maximal in the neonatal period and then declines until the start of the adolescent growth spurt at about age 11.<sup>55</sup> Thereafter, the rate of growth increases to a peak. In girls, this occurs at about age 13 to 14 years and growth ceases a few years later. In boys, the peak is delayed by about 2 years, and growth continues into adulthood.<sup>56</sup> The growth and maturation of specific regions of the lung, however, are not always parallel to changes in body weight.

The number of conducting airways is complete at birth, and they increase only in size with age.<sup>15</sup> Hence, the conducting airways of the infant may be regarded as a miniature version of those in the adult and the growth of the conducting airways in both length and diameter takes place in a symmetrical manner with age, with constant relation to the rest of the lung.<sup>57</sup> However, the size of the pulmonary airways increases from birth to adolescence, primarily by the addition of new alveoli.<sup>11,16</sup> The resulting surface area for respiratory absorption also increases.<sup>17</sup> At birth, the alveolar surface area is approximately 3 m<sup>2</sup>; however, in adults, the alveolar surface area is 75 m<sup>2</sup>, an approximate 25-fold increase.<sup>18</sup> This increase

in surface area for absorption is in rough proportion to body weight, resulting in similar body weight-adjusted values for alveolar surface area. Estimates of alveoli present at birth vary widely from 17 to 71 million, with a mean of about 55 million. This value in the adult lung varies from 200 to 600 million.<sup>16</sup> The maximal number of alveoli is reached at about 10 years of age, and thereafter maturation of the respiratory system accelerates until maximal function is reached, at approximately the age of 20 years for females and 25 years for males.<sup>58</sup> In this respect, the growth pattern of the conducting airways is different from the alveoli, because the latter increases in both number as well as size. Alveolar surface area increases rapidly until 8 years and then more slowly until the adult area of 70 to 80 m<sup>2</sup> is attained by 20 years.<sup>59</sup> Similarly, lung volume increases rapidly in infants and then shows a moderate increase to an average value of 4000 cm<sup>3</sup> at about 20 years.<sup>59</sup> The lung surface area and volume in males increase more rapidly than those in females do during adolescence and adulthood.<sup>60</sup> Females reach their maximum value at 14 to 20 years, while males continue to increase until 18 to 25 years.<sup>58</sup>

Age-related differences in ventilation parameters have been reported, with the greatest differences occurring between neonates and adults.<sup>18</sup> The respiratory ventilation rate in infants is significantly larger in relationship to lung surface area (133 ml/min/kg BW/m<sup>2</sup>), when compared with adults (2 ml/min/kg BW/m<sup>2</sup>). Therefore, in infants there is potentially a greater exposure of the lung surface to airborne compounds on a body weight basis.<sup>18</sup> Differences in minute ventilation normalized to body weight between neonates, infants (1 year) and children up to 10 years of age are small, but the rates are approximately 40 to 50% higher than those of adults.<sup>6</sup> Activity-adjusted values for total intake (L/kg/day) by inhalation are highest for children and lowest for newborns.<sup>6</sup> In comparison, a 25% reduction in the vital capacity and an increase in the residual volume of 50% have been reported in the elderly.<sup>46</sup> The different characteristics of ventilation between infants/children and adults can also impact the pulmonary uptake of volatile compounds. In infants and children, the alveolar ventilation rate ( $V_{alv}$ ), normalized to functional reserve capacity

(FRC) ( $V_{\text{alv}}/\text{FRC}$ ), is much larger than in adults.<sup>21</sup> More rapid turnover of alveolar air allows more rapid equilibrium with blood and potentially higher uptake in infants and children.<sup>21</sup>

Changes in lung parameters are also observed in the elderly. Morphological changes in the senescent adult lung result in a loss of the alveolar surface area. Alveolar ducts in humans increase in diameter and alveoli become wider and more shallow with age. Morphometric studies have consistently found an increase in the average distance between airspace walls and a decrease in surface area of airspace wall per unit of lung volume beginning in the third decade of life. The decrease in airspace wall surface area per unit lung volume is approximately linear and continues throughout life.<sup>19</sup> Furthermore, the flattening of the internal surface of the alveoli is associated with a reduction in alveolar surface ( $75 \text{ m}^2$  at age 30 years and  $60 \text{ m}^2$  at age 70 years, a reduction of  $0.27 \text{ m}^2/\text{year}$ ).<sup>61</sup>

Total lung capacity does not change significantly with age.<sup>20</sup> This constancy in the total lung capacity is maintained due to the complementary change to its constituent volumes: the vital capacity (mobile volume) and the residual capacity (fixed volume). The vital capacity decreases progressively in adults, even after adjusting for height and weight,<sup>62</sup> due to a decrease in the lung elasticity. The progressive narrowing and closing of small lung airways increase the residual volume with age until the mean value at 60 years is approximately 118% of the value at 20 years.<sup>63</sup> Gas exchange efficiency is also reduced progressively with age in adults. Ventilation-perfusion imbalances and alveolar hypoventilation may be factors in the decline of gas-exchange efficiency,<sup>64</sup> but other factors, such as the reduction in the surface area, increase in membrane thickness, reduced membrane permeability,<sup>65</sup> and a reduced capillary blood volume,<sup>66</sup> also contribute significantly.

The most important physiological changes associated with aging are a decrease in the static elastic recoil of the lung, a decrease in compliance of the chest wall, and a decrease in the strength of respiratory muscles. Most of the age-related functional changes can be related to these three phenomena. After the age of 50 years, a proportion of the elastic fibers in the region of the respiratory bronchiole and alveolus degenerate and appear ruptured and coiled.<sup>67</sup>

These changes are most marked around the alveolar ducts. Consequently, dilatation of the alveolar ducts occurs and this is followed by enlargement of the air spaces.<sup>68</sup> A consequence of the reduction in supporting tissues around the airways is a tendency for the small airways (<2 mm) to collapse. Therefore, premature closure of the airways may occur during tidal breathing.

Quantitative relationships between lung surface area, volume, and age have been developed and reported in the literature. Based on morphometric measurements, Thurlbeck<sup>60</sup> developed regression equations for lung surface area (in  $\text{m}^2$ ) as a function of age. The equations for boys and girls, respectively, were

$$21.9+10.7*\ln(\text{age}) \text{ and } 18.5+7.7*\ln(\text{age})$$

Similarly, the regression equations for lung volume (in liters) in boys and girls, respectively, were

$$0.973+\ln(\text{age}) \text{ and } 0.794+\ln(\text{age}).$$

Petrini et al.<sup>69</sup> measured pulmonary capillary blood flow (in L/min) in 176 normal subjects (94 males, 82 females), ranging from 17 to 70 years of age, and expressed blood flow rate as a function of age (in years) in males and females, respectively, using

$$Q = \exp(1.25-0.0089*(\text{age})) \text{ and } Q = \exp(1.25-0.0089*(\text{age})).$$

Changes in the total lung surface area and volume alone are insufficient to predict regional dosimetry of inhaled pollutants as a function of age. Anatomical information on airway dimensions on a generation-to-generation basis is required to compute regional lung dose metrics. Age-dependent lung models have been developed<sup>70</sup> to characterize the dimensions of a maturing lung and these model have been used to predict particulate and vapor dosimetry in the lungs as a function of age.<sup>71,72</sup> Based on airway dimensions in the Weibel model, Hofmann<sup>70</sup> derived a series of empirical relations to determine the length and diameter of the trachea, bronchial airways, and alveoli diameter and total number of alveoli in the pulmonary airways as a function of age (Table 3).

**TABLE 3**  
**Empirical Relations for Airway Length and Diameter for an Age-Dependent Lung**

Trachea Diameter (cm)	$D_0(t) = \frac{D_0(t)}{1.67} [1.271(1 - e^{-0.07t}) + 0.55]$
Trachea Length (cm)	$L_0(t) = \frac{D_0(t)}{12.29} [8.72(1 - e^{-1t}) + 4.0]$
Bronchial Diameter (cm)	$D_i(t) = \frac{D_i(t)}{1.26} [0.863(1 - e^{-12t}) + 0.42]$
Bronchial Length (cm)	$L_i(t) = \frac{D_i(t)}{4.285} [2.931(1 - e^{-1t}) + 1.5]$
Alveoli Diameter ( $\mu\text{m}$ )	$D(t) = [172.76(1 - e^{-2t}) + 100]$
Alveoli Number	$N(t) = 10^6 [286.21(1 - e^{-4t}) + 37.6]$

Source:<sup>70</sup>

The ventilation conditions of humans are also age-related. Although the reported values are highly variable, Hofmann<sup>70</sup> estimated that the resting tidal volume increases from about 22 cm<sup>3</sup> at birth to about 500 cm<sup>3</sup> in adulthood, whereas the resting respiratory frequency decreases from about 42 min<sup>-1</sup> to about 14 min<sup>-1</sup>. The empirical formulas derived by Hofmann<sup>70</sup> for the resting tidal volume and respiratory frequency as a function of age *t* (in years) are summarized in Table 2.

Respiratory tract clearance is accomplished by various regionally distinct processes. In the nasal cavity and the conducting airways, clearance occurs via the mucociliary transport and in the pulmonary region clearance is primarily by macrophages.<sup>73</sup> Nasal mucus flow rate in healthy adults is about 5 mm/min,<sup>74</sup> similar to the tracheal mucus transport rate.<sup>75</sup> There appears to be no clear evidence for any age- or gender-related differences in either mucociliary clearance or macrophage clearance.

#### 4. Observed Differences in Absorption

*Neonates, Infants, and Children.* There is limited experimental evidence of age-specific differ-

ences in gastrointestinal tract absorption of xenobiotics in children. The example of lead, a water-soluble hydrophilic compound, is well known. Neonates through 2 years of age absorb 4 to 5 times more lead (42 to 53%) than adults (10%).<sup>47</sup> From ages 2 through 6, the amount of absorption drops slightly to 30 to 40% and then drops again between the ages of 6 and 7 years.<sup>6</sup> The mechanism responsible for these differences is unknown, but it has been speculated that the gastrointestinal tract develops more selective intestinal absorption processes during maturation<sup>6</sup>. Pulmonary absorption of lead is also greater in children (42%) than in adults (15 to 30%).<sup>76</sup>

Compared with xenobiotics, neonatal pharmacokinetics of pharmaceutical compounds have been studied widely, providing classical pharmacokinetic characterization of water-soluble and moderately lipophilic compounds. The expectations for environmental chemicals of similar characteristics can be built on these characterizations. However, measures of absorption, such as time to peak concentration ( $T_{\text{max}}$ ), which reflects outcomes influenced by other factors (e.g., gastric emptying time) rather than simple absorption alone, are typically reported. The  $T_{\text{max}}$  values for anticonvulsants, several classes of antibiotics and

digoxin for neonates and adults were reviewed by Morselli et al.<sup>26</sup> To the extent that  $T_{\max}$  reflects differences in the rate of absorption, the rate was variable and ranged from higher (phenobarbital, theophylline) or equivalent (digoxin) to lower (rifampicin) for neonates compared with adults.<sup>26</sup> These changes are not predictable by chemical class; phenobarbital, digoxin, and rifampicin are moderately lipophilic and acetaminophen and theophylline are water soluble.

In contrast to rates of absorption, the extent of absorption may be equivalent. Delayed absorption of both moderately lipophilic (phenobarbital) and water-soluble (acetaminophen) compounds in neonates has also been documented.<sup>13</sup> The effect appears to be related to more than reduced gastric emptying time; the enteral absorption rate constant increases with age.<sup>13</sup> These data only support an age-related difference in the extent of gastrointestinal tract absorption. An exception is lead, where it appears that absorption is greater.

Minimal experimental data are available regarding differences in the rate or extent of dermal absorption between adults, children, and infants. This likely reflects the limited use of the dermal route for administration of pharmaceuticals and the inappropriateness of experimental work in humans.

The characterization of age-dependent differences in the pulmonary uptake of anesthetics is likely an indication of expected behaviors for similar volatile organic xenobiotics. The initial uptake rate of volatile anesthetics, both lipophilic (e.g., halothane and cyclopropane) and water soluble (e.g., nitrous oxide), is higher in infants and children than adults.<sup>21</sup> A larger alveolar ventilation rate relative to FRC and body weight, as well as greater perfusion rates and lower fat content, are believed to be responsible for this age-specific difference in pulmonary absorption.<sup>21</sup> To the extent that these data reflect the behavior of water-soluble and lipophilic volatile organics, it appears that the initial rate of uptake of both classes of compounds would be expected to be higher in infants and children.

Inhaled particles deposit in any one of the three regions of the lung depending on the aerosol size, breathing characteristics, and airway morphology. Fractional deposition of particles in the developing lungs has been studied by direct *in*

*vivo* measurement and has been inferred using mathematical models that quantitatively describe the postnatal changes in the human lung.<sup>70</sup> These models provide a basis to evaluate both the age dependence on inhaled particle dosimetry, as well as investigate aerosolized drug dosage extrapolation to children based on formulations in adults.

Swift<sup>77</sup> constructed replica nasal casts of an infant and adult and studied deposition using particles from 1 to 10  $\mu\text{m}$ . Swift observed comparable nasal cavity deposition efficiency in adults and children for equivalent states of activity. Oldham et al.<sup>78</sup> examined the deposition of monodispersed particles, having diameters of 1, 5, 10, and 15  $\mu\text{m}$ , in hollow airway models designed to represent the trachea and the first few bronchial airway generations of an adult, a 7-year-old child and a 4-year-old child. In most cases, the total deposition efficiency was greater in the child-size models than in the adult model.<sup>78</sup> Bennett and co-workers compared the deposition of 4.5  $\mu\text{m}$ , poorly soluble iron oxide particles in children and adults with mild cystic fibrosis and found that children had higher deposition in the extrathoracic airways.<sup>79</sup> However, no significant difference was observed in total respiratory tract deposition between the children and adults. In a subsequent study, Bennett et al.<sup>80</sup> compared the total fractional deposition of 2  $\mu\text{m}$  wax particles in children (7 to 14 years), adolescents (14 to 18 years), and young adults (19 to 35 years). Although no significant age-related difference in fractional deposition, based on smaller lung size and higher minute ventilation in children, was observed, they concluded that children would receive a higher dose of particles per lung surface area compared with adults.

Hofmann<sup>70</sup> developed a theoretical age-dependent lung morphology by scaling airway anatomical parameters and airflow rates and examined their effects on particle deposition. They showed that particle deposition is strongly dependent on age, with children receiving a significantly higher dose than adults. Xu and Yu<sup>81</sup> also modeled particle deposition as a function of age and found higher total deposition in children than adults in the tracheobronchial and alveolar region. Using morphometric measurements taken from 21 replica airway casts of children and adolescents, Phalen et al.<sup>82</sup> constructed mathematical

models to estimate tracheobronchial particle deposition efficiency for infants, children, and adolescents. Their computed particle deposition efficiencies indicated that under most levels of physical activity and for most particle sizes (0.01 to 100  $\mu\text{m}$ ), children exhibited a higher tracheobronchial deposition than adults. More recently, a theoretical lung model incorporating changes in morphology and breathing pattern with growth was used by Musante and Martonen<sup>17</sup> to predict regional particle deposition in children. Based on model-derived estimates they concluded that under both resting and active breathing conditions, tracheobronchial deposition decreased with age. In particular, if ventilation rate and cumulative surface area are considered, children may receive a localized dose that is three times higher than adults. They also concluded that the pulmonary deposition was highest in the 4 to 6 year old for all particle sizes examined.<sup>17</sup> Hence, both *in vivo* experimental measurements and mathematical models predict a higher fractional deposition of inhaled particulates in children compared with adults.

The impact of physiological differences specific to the neonatal and childhood periods on absorption is route specific. The extent of gastrointestinal tract absorption appears to be equivalent for the lipophilic and water-soluble compounds studied, while the rate of absorption varies by chemical but is independent of physicochemical property. The absorption of compounds that enter through the gastrointestinal tract through specialized transport processes, for example, lead, may be higher in neonates, reflecting higher absorptive capacity of the neonatal intestine for some nutrients during this lifestage. Dermal absorption data are lacking, but one study reported no differences in penetration between children and adults.<sup>12</sup> Pulmonary absorption appears to be higher in infants and children for water-soluble and lipophilic volatile organics as does deposition of particulates. Increased uptake and deposition result from age-specific differences in airway geometry increases in ventilation rates relative to body weight and differences in body composition.

*Elderly.* The physiological and biochemical changes that accompany aging have been shown to affect gastrointestinal absorption.<sup>2</sup> Reduced splanchnic blood flow, gastric emptying times,

and increased gastric pH have been reported in the elderly as have reductions in pharmacokinetic parameters correlated with absorption ( $C_{\text{max}}$ ,  $T_{\text{max}}$ ), but attributing decreases in absorption specifically to changes in one or more of these physiological processes is difficult. In addition, based solely on the data presented here, differences in the rate and extent of absorption are not classifiable by physicochemical characteristics.

Decreases in splanchnic blood flow in the elderly increase the apparent absorption of compounds undergoing high hepatic extraction such as nifedipine.<sup>8</sup> The absolute bioavailability of nifedipine is 30% higher in the elderly when compared with young adults.<sup>8</sup> As indicated by a longer  $T_{\text{max}}$ , the rate of absorption of some moderately lipophilic (quinidine, chlorthalidone, nalidixic acid) and hydrophilic (ethambutol, practolol) pharmaceuticals has been shown to be lower in the elderly.<sup>46</sup> The absorption rate constant for practolol is 1.13 L/h in adults and 0.71 L/hr in the elderly.<sup>46</sup> The rate constant for absorption of nalidixic acid is similarly lower in the elderly (0.52 vs. 0.29/h).<sup>46</sup> On the other hand, for a group of water-soluble drugs, clomethiazole, cimetidine and propranolol, the extent of absorption is higher in the elderly.<sup>46</sup>

A recent review reports that there are no data that support an effect of gastric pH on gastrointestinal absorption in the elderly.<sup>2</sup> In addition, evidence suggests that the extent of gastrointestinal absorption of antibiotics in the elderly is comparable to that in younger patients, even when differences in the rate of absorption are apparent.<sup>83</sup>

In summary, the relevant data suggest that in general, the extent of gastrointestinal absorption in the elderly is unaltered, but the rate of absorption is lower for some compounds,<sup>2</sup> with the physicochemical characteristics of the compound playing little role in producing these differences. Differences in gastrointestinal absorption in the elderly result in delayed peak concentrations, but similar AUCs.<sup>83</sup>

Roskos et al.<sup>14</sup> compared the dermal absorption of compounds with aqueous solubilities of  $\sim 0$  (modestly lipophilic, testosterone) to 21.7 g/L (water soluble, caffeine) in young adults 22 to 40 years of age and older adults ages greater than 65 years. The extent of absorption in older adults was lower, but not statistically different for the

modestly lipophilic compounds, and 36 to 52% lower for water-soluble compounds.<sup>14</sup> The tested compounds included acetylsalicylic acid, caffeine, benzoic acid, hydrocortisone, testosterone, and estradiol. This pattern conforms with expectations based on reports of lower hydration of the epidermis,<sup>14</sup> which should have a greater effect on the absorption of hydrophilic compounds. Generally, dermal absorption of water-soluble compounds can be expected to be lower in the elderly compared with younger adults.

*Gender.* A recent review of gender-related differences in pharmacokinetics provides no convincing data that gender differences in gastrointestinal absorption rate constants unrelated to differences in first pass intestinal metabolism exist.<sup>10</sup> The authors concluded that gender differences in absorption and bioavailability are rare and likely are without clinical significance.

No data characterizing gender differences in dermal uptake were available. One study used a PBPK model of dermal absorption from bath water of various temperatures to estimate chloroform skin permeability rates,<sup>84</sup> and reported a sex difference in the rate for one of two tested water temperatures. Because of uncertainties associated with model assumptions (i.e., equivalent skin blood flow, skin thickness, and subcutaneous fat between male and females), the difference in rates could not be solely attributed to a difference in skin permeability between males and females.<sup>84</sup>

Experimental work evaluating gender differences in pulmonary uptake of xenobiotics in humans is similarly lacking. The paucity of available literature evaluating gender difference in pulmonary uptake limits the characterization of any such differences. One study reported no differences in the pulmonary uptake of methylene chloride between males and females.<sup>85</sup>

Unlike vapors, gender-related differences in particle deposition in the respiratory airways have been explored. In general, studies on gender-specific effects on particulate dosimetry are equivocal and do not show any significant gender-related changes to particulate dosimetry in the adult lung. Using 2.5 and 7.5  $\mu\text{m}$  particles, Pritchard et al.<sup>86</sup> concluded that females receive a higher extrathoracic and tracheobronchial deposition and a smaller pulmonary deposition compared with males for the same particles and at similar inspiratory flow rates.

More recently, Bennett et al.<sup>80</sup> measured total respiratory tract deposition in adult males and females aged 18 to 80 years using 2  $\mu\text{m}$  particles and showed comparable deposition efficiency under normal breathing conditions. Dosimetry studies by Kim and Hu<sup>87</sup> and Jaques and Kim<sup>88</sup> assessed regional deposition efficiency in healthy adult males and females using a bolus delivery technique with 1, 3, and 5  $\mu\text{m}$ . Deposition was comparable in males and females for 1  $\mu\text{m}$  particles, but a higher deposition (15%) was noted in females compared with males for the 3 and 5  $\mu\text{m}$  particles.<sup>87,88</sup>

## B. Distribution

The distribution of a chemical throughout the body is influenced by physiological processes and constructs, as well as the physicochemical properties of a chemical. The distribution of chemicals is typically described as the volume of distribution,  $V_d$ , or volume of distribution at steady state  $V_{d_{ss}}$ . As a human ages, the extent to which a chemical distributes throughout the body is influenced by five main physiological properties, which include body composition (body water, fat, lean body mass), blood flow, composition and concentrations of plasma binding proteins, tissue-protein concentration, and fluid pH.<sup>2</sup> In addition, plasma lipoprotein concentrations have an impact on the distribution of lipophilic compounds.<sup>22-24</sup> Changes in these physiological properties during development, maturation, and aging have demonstrable influence on distribution.<sup>2,24,46,89</sup> Gender differences, predominantly in lean body mass, and percent body fat can also result in differences in volumes of distribution.<sup>8,10</sup>

Changes in each of these parameters with age or gender have an individual impact on the volume of distribution. However, changes in overall chemical distribution with age or gender may be the result of a combination of changes in these parameters, depending on the physicochemical properties of a chemical. The following sections provide information on the age- and gender-related differences in each of these physiological processes and their potential impact on distribution, followed by a discussion of measured overall differences in distribution of specific chemicals by age or gender.

## 1. Body Composition

While the absolute volumes of some tissues increase from birth to stable adult levels (Figure 4) in rough proportion to height<sup>90</sup> or body weight<sup>4</sup> (Figure 5), the relative volumes of total body water, extracellular water, body fat and lean body mass do not increase in proportion to height<sup>24,89,90</sup> (Figure 6 and Table 4). The relative volume of lean body mass and fat undergo additional changes during aging, and are notably different in the elderly<sup>2</sup> (Figure 6). In women, lean body mass decreases from 76% to 52%, and percent body fat increases from 33% to 49% between ages of 25 and 65 to 70. In men, lean body mass decreases from 80% to 64%, and percent body fat increases from 19% to 34% during the same periods (Figure 6).

Body water constitutes approximately 77% of body weight in full-term infants, 73% at 3 months, and decreases to adult levels of 55% by age 12.<sup>89</sup> Similarly, extracellular water is 45% of body weight in full-term neonates, dropping to 33%, 28%, and 20% after 3 months or 1 year, respectively, before reaching the adult level of 20.<sup>24</sup>

Body fat increases in a gender-dependent fashion during development and maturation. Fat content increases between the ages of 5 and 10 in boys, dropping afterward until age 17. In women, fat content increases rapidly during puberty, after which women maintain body fat levels approximately twofold higher than men.<sup>89</sup> Body fat increases in women from an average of approximately 33% at age 20 to approximately 48% on average in women over 70 years of age. The corresponding age-dependent increase in men is from an average of 18 to 36%.<sup>91</sup> In neonates, the average percent body fat is lower than that observed in adults, representing 15% of body weight.<sup>26</sup>

## 2. Serum Binding Proteins

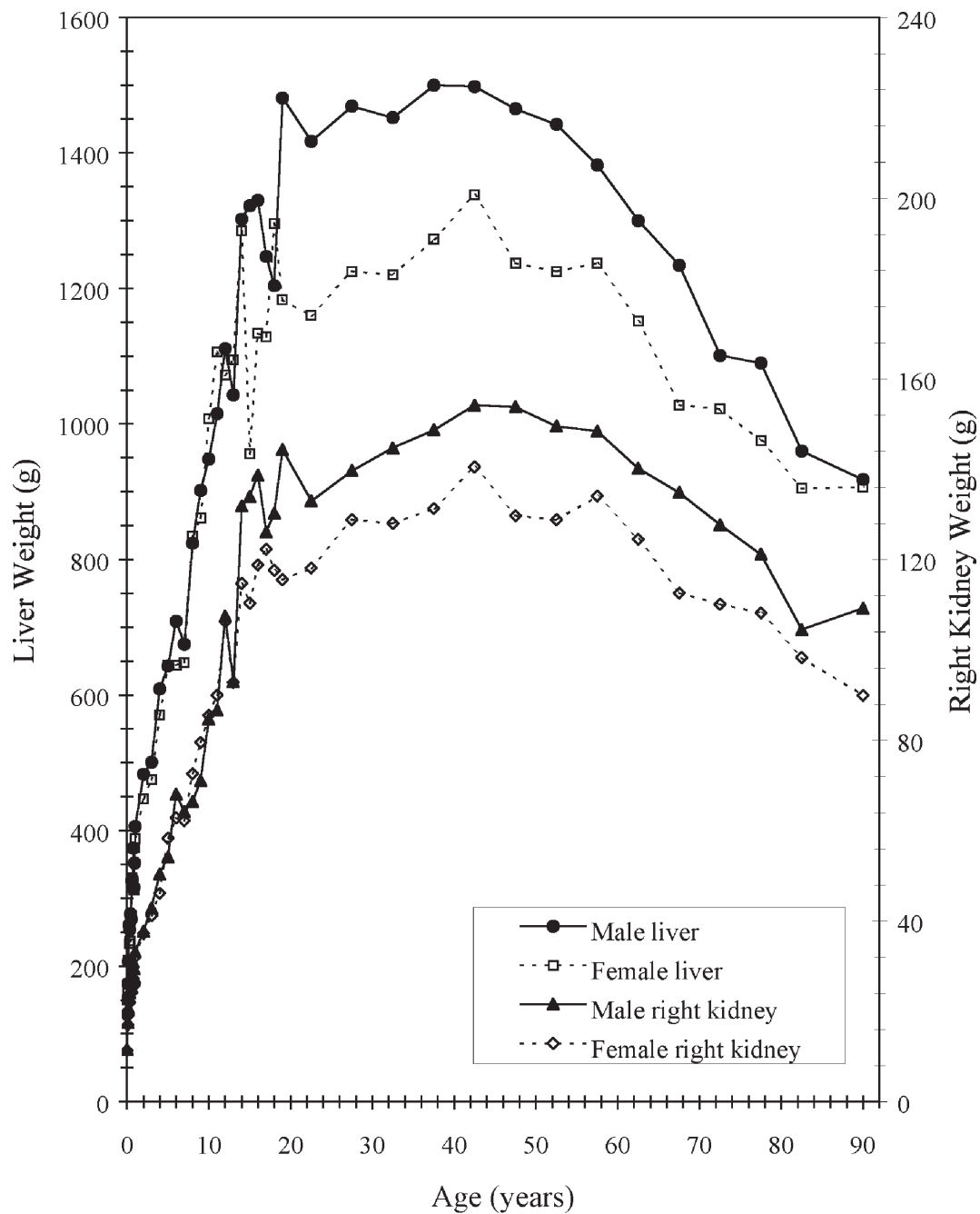
The extent of binding to serum proteins influences the volume of distribution by changing the storage capacity of the blood compartment for a chemical. Both the composition and concentrations of plasma proteins have the capability to

influence the volume of distribution of a compound.<sup>24</sup>

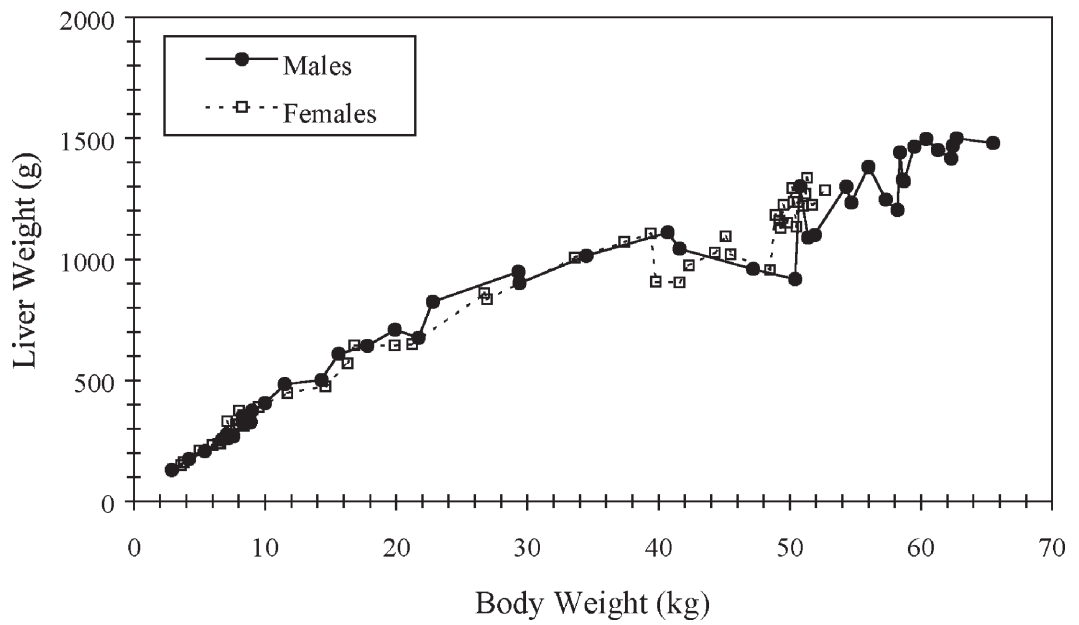
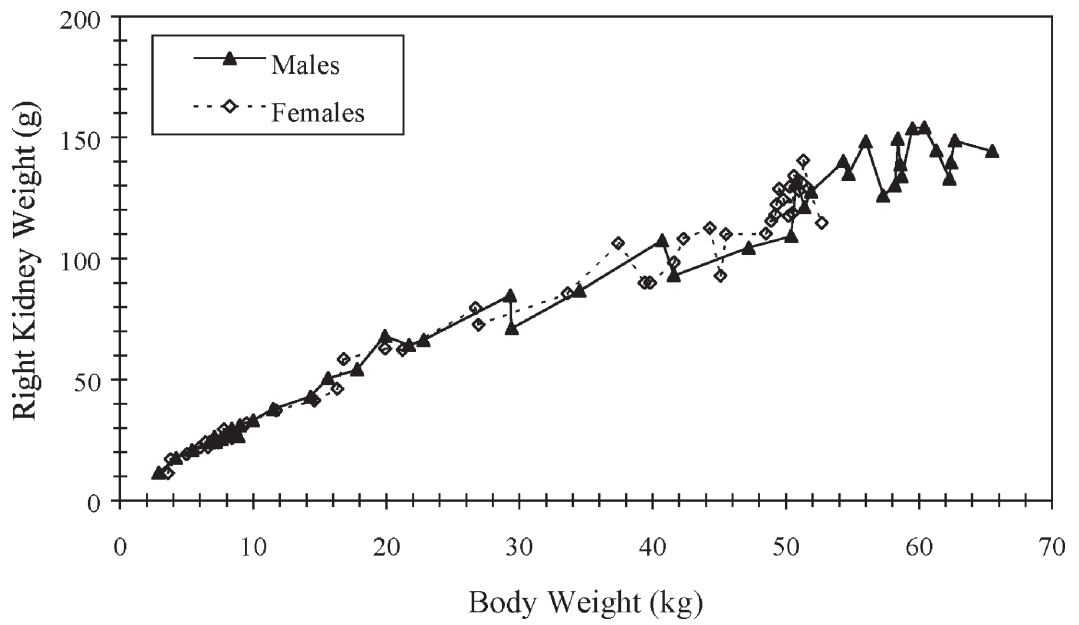
In humans, there are four serum-binding proteins that are known to impact the bound/free concentrations of pharmaceutical agents or xenobiotics, and hence the volume of distribution of these compounds.<sup>22,23,92-94</sup> These include the steroid hormone binding protein (SHBG),<sup>92-94</sup> albumin,  $\alpha_1$ -acid glycoprotein (AIG),<sup>24,46,95</sup> and the serum lipoprotein family (SLPF), which includes chylomicrons, high-density (HDL), and low-density (LDL) lipoproteins.<sup>22,23</sup> Other serum-binding proteins that impact the free fraction of endogenous hormones are also present in human blood.<sup>96,97</sup> but they are not addressed here because characterization of their influence on drug and chemical binding and distribution is very limited.

Total plasma protein concentration does not seem to change significantly with age, but changes in the concentrations of specific binding proteins do occur.<sup>46</sup> Changes in the concentrations of binding proteins are developmental stage specific, typically showing the largest change between birth and full maturation. Total protein (7.1 vs. 5.5 g/100 ml), albumin (4.5 vs. 3.7 g/100 ml), and globulins (2.58 vs 1.6 g/100 ml) are higher in adult plasma, compared with newborn cord plasma.<sup>98</sup> Bilirubin concentrations are higher in the newborn (2.0 vs. 0.2) compared with adults. Plasma protein concentrations do not change significantly in healthy children between the ages of 2 and 18.<sup>89</sup> Differences in serum binding protein concentrations, particularly in SHBG, which binds estrogen, show gender differences as well.

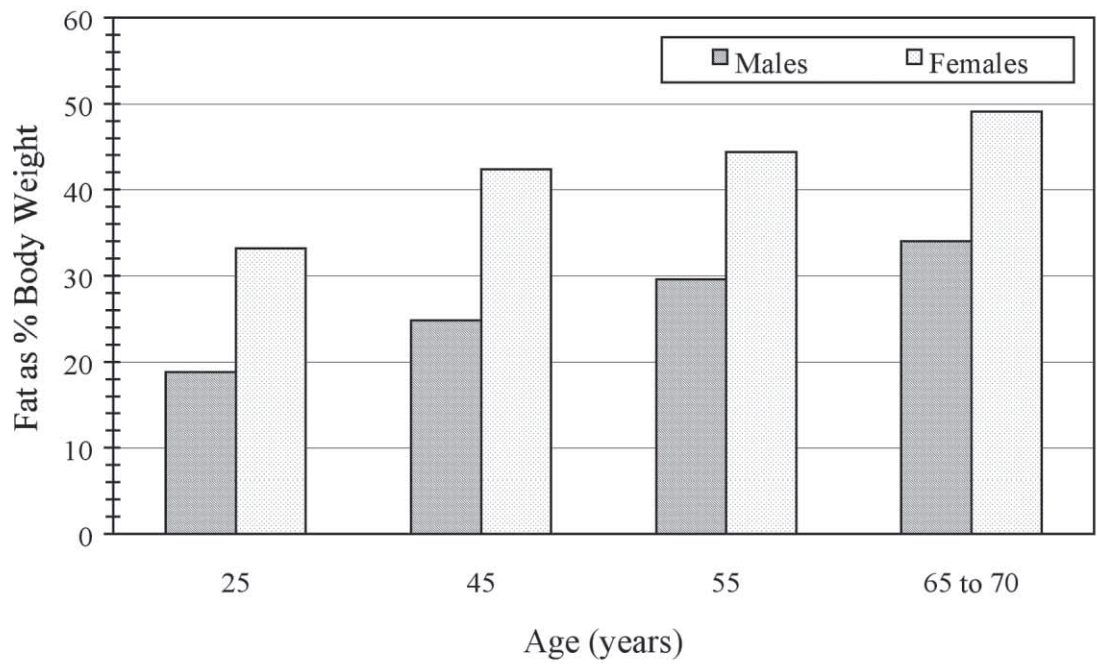
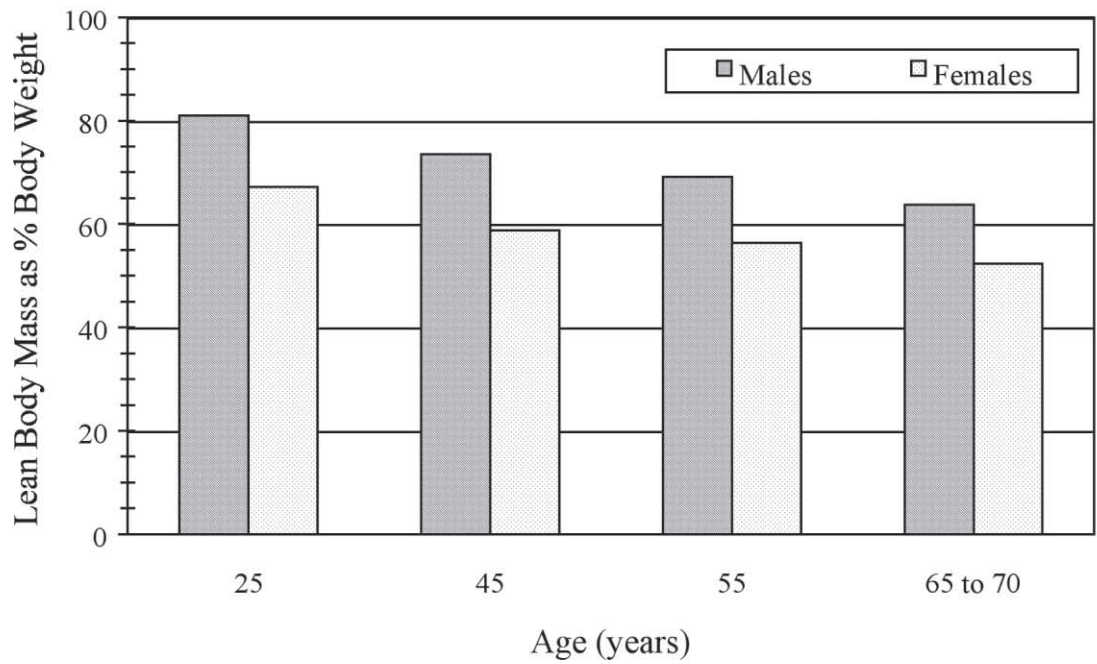
*Albumin.* Albumin is the major drug binding protein in the serum.<sup>2</sup> Plasma albumin is reduced in the elderly, compared with younger adults.<sup>46</sup> The decrease is modest.<sup>95</sup> The free fraction and free serum concentrations of benzodiazapines and most neuroleptics depend primarily on the serum albumin levels<sup>95</sup>. Hepatic extraction of the benzodiazapines is limited by protein binding. Age-related reductions in serum albumin concentrations can increase hepatic extraction of these compounds.<sup>95,99</sup> Changes in the free fraction could cause changes in the volume of distribution as well. Albumin concentrations in the neonate are equivalent or modestly lower than adult values.<sup>24,98</sup> At birth and to 3 months of age, the range of normal albumin concentrations is 3.2 to 4.8 g/dL,



**FIGURE 4.** The relationship between right kidney or liver weight and age in Japanese from birth to old age. (Adapted from Ogiu *et al.*<sup>4</sup>)



**FIGURE 5.** The relationship between right kidney or liver weight and body weight in Japanese from birth to old age. (Adapted from Ogiu *et al.*<sup>4</sup>)



**FIGURE 6.** The relationship between lean body mass or body fat as percent of body weight and age. (Adapted from Mayersohn.<sup>2</sup>)

**TABLE 4**  
**Fractional Organ Weight as a Function of Age<sup>a</sup>**

<b>Organ Weight as a % of Body Weight</b>		
<b>Organ</b>	<b>Neonate</b>	<b>Adult</b>
Skeletal Muscle	25.0	40.0
Skin	4.0	6.0
Heart	0.5	0.4
Liver	5.0	2.0
Kidneys	1.0	0.5
Brain	12.0	2.0

<sup>a</sup>Adapted from<sup>144</sup>.

increasing only slightly to 3.7 to 5.7 g/dL at 1 year of age.<sup>100</sup> Neonatal albumin has different physiochemical properties (amino acid sequence) than the adult protein and shows a lower affinity for drugs.<sup>101</sup>

**Steroid Hormone Binding Globulin.** One-year-old boys have a blood SHBG concentration of ~110 nM. The concentration declines steadily from age one, through puberty, until reaching adult levels of ~30 nM.<sup>102</sup> Similarly, SHBG levels are higher in young female children compared with adults.<sup>103</sup> SHBG levels are ~70 nM in young girls between ~3.7 and 6.5 years of age, and drop during puberty (~13.4 years) to adult levels of 40 nM.<sup>103</sup> Levels of SHBG are higher in adult women (40 nM) compared with men (20 nM) and rise significantly during pregnancy to 400 nM.<sup>93,94</sup> SHBG concentrations are reported to be higher in the elderly compared with young adults. Mean SHBG levels are ~53 nM and 70 nM in elderly men and women of approximately 70 years of age, respectively.<sup>104</sup>

**Glycoproteins and Lipoproteins.** A1G levels are decreased in the neonate and do not reach adult levels until about 1 year of age.<sup>13</sup> There appears to be a moderate increase in A1G with age,<sup>95</sup> although concentrations of this acute phase protein can also rise in response to disease states.

However, another study reported no change in A1G with age.<sup>2</sup> The tricyclic antidepressants bind both albumin and A1G, but free concentrations were determined primarily by the serum concentrations of A1G.<sup>95,99</sup>

The concentration of globulins is lower in newborns. The concentrations of  $\alpha_1$ -globulin remains constant, while  $\alpha_2$  and  $\beta$  globulins increase with development. At birth,  $\alpha_1$ -globulin concentrations are 0.2 to 0.03 g/dL, and increase to 0.5 to 1.1 g/dL by 1 year of age. Similarly,  $\beta$ -globulins increase from 0.3 to 0.6 g/dL at birth to 0.4 to 1.0 g/dL at 1 year of age.<sup>100</sup> In addition, the binding properties of the globulin fraction in newborn cord blood are different from those of the adult globulins.<sup>105</sup> Lipoprotein concentrations are lower in newborns.<sup>98</sup>

### **3. Tissue Binding**

Digoxin binding to erythrocytes shows age dependence. The erythrocyte/plasma digoxin ratio is reported to be 3.6 in infants on long-term treatment and 1.3 in adults.<sup>24</sup> This is consistent with findings that neonatal erythrocytes have 2.5 times the digoxin binding sites found in adult erythrocytes.<sup>24</sup> Because measurement of tissue

binding of drugs is not typically made directly, but rather inferred from compartmental analysis of plasma-concentration time course data, it is difficult to conclude with confidence that increases or decreases implied by the model are attributable to actual changes in tissue binding.<sup>2</sup>

#### 4. Observed Differences in Distribution

*Neonates, Infants and Children.* In general, age-related differences in distribution occur in the first 10 to 12 months of life, after which distribution is similar to adults.<sup>24</sup> Hattis and co-workers have compiled a database containing volumes of distribution for compounds in neonates, children, and adults.<sup>25</sup> The volume of distribution was higher in neonates or children compared with adults for 10 of the 12 compounds with apparent differences in the volume of distribution (Table 5). The ratio of the perinatal Vd/adult Vd was calculated for these compounds (Table 5). The majority of the compounds had a ratio between 1.3 and 2.8. This range may be reasonable bounds for the increase in Vd for water soluble and moderately lipophilic compounds in neonates and children. Only two compounds, one of that is lipophilic, presented a Vd that was lower in the perinatal period. The volume of body water in children (<12 years) results in larger volumes of distribution (35% of body weight) for the water-soluble aminoglycoside antibiotics when compared with adults (20 to 30% of body weight).<sup>89</sup> Volumes of distribution in neonates and children are also higher for modestly lipophilic compounds. The volume of distribution of the diuretic furosemide is increased in newborns (0.25 to 1.13 L/kg) compared with adults (0.07 to 0.18 L/kg).<sup>26</sup> The volume of distribution of the anticonvulsant phenytoin is higher in newborns (0.8 to 2.0 L/kg) compared with infants (0.3 to 1.0) and adults (number not given). The volume of distribution of two local anesthetics, lidocaine and mepivacain, is larger in the neonate than the adult.<sup>26</sup> In light of the larger volume of body water in the neonate, the higher volumes of distribution for lipophilic compounds are at first surprising. The increase may be attributable to lower serum protein content, including albumin, which has a lower affinity for drugs than the adult form, lower lipopro-

tein concentrations, and higher bilirubin levels. These factors would reduce the carrying capacity of blood and increase the volume of distribution of lipophilic or other compounds transported in the blood bound to these proteins or lipoproteins.

Generally, reduced plasma protein binding relative to adult levels is observed in newborns.<sup>26</sup> Morselli et al. attributed reduced binding to several factors, including reduced total plasma proteins concentration, presence of fetal albumin, which has lower affinity for drugs, and lower concentrations of gamma globulins and lipoproteins. Gamma globulins are lower in newborns as well as infants.<sup>26</sup> Decreased binding to plasma proteins in neonates is responsible for a parallel increase in the volume of distribution of two modestly lipophilic compounds, phenobarbital, and phenylbutazone.<sup>101</sup> Similarly, reduced levels of serum protein binding of salicylate in newborns have been reported.<sup>24</sup> The volume of distribution of the hydrophilic compound, theophylline (adult 0.44 to 0.55, neonate 0.2 to 2.8), is ~three-fold higher in the neonate, attributable to a 30% lower level of protein binding in the neonate.<sup>13</sup> A lower fraction of protein-bound phenytoin (modestly lipophilic) in the neonate similarly leads to a two-fold increase in the volume of distribution.<sup>13</sup> The volume of distribution of gentamicin, a hydrophilic, weakly basic antibiotic with similar free fractions (or amount bound) in the neonate and adult, is ~2.5-fold higher in the neonate than the adult.<sup>13</sup> Amikacin, also hydrophilic, also has a Vd, which is ~2 times higher in infants and children compared with adults.<sup>26</sup> Other water-soluble compounds, the cephalosporins and penicillins, also have a higher volume of distribution in infants and children compared with adults. The increases are attributed to increases in body water, and in the case of penicillins additional reductions in protein binding.<sup>26</sup>

Kurz et al.<sup>98</sup> measured the adult and newborn cord plasma binding of 20 drugs composed of several drug classes (antibiotics, barbiturates, antihistamines, opiates, sulfonamides, benzodiazepines), which included lipophilic and hydrophilic compounds, to determine if the measured differences in blood constituents affected the free fraction. Differences were small, ranging from adult/newborn ratio of fraction bound of 1 to 2.4.<sup>98</sup> The differences in the extent of binding

**TABLE 5**  
**Ratio of Perinatal to Adult Volume of Distribution of Selected Pharmaceuticals**

Compound	$Vd_{\text{perinatal}}/$ $Vd_{\text{Adult}}$	Physicochemical Characteristic	Age of Perinatal Group
Fentanyl	1.3	Highly Lipophilic	Neonate < 1 mo
Fentanyl	1.9	Highly Lipophilic	Child 1-5 yr
Fentanyl	2.8	Highly Lipophilic	Infant 1-12 mo
Lorazepam	0.7	Moderately Lipophilic	Full term neonates
Ticarcillin	1.5	Moderately Lipophilic	1 mo - 2 yr
Ketamine	1.6	Moderately Lipophilic	3-12 mo
Metoclopramide	2.0	Moderately Lipophilic	Premature neonates, 1-7 weeks post-natal
Furosemide	2.1	Moderately Lipophilic	Premature neonates
Lignocaine	2.5	Moderately Lipophilic	Premature neonates, 9-42 days
Ketamine	4.6	Moderately Lipophilic	< 3 mo
Furosemide	7.3	Moderately Lipophilic	Neonates
Vancomycin	0.7	Not Classified	Premature, 4-17 days postnatal
Theophylline	1.4	Water Soluble	Neonates, 3-36 d
Theophylline	1.4	Water Soluble	Infants
Caffeine	1.6	Water Soluble	Premature neonates
Theophylline	1.6	Water Soluble	Neonates, 1-26 d
Clavulanic Acid	1.6	Water Soluble	1 mo - 2 yr
Tobramycin	2.3	Water Soluble	10-14 yr
Theophylline	2.3	Water Soluble	Neonates

were not due to differences in protein concentrations or the presence of ultrafiltrable plasma constituents, but may have been the result of higher bilirubin levels, which can compete effectively for drug binding sites on albumin. Kurz et al. extended this analysis by measuring differences in drug binding to the albumin and globulin fractions of adult and newborn plasma in the presence and absence of bilirubin. Of the six tested compounds, only promethazine, a highly lipophilic compound, had a difference in binding to the albumin fraction. Binding was higher to cord blood albumin. Bilirubin had no influence on the binding of three lipophilic compounds, promethazine, thiopental, or desipramine, or did it influence binding of water-soluble salicylic acid to albumin. Nevertheless, decreased binding of two hydrophilic compounds, nitrofurantoin and sulfamethoxydiazine, was observed. The effect was greatest in cord albumin from newborns. Binding of the lipophilic compounds (promethazine, thiopental, and desipramine) to newborn globulins was lower than to adult globulins. These three drugs have the highest octanol water partition coefficients (~14 to 600) of the test drugs. Reduced binding of these drugs to globulins in the newborn can be attributed to lower concentrations of lipoproteins in the globulin fraction. The water-soluble compounds (nitrofurantoin and meticillin) were not bound to adult or newborn globulins, reflecting their low octanol water partition coefficients (0.01 to 2). Collectively, these binding data demonstrate the quantitative differences in binding to plasma proteins that can lead to differences in measured volumes of distribution and clearance. Such differences are influenced by the physiochemical characteristics of the compound and the composition and concentration of plasma constituents, including albumin, globulins, and bilirubin. In addition, it has been suggested that competition between bilirubin and other toxicants may result in release of these compounds from albumin binding sites, unexpectedly high serum free concentrations and associated toxicity.<sup>26</sup> The volume of distribution of digoxin is 5 to 12 times lower in newborns than adults.<sup>26</sup> This difference has been attributed to several factors, including lower plasma protein binding, higher tissue binding, and larger extracellular fluid volume.<sup>26</sup>

*Elderly.* Searching a large database of pharmacokinetic parameters of drugs in the young and elderly developed by Ritchel et al.<sup>46</sup> revealed several drugs with significant differences in volumes of distribution. Consistent with the observed decrease in lean body mass in the elderly, changes in the volume of distribution correlated with lipophilicity. Lipophilic compounds, such as the benzodiazepines chlordiazepoxide, clobazam, and diazepam, as well as the aminoglycoside antibiotic, amikacin, had higher volumes of distribution in the elderly. Water-soluble drugs, such as propicillin, had a lower volume of distribution in the elderly reflecting a reduction in the relative size of the body water compartment. Amantadine, a tricycloamine of modest lipophilicity, had a lower volume of distribution in the elderly,<sup>106</sup> in contrast to expectations.

Age-associated reductions in serum-albumin concentrations increase the serum free fraction of the lipophilic benzodiazepines, chlordiazepoxide, diazepam, lorazepam, and alprazolam.<sup>95</sup> Proportionate increases in their hepatic clearance are observed, resulting in minimal changes in free concentrations at steady state.<sup>95</sup> For compounds whose hepatic clearance is restricted by protein binding, increases in free fractions resulting from reductions in binding proteins may not affect a change in serum-free concentrations at steady state because with the increases in free fraction comes a proportionate increase in clearance.<sup>46,95</sup> Chemicals that bind A1G or albumin, such as the lipophilic tricyclic antidepressants, have the capacity to free other bound compounds, increasing pharmacologically active free concentrations.<sup>95</sup> This has been observed for both water-soluble (salicylate, and sulfadiazine) and lipophilic drugs (phenylbutazone) in elderly patients undergoing multidrug treatments.<sup>46</sup>

Increases in A1G may increase binding of propranolol, which is significantly bound by A1G in the elderly,<sup>2</sup> although several studies found no difference in the amount of serum A1G, or the free or total concentrations of propranolol between healthy, young adults (25 to 33 years) and elderly adults (62 to 79 years).<sup>99,107-109</sup> Disease states common in the elderly induce increases in A1G and cause reductions in free fraction of propranolol.<sup>110</sup> Increases in the free fraction of propranolol can be attributed to disease-induced

changes in AIG, not to age-dependent changes in AIG. The volume of distribution of the hydrophilic antibiotic gentamicin is between 0.2 and 0.37 L/kg in both the young and elderly.<sup>111</sup> This is one of many water-soluble or modestly lipophilic pharmaceuticals (e.g., aspirin, digoxin, lidocain, theophylline) that present no difference in the Vd between the young and elderly,<sup>106</sup> demonstrating the influence of physiological changes in the elderly on distribution is chemical specific.

**Gender.** Gender differences in lean body mass, body fat, and to a lesser extent plasma protein concentrations are expected to lead to parallel differences in the disposition of drugs between males and females. Wilson's summary of gender differences in drug disposition indicates that for two lipophilic compounds, chlordiazepoxide and diazepam, the Vd is 27 to 28% higher in women.<sup>112</sup> In contrast, the Vd of more water-soluble, less lipophilic paracetamol is 36% lower in women compared with men. These changes are statistically significant. While the direction of the difference depends on the properties of the chemical, the magnitude of the difference appears to be reasonably similar. Gender differences for vancomycin, fluoroquinolones, and theophylline have also been documented<sup>10</sup>. The Vd of vancomycin (solubility not reported) is 11% higher in women.<sup>10</sup> The Vd of the hydrophilic fluoroquinolones, fleroxacin, levofloxacin and ofloxacin are each lower in the female, levofloxacin, being 15% lower. Similarly, theophylline, which is hydrophilic, has a Vd that is 10% or 22% smaller in young and elderly women, respectively, when compared with corresponding male groups.<sup>10</sup> The observed differences in the Vd for these compounds, both lipophilic and hydrophilic, are consistent with gender-specific differences in lean body mass. In fact, normalization of the Vd for lean body mass minimizes gender-specific differences in Vd for some chemicals.<sup>10</sup>

### C. Metabolism

The terms metabolism and biotransformation are often used interchangeably to refer to the enzymatic transformation of xenobiotics to metabolites. Metabolism may also refer to the total fate of a chemical in the body, including absorp-

tion, distribution, biotransformation, and excretion. However, in this section, metabolism will be used to refer to the process of enzymatic transformation of xenobiotics. There are two types of metabolic reactions catalyzed by enzymes: Phase I and Phase II.<sup>28</sup> Phase I reactions involve hydrolysis, reduction, or oxidation of the xenobiotic to expose or introduce a functional group. Phase II reactions involve glucuronidation, sulfation, acetylation, methylation, and conjugation with glutathione or amino acids. The Phase I and Phase II reactions and the enzymes involved in each are listed in Table 6. Some xenobiotics undergo both Phase I and Phase II reactions; however, a Phase II reaction does not have to be preceded by a Phase I reaction.<sup>28</sup> Both types of reactions may be influenced by variations in age and gender.

Metabolic enzymes are present throughout the body in several subcellular compartments.<sup>28</sup> In humans, the liver is the primary source of metabolic enzymes. However, metabolic enzymes can also be found at the major routes of entry for environmental contaminants, including the skin, lung, nasal mucosa, eye, and gastrointestinal tract.<sup>28</sup> In addition, enzymatic activity can also be seen in the kidney, adrenal glands, pancreas, spleen, heart, brain, testis, ovary, placenta, plasma, erythrocytes, platelets, lymphocytes, and aorta.<sup>28</sup> Chemicals may undergo metabolic transformation in these tissues, although the metabolic capacity of these tissues is limited when compared with the liver.

Both Phase I and Phase II reactions undergo maturation during the time between infancy and early childhood.<sup>24,89</sup> The process of aging, however, does not uniformly affect the hepatic metabolic capacity.<sup>8</sup> The Phase I and II metabolic pathways develop at different rates, vary among individuals, and can be induced *in utero*, making age-related changes in biotransformation complex and difficult to predict.<sup>24</sup> Total enzyme and esterase activity is decreased during the neonatal and postnatal periods.<sup>13</sup> Phase I activity is present at birth and gradually increases to adult levels by about 6 months of age. Phase II conjugation reactions are reduced at birth but activity does vary. For example, glucuronic acid is decreased at birth; however, sulfate conjugation is well expressed. Glycine conjugation in neonates is reported to be comparable to that of adults. Enzymes responsible

**TABLE 6**  
**Phase I and Phase II Metabolic Reactions and Associated Enzymes**

TYPE OF REACTION	ENZYME
Phase I	
Hydrolysis	Carboxylesterase
	Peptidase
	Epoxide hydrolase
Reduction	Axo-and nitro-reduction
	Carbonyl reduction
	Disulfide reduction
	Sulfoxide reduction
	Quinone reduction
	Reductive dehalogenation
Oxidation	Alcohol dehydrogenase
	Aldehyde dehydrogenase
	Aldehyde oxidase
	Xanthine oxidase
	Monoamine oxidase
	Diamine oxidase
	Prostaglandin H synthase
	Flavin Monooxygenase
Cytochrome P450	
Phase II	
	Glucoronide conjugation
	Sulfate conjugation
	Glutathione conjugation
	Amino acid conjugation
	Acylation
	Methylation

for theophylline oxidation and caffeine methylation are found in premature infants; however, enzymes responsible for oxidative demethylation do not develop until several months after birth. In general, drugs and chemicals that undergo Phase I-type reactions tend to have a slower clearance in the elderly, while Phase II-type reactions are not affected by the aging process.<sup>8</sup>

A review of the scientific literature indicated that data regarding age and gender differences in metabolism have been collected for several pharmaceutical agents, with considerably less data available for environmental chemicals. However, the data for the pharmaceutical agents can be used to demonstrate any age- and gender-specific dif-

ferences in metabolism. For an enzyme that acts on a particular chemical class or substrate (e.g., an enzyme that acts by adding hydroxyl groups to benzene rings), any quantitative age- and gender-specific difference in metabolism applicable to these pharmaceutical agents would also be relevant for structurally similar environmental chemicals.

### **1. Physiological and Biochemical Determinants of Metabolism**

While the majority of tissues have the capacity to metabolize some xenobiotics, the enzyme sys-

tems responsible for metabolism of foreign compounds are primarily present in the liver. While extrahepatic metabolism can contribute significantly to local metabolism and toxicity, this section focuses on hepatic metabolism because the liver has the highest metabolic capacity for xenobiotics, it is the central organ for systemic metabolism, and it is the most completely understood.

Two sources of blood deliver xenobiotics to the liver, arterial blood through the hepatic artery and venous flow through the portal vein. Xenobiotics absorbed through the intestinal tract may be delivered directly to the liver through the portal vein or transported within the triglyceride pool in chylomicrons to the vena cava via the thoracic duct. The delivery of compounds through the portal vein to the liver before entering the general venous blood compartment results in a first pass effect for this route of exposure. Compounds delivered to the liver enter hepatocytes primarily by passive diffusion, where they are subject to metabolism. The Phase II reactions typically increase transfer of the conjugated product across hepatic, intestinal, and renal membranes and, as a result, elimination. Phase I enzymes include the P450 family of proteins, esterases, epoxide hydrolase, and alcohol and aldehyde dehydrogenases, among others. Phase II enzymes include glucuronosyltransferases, sulfotransferases, and glutathione-S-transferases, among others.

The extent of metabolism depends on the concentration, composition, and metabolic constants ( $V_{\max}$ ,  $K_m$ ) of the hepatic enzymes, as well as subcellular localization and the rate of delivery (equal to the perfusion rate \* concentration). The maximum capacity ( $V_{\max}$ ) and Michaelis Constant ( $K_m$ ) are intrinsic properties of the enzyme and are not expected to change with age. The expression and concentration of Phase I and II enzymes in the liver are regulated by various factors, hormone status, for instance, that may change with age, increasing or decreasing the total metabolic capacity of the liver. The size of the liver also changes with age, leading to changes in total metabolic capacity, which in the absence of disease is proportional to body weight raised to the  $\frac{2}{3}$  power. Changes in hepatic blood flow can affect changes in the rate of delivery, and under some conditions (perfusion limited metabolism) changes in metabolism.

During aging and development changes in the size of the liver, the composition and concentration (collectively “expression”) of the Phase I and Phase II enzymes, as well as the hepatic blood flow, have the potential to influence the extent of metabolism. Age-dependent changes in these processes do occur and are described in the following sections, organized by enzyme system.

## **2. Influence of Age on Phase I Enzyme Systems**

Phase I reactions are catalyzed by the mixed function oxidase (MFO) system, including cytochrome P450, cytochrome b5, and NADPH cytochrome c reductase, which are present primarily in the adult, fetal, and neonatal liver.<sup>24</sup> In the fetus, premature, infant, and full-term neonate, cytochrome P450 is approximately 50 to 70% of the measured levels in adults.<sup>24</sup> NADPH cytochrome c reductase activity is lower in premature infants than in full-term infants, and both are lower than adult values.<sup>24</sup> Milsap and Jusko<sup>13</sup> reported that esterase activity in premature infants does not reach normal infant levels until 10 to 12 months of age.

*Cytochrome P450.* The cytochrome P450 family of enzymes is the primary enzyme system involved in Phase I metabolic reactions.<sup>28</sup> The P450 family consists of several different isoenzymes with varying degrees of substrate selectivity. Total cytochrome P450 activity in humans appears to remain stable from fetal development (both premature and full-term) through the first year of life.<sup>24,30</sup> One study reported similar cytochrome P450 activity between fetal and adult human liver.<sup>113</sup> However, other studies have indicated that total cytochrome P450 activity varies significantly between children and adults.<sup>113,114</sup> Hepatic cytochrome P450 activity levels in the fetus and neonate are reported to be approximately 50 to 70% of measured values in the adult.<sup>24</sup>

The influence of age on the activity of independent isoenzymes has been studied. Isoenzymes of the CYP4A and CYP3A families are the major isoenzymes detected readily in the fetal liver.<sup>115</sup> The relative concentration of CYP4A1 in the fetal liver was 40% or more of the adult concentration during the first week after birth.<sup>115</sup> The CYP3A

family of isoenzymes, consisting of CYP3A4, CYP3A5, and CYP3A7, is the most abundant P450 enzyme in human liver microsomes.<sup>30</sup> and is age dependent, with newborns having 25 to 50% less activity than adults.<sup>27,28</sup> CYP3A5 isoenzymes are present in embryonic liver tissue, and to a lesser extent, fetal liver tissue. Reports of one study showed 50% of infant liver expressed CYP3A5 activity, while only 29% of adult livers expressed CYP3A5 activity.<sup>30</sup> While variations have been reported in the concentrations of the isoenzymes in the CYP3A family, total CYP3A concentrations appear to remain constant from early gestation to adulthood.<sup>115,116</sup>

CYP3A7 has been detected in the fetal liver at 17 to 32 weeks of gestation and accounts for 36 to 85% of the total P450 present in the fetal liver.<sup>29</sup> The activity of CYP3A7 is positively correlated with testosterone 6-hydroxylase and dehydroepiandrosterone 16-hydroxylase activities.<sup>29</sup> Levels of CYP3A7 decline to adult levels at 3 to 12 months of age and CYP3A4, which is not present in the fetal liver, becomes the major P450 isoenzyme in the newborn and adult liver.<sup>29-31</sup> Levels of CYP3A4 begin to rise in the newborn around day 7 and peak at about 1 year of age.<sup>30</sup> LaCroix et al.<sup>116</sup> reported that CYP3A4 levels are very low at birth and reach 30 to 40% of adult levels by 1 month of age.

There is evidence that CYP3A4 levels are lowest in newborns and peak during adulthood.<sup>32</sup> Decreases in the levels of CYP3A4 have been noted in adults from the ages of 20 to 80 years.<sup>32</sup> However, other investigations concluded that there was no decrease in the activity of CYP3A4 with age in the human liver.<sup>33</sup> There is, however, a significant decrease in liver mass (35%), liver blood flow (35%), and volume (24 to 44%) between adulthood and late old age that could account for the decline in total systemic clearance of CYP3A4 prototype compounds.<sup>33</sup> Hepatic regional blood flow decreases by 0.3 to 1.5% per year after age 25.<sup>46</sup>

In addition to its presence in the fetal liver, CYP3A7 mRNA and protein have been detected in the endometrium and placenta.<sup>30</sup> The levels of CYP3A7 are higher during pregnancy and increase significantly from the first to the second trimester.<sup>30</sup> Compared with CYP3A7 levels in the fetal liver, the amount of CYP3A7 in the placenta

and the endometrium per gram of tissue ranged from 0.6% to 5.5%.<sup>30</sup>

The CYP3A family of isoenzymes is involved in the metabolism of a large group of xenobiotics and steroids.<sup>28</sup> In order to understand the effects related to the age-dependent variation of the CYP3A isoenzymes, one must be aware of the magnitude and variety of substances and compounds that are metabolized by the CYP3A isoenzyme family (Table 7).

Most of the interactions between CYP3A isoenzymes and its substrates are the result of either induction or inhibition of the CYP3A enzyme. Induction increases the content of the isoenzyme and enhances drug clearance. Substrates known to induce CYP3A4 activity *in vivo* and *in vitro* include corticosteroids, anticonvulsants, and several antimicrobials.<sup>30</sup> Compounds that are known to inhibit CYP3A4 both *in vivo* and *in vitro* include imidazole derivatives, erythromycin, clarithromycin, troleandomycin, gestodene, ritonavir, fluvoxamine, and grapefruit juice. Dexamethasone, rifampicin, and phenobarbital are known to induce CYP3A4 but do not appear to induce CYP3A5 activity. However, the CYP3A5 gene does contain the sequence information needed to corticosteroid regulation of transcription.<sup>30</sup> Triazolam, gestodone, ketoconazole, and fluconazole have been shown to inhibit CYP3A5 activity, as well as CYP3A4 activity. Rifampicin induces CYP3A7 expression in adult hepatocytes and gestodene inhibits its activity. Dose-dependent increases have been noted following pretreatment of HepG2 cells expressing CYP3A7 with dexamethasone, rifampicin, troleandomycin, erythromycin, phenobarbital, or lovastatin.<sup>30</sup>

When one considers the age-dependent variation of the CYP3A family and the magnitude of substrates related to this family of isoenzymes, it is obvious that age-dependent effects are important. This is especially true when treating infants and children with pharmaceutical substrates of the CYP3A family. Nifedipine, a CYP3A substrate and antihypertensive drug, has a shorter half-life in children age 5 to 68 months when compared with adults. Cyclosporin and tacrolimus, both CYP3A substrates, are immunosuppressants used in pediatric transplant patients to prevent rejection. Both drugs have a higher plasma clearance rate in children, requiring higher doses per kilogram body weight in younger children to maintain plasma concentrations equal to adults. Some

**TABLE 7**  
**Important Substrates of CYP3A Isoenzymes**

---

**Pharmaceuticals in the following categories:**

Antihistamines  
Antireflux  
Anti-emetic  
Anticonvulsants  
Anti-HIV  
Antimicrobials  
Antifungals  
Immunosuppressants  
Chemotherapeutics  
Benzodiazepines  
Anaesthesia-analgesics  
Antihypertensives  
Anti-arrhythmics  
Antidepressants

**Xenobiotics:**

Aflatoxin B1  
Benzphetamine  
Benzopyrene  
Heterocyclic amines  
Sterigmatocystin

**Endogenous substrates:**

Androstenedione (6 $\beta$ -hydroxylation)  
Cortisol (6 $\beta$ -hydroxylation)  
Dehydroepiandrosterone  
Dehydroepiandrosterone sulfate  
Estradiol  
17 $\beta$ -Ethinylestradiol  
Progesterone (6 $\beta$ -hydroxylation)  
Testosterone (2 $\beta$ -hydroxylation, 6 $\beta$ -hydroxylation, and 15 $\beta$ -hydroxylation)

---

Source: <sup>30</sup>.

of the variation in plasma clearance could be due to changes in body weight; however, it is also suggestive of increased hepatic and intestinal CYP3A activity in younger children.<sup>30</sup>

In studies performed by Jacqz-Aigrain and Cresteil,<sup>117</sup> results indicate that in adults, CYP3A4 is the major enzyme involved in the *N*-demethylation of dextromethorphan, and *O* and *N*-demethylations of dextromethorphan are carried out by isoenzymes in the CYP2D and CYP3A subfamilies, respectively. Using dextromethorphan as an indicator of the levels of CYP3A and CYP2D6 present, concentrations in the fetal and adult liver were compared. *O*-demethylation ac-

tivity was present in the fetal liver, but it did not exceed 5 to 10% of the adult values. During the first week after birth, *O*-demethylation rates increased to 25% of the adult levels. However, all of the fetal and neonatal liver preparations were active in the *N*-demethylation, reaching 30% of the adult values. Immunoinhibition studies using anti-CYP3A IgG showed that the anti-CYP3A IgG had only a small inhibitory effect on methoxymorphinan formation in fetuses. These results indicate the fetal liver contains a specific form of CYP3A with different catalytic properties for dextromethorphan than those in the adult liver.

CYP2D6 protein levels in fetuses and neonates of less than 24 h of age were reported to be less than 5% of adult values.<sup>117,118</sup> The levels of CYP2D6 begin to rise steadily during days 1 through 28 following birth. The increase observed in CYP2D6 was independent of gestational age at birth and reaches 2/3 of the adult value in infants aged 1 month to 5 years. A similar pattern was reported for CYP2C (Figure 7). Little or no protein content was reported in infants less than 24 h of age. However, CYP2C levels steadily increased during the first week of birth and reached 1/3 of the adult rate by the end of the first month.<sup>119</sup>

Other studies have shown variations in the CYP1A, CYP2C, and CYP2E isoenzyme family. Sonnier and Cresteil<sup>120</sup> examined the ontogenesis of CYP1A proteins in human fetal, neonatal, and adult liver samples. Results indicated that there was no CYP1A2 protein present in the fetal and early neonatal liver samples. In the first month of life, CYP1A2 was about 3% of the adult values. The levels began to increase during the next 3 months and were approximately 50% of adult values at the end of 1 year (Figure 8).

The developmental expression of CYP2E1 in the fetal and newborn liver was studied by Vieira et al.<sup>121</sup> Results showed that the CYP2E1 isoenzyme was not present in the fetal liver; however, a surge in the isoenzyme occurred during the first few hours after birth regardless of gestational age. At 1 year of age, the CYP2E1 levels reached approximately 40% of the adult levels (Figure 9). In contrast to the abrupt increase in CYP2E1 protein content, the CYP2E1 RNA moderately increased throughout early postnatal development. The concentration of CYP2E1 RNA was 15 times lower in early neonates when compared with adults with major increases occurring in newborns 1 to 3 months of age. At 3 to 12 months of age the infants reached 50% of the adult levels of CYP2E1 RNA.

In epilepsy patients ranging from 3 months to 29 years old, there is a significant correlation between age and the dose ratio of carbamazepine, an anticonvulsant drug. The metabolism of carbamazepine to carbamazepine-10, 11-epoxide is catalyzed by CYP3A4. In addition, there is some evidence that CYP3A7 also catalyzes this reaction based on the presence of the epoxide metabolite in stillborn fetuses of mothers receiv-

ing carbamazepine during pregnancy. An inverse relationship between the epoxide metabolite and carbamazepine also exists in children from 2 weeks to 15 years old being treated with carbamazepine.<sup>30</sup> Cisapride is a prokinetic drug metabolized primarily by CYP3A4. When given with other CYP3A4 inhibitors, cisapride has been shown to cause prolonged QTc intervals and serious ventricular arrhythmias, suggesting that low levels of CYP3A4 in infants predisposes them to concentration-related effects of the drug.

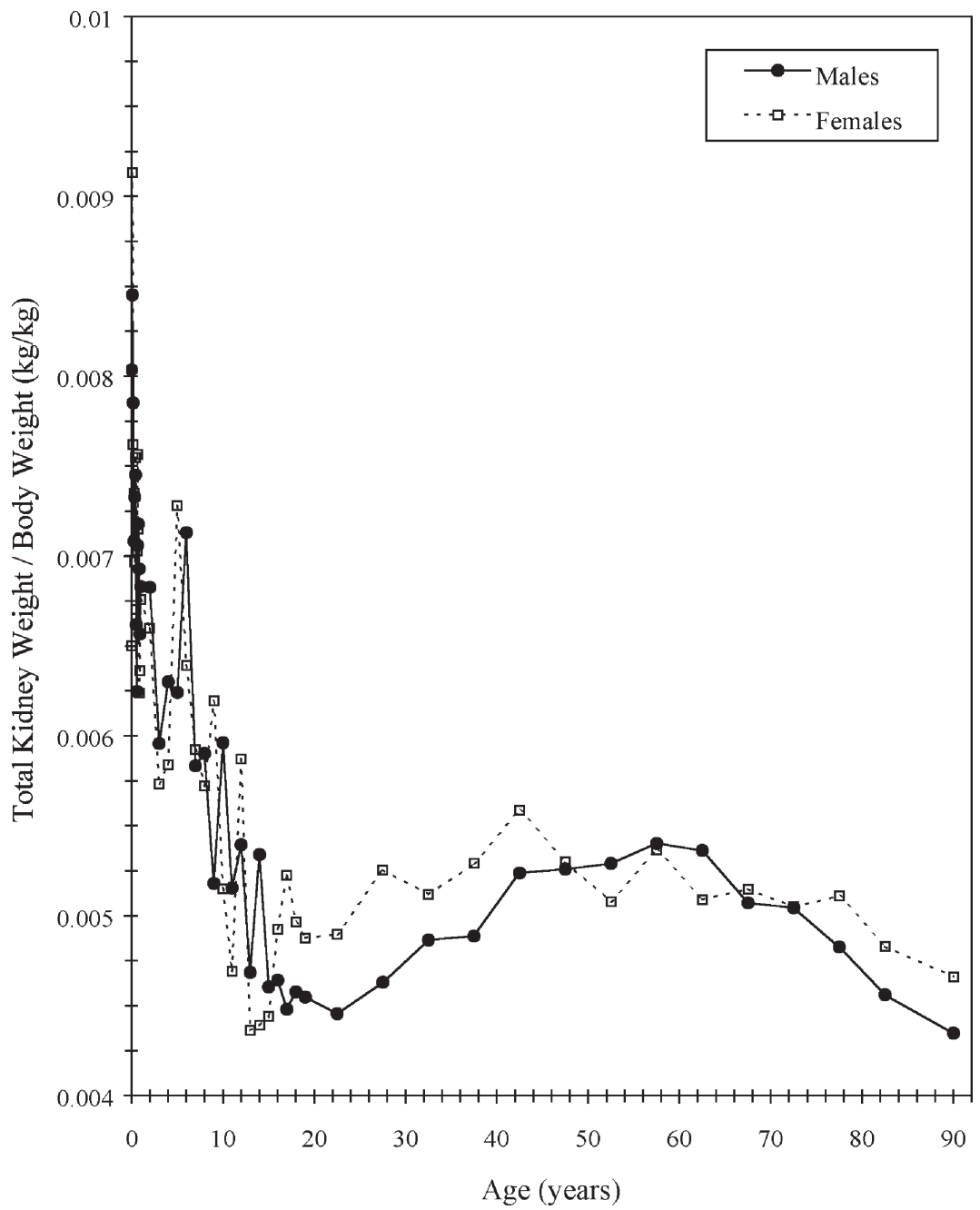
Although the compounds used in this example are pharmaceutical agents, a similar scenario could also be observed with environmental chemicals that are metabolized by CYP3A. Consequently, depending on the chemical and if the parent or metabolite is the toxic moiety, the fetus and neonate may be more or less sensitive to a toxic chemical that is metabolized by CYP3A.

*Influence of Age on Other Phase I Reactions.* Hydrolysis is a major biotransformation reaction for certain substrates, such as procaine, pethidine, meprobamate, paroxone, and acetylsalicylic acid.<sup>113</sup> Hydrolytic activity develops after birth in animals, in all organs, and in humans.

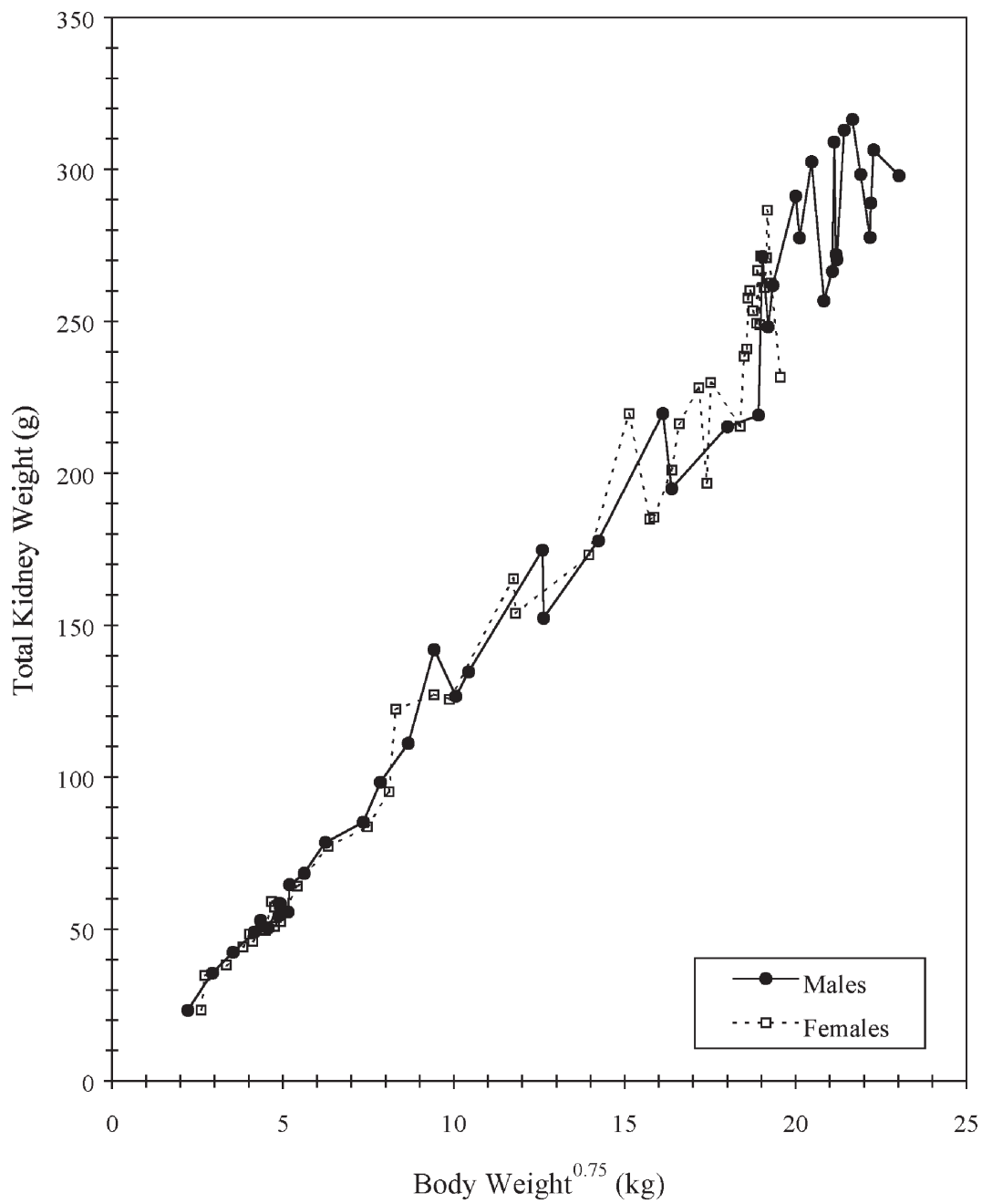
Esterases, which are involved in Phase I hydrolysis reactions, catalyze hydrolytic cleavage of ester bonds to produce carboxyl groups and an alcohol. The carboxyl groups and alcohols can then undergo Phase II conjugations. There are four main groups of esterases:<sup>122</sup>

- Arylesterases, which hydrolyze aromatic esters,
- Carboxylesterases, which hydrolyze aliphatic esters,
- Acylesterases, in which the acid group of the ester is acetic acid,
- Cholinesterases, which hydrolyze esters where the alcohol group is choline.

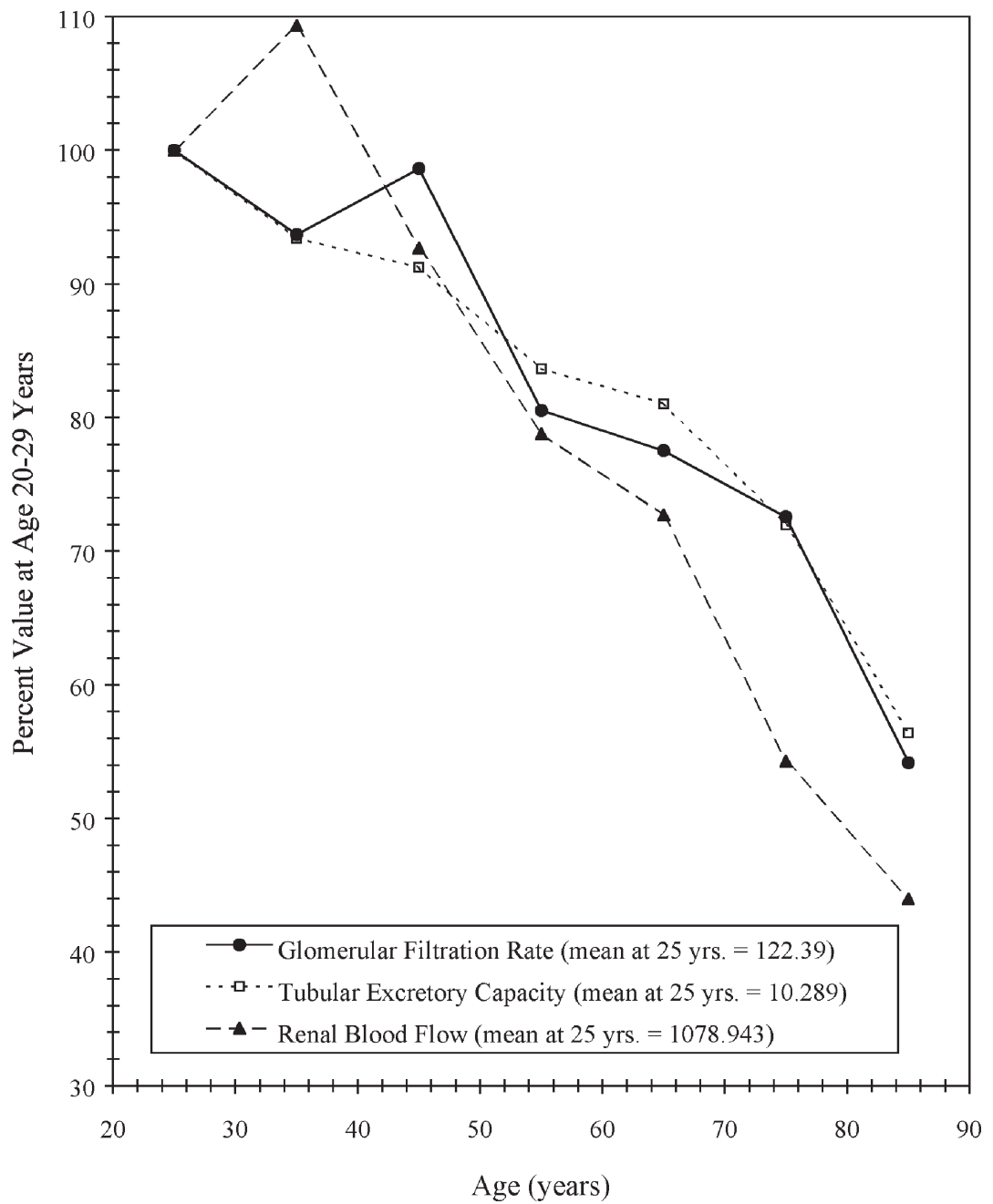
Many drugs and exogenous chemicals undergo esterase-catalyzed hydrolysis, including analgesics, steroids, local anesthetics, organophosphorous ester insecticides, and phthalates.<sup>34</sup> Studies have been performed to determine the age-related effects and perinatal development of red cell acetylcholinesterase (ACHE), plasma proteins, plasma pseudocholinesterase (ppACHE), and arylesterases (ArE).<sup>34</sup> Results showed an increase in ACHE plasma protein con-



**FIGURE 7.** The relationship between relative total kidney weight and age in Japanese from birth to old age. (Adapted from Ogiu *et al.*<sup>4</sup>)



**FIGURE 8.** Total kidney weight scales with body weight to the 0.75 power ( $BW^{0.75}$ ) from birth to old age. (Adapted from Ogiu *et al.*<sup>4</sup>)



**FIGURE 9.** Age-related decrease in renal function parameters. Numbers are reported as fraction of the value at maturity (20 to 29 years). (Adapted from Mayersohn.<sup>2</sup>)

centration between 28 and 40 weeks of gestation and a slower rate of increase until 1 year of age. Rapid increases in ppACHE and ArE occurred between 28 weeks of age to 1 year, with no changes occurring after 1 year. Red cell ACHE levels in infants were approximately 55% of adult values and ppACHE levels were 50 to 60% of adult values. At 34 weeks gestation ppACHE levels were comparable to infant values; however, at 28 to 31 weeks of gestation ppACHE levels were about 41% and 65% of the adult levels, respectively.

Plasma pseudocholinesterase is responsible for the hydrolysis of local anesthetics, including procaine. In an experiment to compare ppACHE activity with the rate of procaine hydrolysis, results indicated low hydrolysis activity at 28 weeks of gestation. These results correspond to the low levels of ppACHE at 28 to 31 weeks of gestation reported in the earlier study.<sup>34</sup>

Lower esterase activity has also been reported in premature infants, when compared with full-term infants. The decreased blood esterase levels and corresponding low hydrolysis rate could lead to an increase in the volume of distribution. This could account for the prolonged effect and cardio-respiratory depression in preterm infants administered drugs containing ester bonds.<sup>26</sup>

Dehydrogenation is another Phase I reaction important in the metabolism of certain alcohols. Alcohol dehydrogenase (ADH) is detectable in the human fetal liver in the second month of gestation at approximately 3% to 4% of the adult levels.<sup>113</sup> However, adult levels of ADH were not detected until 5 years of age (Table 8). Studies have shown that one, two, and four isoenzymes have been detected in the fetal, newborn, and adult liver, respectively.

### **3. Influence of Gender on Phase I Enzyme Systems**

*Cytochrome P450.* The cytochrome P450 system includes a subfamily of approximately 30 isoenzymes. Gender differences have been noted in some of these isoenzymes, including CYP1A2, CYP2A1, CYP2C19, CYP2D1, CYP2D6, CYP2E1, CYP3A4, CYP2C7, CYP2C12, CYP2C13, and CYP2C11. It has been suggested that the activity of most of these isoenzymes is greater in males when compared with

females.<sup>10,32,37,123</sup> However, conflicting information has been reported for CYP3A4 and CYP2C19 activity in males vs. females.<sup>32</sup> Other studies have shown that some isoenzymes are gender specific.<sup>124</sup> Several different factors have been investigated to determine the reason(s) for these gender differences. Some factors suggested to contribute to the gender differences include sex hormones, body weight, body water content, and diet.

One isoenzyme that has been shown to have gender-specific differences in activity is CYP1A. The CYP1A subfamily plays a primary role in the oxidative metabolism of some critical environmental contaminants, including polycyclic hydrocarbons, heterocyclic amines, and aromatic amines. The CYP1A2 isoenzyme is expressed in human tissues, with the liver being the main site of CYP1A2 expression.<sup>123</sup> Human CYP1A2 is an activator for a series of aromatic amines and heterocyclic amines during the metabolic activation of various procarcinogens and plays a major role in the activation of cigarette smoke condensate.<sup>35</sup> Human CYP1A2 has been shown to catalyze phenacetin *O*-deethylation, 4-aminobiphenyl (4-ABP) *N*-oxidation, ethoxyresorufin *O*-deethylation, and caffeine 3-demethylation.<sup>35</sup>

At least two mechanisms are suggested to regulate CYP1A2 activity, one that regulates constitutive levels of expression and one that regulates inducibility.<sup>123</sup> Both aryl hydrocarbon receptor (AhR)-dependent and AhR-independent pathways may be involved in CYP1A2 induction. It has been suggested that individual susceptibility to arylamine-induced cancers in humans is highly dependent on levels of hepatic CYP1A2.<sup>35</sup> Therefore, for metabolic activation of carcinogenic arylamines and arylamides, CYP1A2 isoenzyme is thought to be the primary P450 contributor.<sup>35</sup>

Studies have shown increased CYP1A2 activity in males, when compared with females.<sup>10,32,35,36</sup> Some believe this gender difference is due to the involvement of female hormones as demonstrated by decreased CYP1A2 activity in females using oral contraceptives vs. females not using them.<sup>123</sup> A lower CYP1A2 activity level has also been observed in females with caffeine toxicity; however, this decreased activity was not observed in males.<sup>123</sup> The half-life of theophylline, which is metabolized in part by CYP1A2,

**TABLE 8**  
**Development of Alcohol Dehydrogenase Activity in Fetal and Postnatal Human Liver**

	Age	ADH Activity	
		mU/g liver wet weight	mU/100 mg soluble protein
Fetal	2 - 3 months	111	97
	3 - 4 months	145	135
	3 - 4 months	155	147
	4 months	163	155
	4 months	211	201
	4 - 5 months	246	236
	4 - 5 months	239	228
	4 - 5 months	411	318
	5 - 6 months	328	321
Postnatal	0.3 months	495	550
	2 months	444	555
	7 months	797	1025
	2 years	620	1030
	5 years	3170	2830
	10 years	945	2360
	15 years	1940	3880
Adult	20 years	1625	2030
	50 years	2040	2550
	50 years	6530	5430

Source:<sup>145</sup>.

was approximately 1.5 times less in male subjects compared with females.<sup>37</sup>

Another theory noted for gender differentiation in CYP1A2 activity is diet.<sup>123</sup> Studies have shown decreased CYP1A2 activity in females consuming their normal diet when compared with males. However, following consumption of a controlled diet in males and females for 1 week, no gender difference in CYP1A2 activity levels was noted between males and females.<sup>123</sup>

The results of drug metabolism studies have indicated greater CYP1A2 activity in males when compared with females.<sup>10</sup> In one study, decreased CYP1A2 activity was suggested, based on two-fold higher plasma levels of fluvoxamine in females who received daily oral doses of 100 or 200 mg, when compared with plasma levels in males who received the same doses for the same time period.<sup>10</sup> In a second study, the same results were noted in the 100 mg/day group, but not in the 200 mg/day group. Because fluvoxamine is both metabolized in part by CYP1A2 and is a potent inhibitor of the CYP1A2 isoenzyme, autoinhibition of fluvoxamine metabolism could occur with increasing dose. The concentrations of clozapine and norclozapine were 35% higher in females undergoing steady-state therapeutic treatment for schizophrenia when compared with males involved in the same treatment regime. Again, these results suggest decreased CYP1A2 activity in females, because clozapine is mainly metabolized by the CYP1A2 isoenzyme.<sup>10</sup>

The CYP3A4 isoenzyme is also involved in the Phase I metabolism of numerous pharmaceutical agents. Over 50% of therapeutic drugs, including midazolam, triazolam, carbamazepine, lidocaine, erythromycin, and cyclosporin, are metabolized by CYP3A4.<sup>10,37</sup> The CYP3A4 isoenzyme is considered one of the most important isoenzymes in the P450 subfamily because it is found in critical tissues, including the gastrointestinal tract and liver.<sup>37</sup> The CYP3A4 isoenzyme accounts for 60% of the cytochrome enzymes located in the liver and 70% of those in enterocytes.<sup>10</sup> Conflicting gender differences in CYP3A4 activity have been reported. CYP3A4 activity in young females has been reported as approximately 1.4 times that of males, with this difference attributed to steroid hormones. Results of *in vitro* studies have shown that progesterone

induces CYP3A4 activity, while estrogen and progesterone can be competitive inhibitors of CYP3A4 activity, suggesting that the higher levels of CYP3A4 in women may be due to higher levels of progesterone in the females.<sup>10</sup> However, the results of other *in vitro* and clinical studies have also shown decreased CYP3A4 activity in males when compared with females.<sup>32</sup> Following multiple oral doses of 12 mg of sertindole, an antipsychotic drug primarily metabolized by CYP3A4 and CYP2D6, plasma sertindole concentrations were 20% higher in young females and 31% higher in elderly females compared with males, suggesting increased CYP3A4 and CYP2D6 activity in males.<sup>10</sup>

The CYP2D6 isoenzyme is another member of the P450 family that plays a role in Phase I metabolism of several compounds, such as debrisoquine, sparteine, imipramine, propranolol, and codeine.<sup>37</sup> The CYP2D6 isoenzyme displays a polymorphic pattern in the metabolism of these compounds. There is limited information on the influence of gender for the CYP2D6. Some studies have suggested that males have higher CYP2D6 activity than females. Males were reported to have a higher rate of clomipramine metabolism, which is mediated by CYP2D6 and CYP2C19 when compared with females.<sup>37</sup> Racemic propranolol, metabolized through ring oxidation by CYP2D6 and side chain oxidation by CYP2C and CYP1A and glucuronidation, was metabolized faster in males compared with females. In addition, the oral clearance of propranolol was 63% higher in males compared with females and CYP-mediated side-chain oxidation clearance was 137% higher in males compared with females. The mechanisms for these differences are not clear; however, they suggest that men have higher levels of CYP2D6 activity than women.

The isoenzyme CYP2C19 also demonstrates a polymorphic pattern of metabolism and greater activity has been reported in males vs. females.<sup>10,37</sup> CYP2C19 is responsible for the aromatic ring hydroxylation of *S*-mephenytoin, an anticonvulsant. CYP2C19 is stereoselective for the *S*-enantiomer.<sup>125</sup> Chemicals may also undergo *N*-demethylation by CYP2C19, including diazepam, citalopram, clomipramine, amitriptyline, imipramine, *S*-mephenytoin, methylphenobarbital, and propranolol.<sup>37</sup> An increased rate of mephobar-

bital metabolism was reported in males who received a single oral dose (400 mg) of racemic mephobarbital when compared with females. Males were also reported with greater CYP2C19 activity in piroxicam metabolism, because females exhibited higher plasma concentrations of piroxicam when compared with males. Also, in a phenotypic study involving 166 individuals, males had lower 5-mephenytoin 4'-hydroxylation than females, following a single oral dose of mephenytoin (100 mg), therefore suggesting greater CYP2C19 activity in males. However, some reports have shown an increased CYP2C19 activity in females when compared with males.<sup>32</sup>

The isoenzyme CYP2E1 is responsible for the metabolism of ethanol and a large number of other halogenated alkanes.<sup>28</sup> Increased activity of the CYP2E1 isoenzyme has been reported in males when compared with the activity levels determined in females.<sup>37</sup> The metabolism of chlorzoxazone was reported to be one-third greater in males than females, indicating a higher rate of CYP2E1 activity in males; however, this difference was minimized when normalized according to body weight.<sup>37</sup>

*Influence of Gender on Other Phase I Reactions.* It has been suggested that aldo-ketoreductase activity is higher in males when compared with females.<sup>10</sup> This suggestion was based on an approximate two-fold greater clearance of doxorubicin in males (59 L/h/m<sup>2</sup>) when compared with females (27 L/h/m<sup>2</sup>), with gender accounting for half of the variability as denoted by a multivariate analysis of the data. This was also confirmed by the higher proportion of doxorubicinol, the major metabolite of doxorubicin, detected in males when compared with females.

Higher ADH activity has also been suggested to occur in males when compared with females.<sup>10</sup> Following ethanol dosing, females had greater blood alcohol levels when compared with males. Because ADH oxidizes simple alcohols, such as ethanol, a greater ADH activity is suggested to occur in males when compared with females. However, other factors that may contribute to the differences observed in blood alcohol levels include body weight, body water content, rate of metabolism, and sex hormones.

Recent investigations have suggested that females have a greater rate of methylation of

drugs than males.<sup>126</sup> In a population exposed to inorganic arsenic in the drinking water, females had approximately 3% more dimethylarsinic acid (DMA) in the urine when compared with males. This suggests that females have a higher rate of methylation of arsenic when compared with males.

The results of the pharmacokinetic studies conducted with various pharmaceutical agents have demonstrated age- and gender-specific differences in the activity of several different P450 isozymes. Although the examples provided above are for drugs, it is likely that these observed differences would also apply to environmental chemicals. Consequently, when exposed to an environmental chemical metabolized by one of these isozymes, differences in sensitivity to the toxicity of that chemical could result. For example, the data suggest that CYP1A2 levels are higher in males than females. Therefore, males could metabolize chemicals that are CYP1A2 substrates faster than females. Depending on the chemical, this could serve to detoxify the parent or activate a metabolite.

#### **4. Influence of Age and Gender on Phase II Enzyme Systems**

*Glutathione Transferase.* The tripeptide glutathione is involved in the conjugation of an enormous array of electrophilic xenobiotics and xenobiotics that can be biotransformed to electrophiles.<sup>28</sup> Glutathione transferase catalyzes the conjugation of xenobiotics with glutathione by converting glutathione to the glutathione thiolate anion through deprotonation. Substrates for glutathione transferase are hydrophobic, contain an electrophilic atom, and react nonenzymatically with glutathione.<sup>28</sup>

Rane and Pacifici<sup>41</sup> studied the metabolism of styrene oxide in human fetal livers and different extrahepatic human fetal tissues obtained from legal abortions. In liver preparations, microsomal hydrazase and glutathione-S-epoxide transferase activity were detected in 7 to 10 aborted fetuses, with activity levels in fetal livers ranging from 1.5 to 7.8 nmol/min/mg protein. The microsomal styrene oxide hydrazase activity in the lungs, kidneys, gut, and the placenta was less than 10% of the hepatic activity. However, glutathione-S-epoxide

transferase activity levels were similar in all tissues examined.

Glutathione-*S*-transferase activity toward styrene oxide in adults has been reported by several authors and is higher than in fetal/neonatal tissue.<sup>39,40</sup> Adult liver hepatic glutathione-*S*-transferase activity toward styrene oxide was reported by Mendrala et al. to be from 3 to 40 nmol/min/mg protein, and by Pacifici et al. to be 25 nmol/min/mg protein.<sup>39,40</sup>

*Sulfotransferase.* Sulfation is catalyzed by sulfotransferases, which are soluble enzymes found in the liver, kidney, intestine, lung, platelets, and brain.<sup>28</sup> The process of sulfation transfers the SO<sub>3</sub> group from 3'-phosphoadenosine-5'-phosphosulfate to the xenobiotic, producing a highly water-soluble sulfuric acid ester.<sup>28</sup> Sulfate conjugation primarily involves the biotransformation of phenols, alcohols, and primary amines, which are often products of Phase I reactions.<sup>28,34</sup> However, some compounds are sulfated without prior Phase I transformation and include compounds such as primary alcohols, secondary alcohols, catechols, nitrogen oxide, aliphatic amines, aromatic amines, aromatic hydroxylamine, and aromatic hydroxyamide. It is not uncommon for sulfate conjugation to precede or occur simultaneously with glucuronidation, another type of Phase II biotransformation.<sup>28</sup>

No data were located noting gender differences in the sulfation process. However, age-related differences in sulfation rates have been noted in children and adults and are probably best illustrated in the metabolism of acetaminophen. Acetaminophen undergoes both glucuronidation and sulfation during biotransformation.<sup>34</sup> The overall elimination of acetaminophen does not appear to change from infancy to adulthood; however, the dominant metabolic pathway does appear to change.<sup>42</sup> Acetaminophen appears to be primarily metabolized via the sulfation pathway until approximately age 9, and the glucuronide pathway becomes the primary metabolic route at around age 12.<sup>34</sup> Studies show that older children (>12 years old) and adults eliminate about 50% of the acetaminophen dose as glucuronide conjugates and about 30% as sulfate conjugates. In infants and children (<12 years old) about 45 to 55% of the acetaminophen dose is eliminated as a sulfate conjugate and 18 to 30% as glucuronide conju-

gates.<sup>42</sup> This suggests that in neonates and young children the glucuronidation rate is lower and the sulfation rate is higher compared with adults.

*Glucuronyl Transferase.* Glucuronidation is one of the most important Phase II conjugation reactions, both quantitatively and qualitatively.<sup>28</sup> This is due to the vast number of substrates that are able to participate in this reaction and the diversity of the acceptor groups. During this process glucuronyl transferases transform endogenous and exogenous compounds to polar, water-soluble compounds that are then eliminated in the urine or bile.<sup>28</sup>

Glucuronidation is decreased in children when compared with adults,<sup>27,38,127</sup> however, adult values are reached by the third or fourth year of life.<sup>24</sup> In the human fetus, the liver has been reported as the most active site of glucuronidation.

Low levels of glucuronidation activity are seen in fetal and newborn livers. It has been reported that levels of UDP-glucuronyltransferase, a catalyst for conjugation with glucuronic acid, are undetectable or 20% less than that of adult levels in fetal tissues during the first half of gestation.<sup>38</sup> Prior to birth, UDP-glucuronyltransferase activity was shown to be higher than that of adults for substrates such as *p*-nitrophenol, aminophenol, *o*-aminobenzoate, and 1-naphthol. This activity then decreased to adult levels following birth. However, for substrates such as bilirubin, morphine, and a number of steroids, UDP-glucuronyltransferase activity was shown to develop after birth and did not exceed adult levels.

In three premature (25 to 32 weeks of gestation) infants who died 5 min to 98 h after birth, UDPG-T was virtually absent in the liver.<sup>38</sup> Hepatic bilirubin UDPG-T activity was reduced in living newborns 2 to 42 days old, with congenital gastrointestinal obstruction. It was also reported that human fetuses aged 8 to 22 weeks had markedly decreased hepatic activities of both bilirubin UDPG-T and UDPG-D when compared with normal adults.

Calabrese<sup>34</sup> reported that adult levels of glucuronidation activity are reached by 3 to 4 months of age; however, other studies have reported that adult levels of glucuronic acid are not reached until 3 to 4 years of age.<sup>24,26</sup> Klinger<sup>113</sup> reported that at 4 months gestation, glucuronyltransferase activity is detectable and glucuronidation capacity is fully developed in

the human fetal liver. The low glucuronidation activity in fetuses and newborns decreases bilirubin excretion, which could lead to hyperbilirubinemia in infants, especially premature infants.<sup>34</sup> The effects of low levels of glucuronic acid in the fetus and newborn can also be seen following exposure to trichloroethylene (TCE), a widely used halogenated hydrocarbon.<sup>128</sup> The metabolic pathway of TCE involves the CYP2E1 isoenzyme, ADH, aldehyde dehydrogenase (ALDH), and glucuronidation. The presence and activity of CYP2E1 in the young are still unclear, and study results are contradictory. However, it is known that glucuronidation and ADH activity are reduced in newborns. Decreases in glucuronidation in infants exposed to TCE could increase the formation of the toxic metabolites, trichloroacetic acid and dichloroacetic acid.<sup>128</sup> In addition, the decrease in ADH activity affects the clearance of chloral hydrate. The reported half-life of chloral hydrate was 39.8 h in the preterm fetus, 27.8 h in the neonate, and 9.7 h in children ages 1 to 13 years.<sup>128</sup>

In the case of chloramphenicol, decreased glucuronidation resulted in the accumulation of the parent compound in children and led to toxicity.<sup>24,27,34</sup> Chloramphenicol has been known to produce death in premature infants being treated for infections with the antibiotic.<sup>34</sup> In the case of morphine, which is also metabolized by glucuronidation, the metabolite has greater toxicity. Therefore, children show lower plasma levels of the toxic metabolite than adults exposed to morphine.<sup>27</sup>

Gender differences are more easily detected in drugs that are directly metabolized by a Phase II reaction.<sup>28</sup> Reports have suggested that glucuronyl transferase activity is higher in males when compared with females.<sup>10</sup> However, this is only true for some isoenzymes, and therefore only for certain drugs.

**Glycine Conjugation.** Glycine conjugation involves the conjugation of xenobiotics containing a carboxylic acid group with the amino group of glycine.<sup>28</sup> Klinger<sup>113</sup> reported that by 8 weeks of age, normal children reach adult activity levels of glycine conjugation. Conjugation between aromatic carboxyl groups and an  $\alpha$ -amino group of amino acids (mainly glycine) has been observed in neonates, with adult values reached by 6 months

of age.<sup>24</sup> Following aspirin administration, concentrations of salicylic acid were greater in females when compared with males, therefore suggesting a higher metabolic activity for glycine conjugation in males.<sup>10</sup>

**Acetylation.** Acetylation involves the biotransformation of xenobiotics containing an aromatic amine or hydrazine group.<sup>28</sup> The aromatic amine group is transformed to an aromatic amide and the hydrazine group is transformed to a hydrazide. *N*-acetylation is catalyzed by *N*-acetyl transferase and requires the cofactor acetyl-coenzyme A. The biotransformation occurs in two steps. First, the acetyl group from the coenzyme is transferred to an active site cysteine residue within an *N*-acetyl transferase with release of coenzyme A. Then the acetyl group is transferred from the acetylated enzyme to the amino group of the substrate with regeneration of the enzyme.<sup>28</sup> Premature and mature newborns have been reported with a low acetylation rate for sulfonamides.<sup>113</sup> However, this hepatic acetylation rate increases postnatally.

## D. Elimination

Elimination of chemicals can occur through the kidney (urine), liver (bile), lungs (air), oral cavity (saliva), mammary glands (breast milk), hair, and skin. For drugs and other xenobiotics, routes other than the kidney are generally of minor importance.<sup>43,46</sup> Fecal elimination, which is dominated by biliary excretion, although typically less important than renal elimination, can be important for some classes of compounds.<sup>43</sup> The kidney is the main route of excretion of water-soluble compounds and the water-soluble metabolites of lipophilic compounds.<sup>46,129</sup> Lipophilic compounds, presumably the nonbound serum fraction, can also be excreted by the renal system.<sup>3</sup> However, because clearance is typically reported as total clearance rather than  $Cl_{ren}$ , attributing changes in total clearance ( $Cl_{tot}$ ) to changes in  $Cl_{ren}$  should be done only when  $Cl_{ren}$  dominates  $Cl_{tot}$ .<sup>46</sup> Lactational elimination occurs by passive diffusion of non-ionized or lipophilic compounds. Lipophilic compounds diffuse with dietary fats into milk, where the excreted compound becomes a source of exposure for the neonate. While some patterns of biliary excretion have been described,

for instance, higher probability of biliary excretion for compounds with molecular weights greater than 325 g/m compared with lower molecular weight compounds, predicting biliary excretion by class of compound is not possible. The chemical properties that determine whether a compound is excreted into the bile are poorly understood.<sup>43</sup> In addition, biliary excretion does not equate directly with elimination because reuptake of the compound from the intestine, particularly those that are sufficiently lipophilic, is possible. This section focuses on renal and lactational elimination, reflecting the importance of the renal system for elimination of xenobiotics and the importance of lactational elimination for perinatal exposure. Other routes of elimination are not addressed because of limited information, and in the case of biliary excretion the difficulty in predicting biliary excretion by physicochemical characteristics and with equating excretion by this route with elimination.

### **1. Physiological Determinants of Renal Clearance**

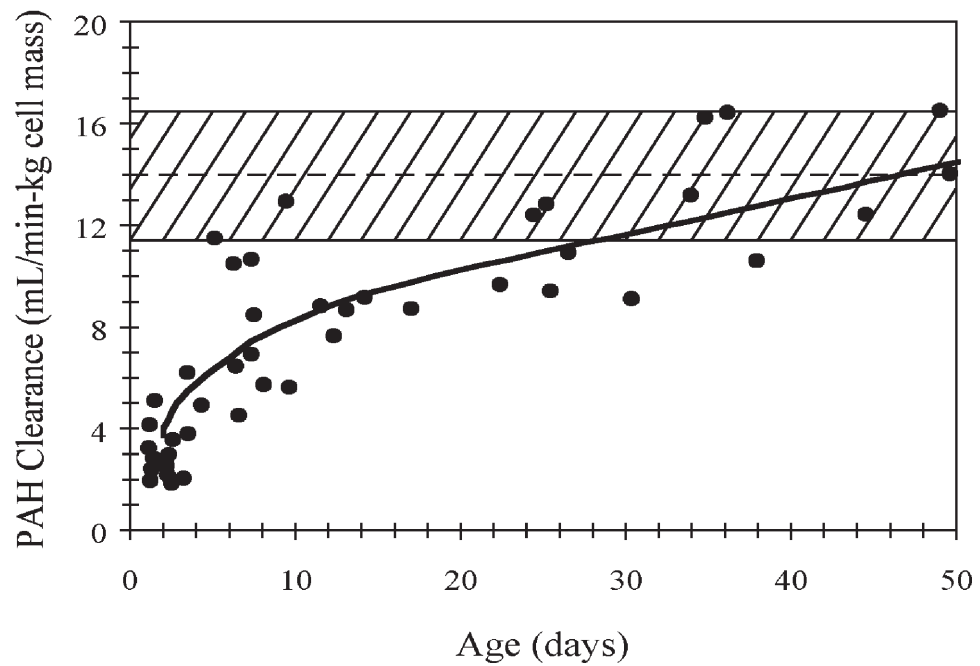
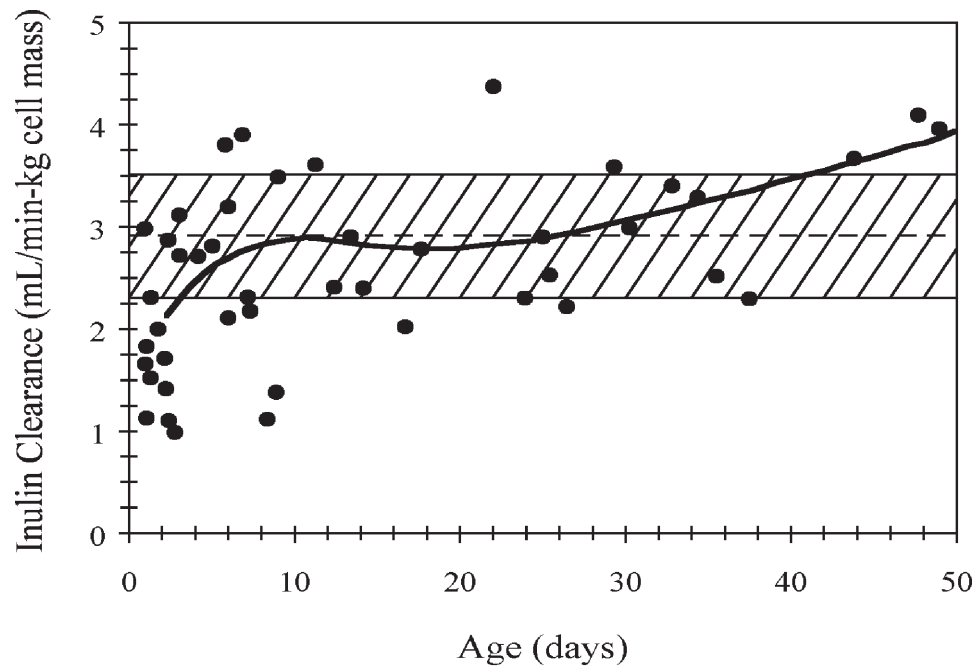
Renal clearance is a function of three major processes, glomerular filtration, tubular secretion, and tubular reuptake. Two processes, glomerular filtration and tubular secretion, move compounds to the proximal tubule and eventually to the urine, while tubular reuptake moves compounds from the lumen of the proximal tubule back to the tubular cell. This latter process occurs by passive diffusion and is limited to nonionic compounds.<sup>3</sup> Binding of compounds, the best studied of which are pharmaceuticals, to serum proteins diminishes renal elimination.<sup>3</sup> The net renal clearance is the difference between the rates of glomerular filtration, tubular secretion, and tubular reuptake.

Glomerular filtration is the transport of material from the renal blood flow, through the pores of the glomerulus into the proximal tubule. Renal blood flow, size of the kidney (number of nephrons), and maturity of the glomerulus all influence the effective GFR. This process accounts for a maximum of 20% of the total excretion capacity of the renal system under “conditions of maximum load”.<sup>3</sup> Tubular secretion is an energy-dependent, active-transport process with unique

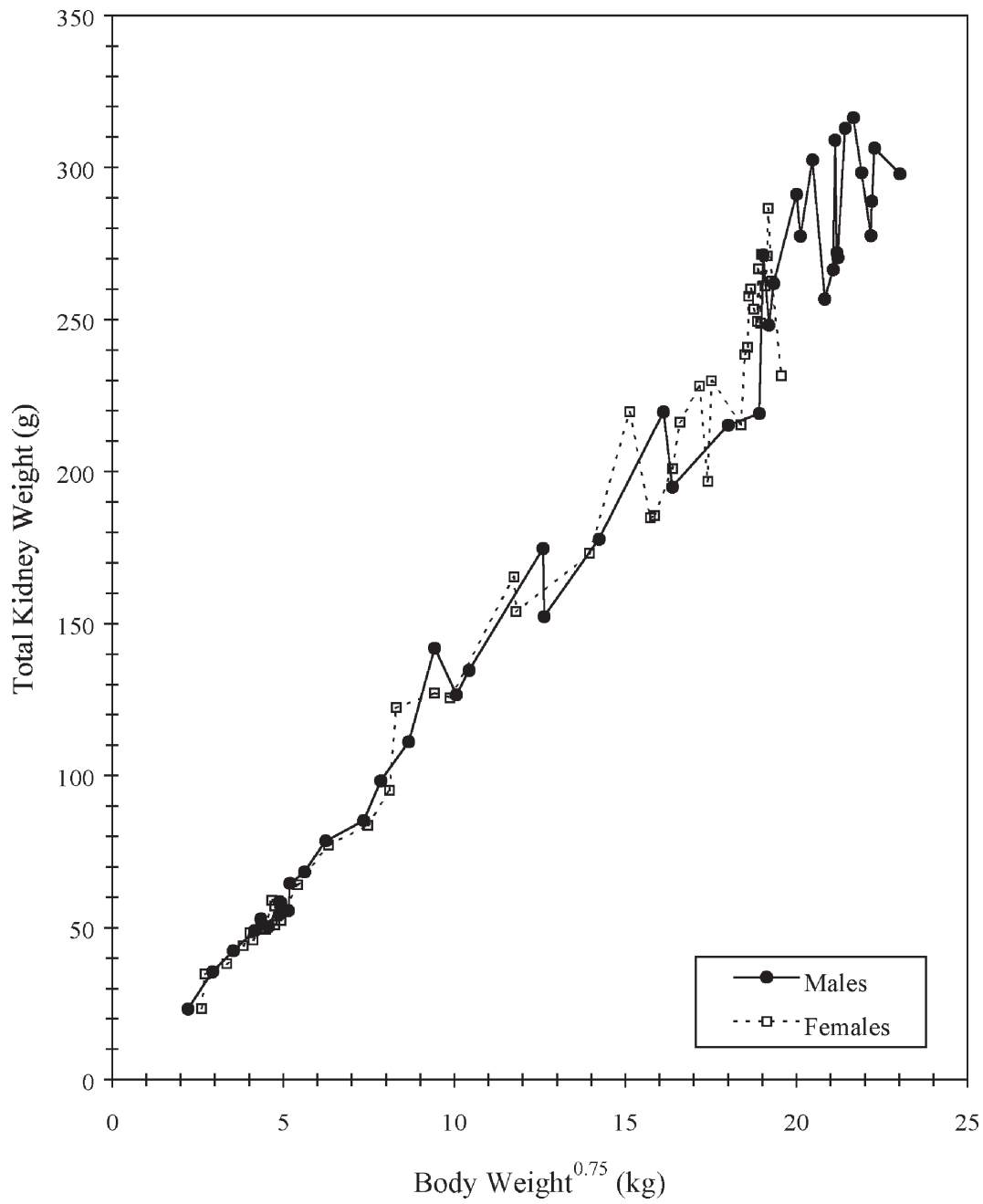
systems for weak organic acids and weak organic bases.<sup>3</sup> Tubular secretion is activated when blood/plasma concentrations of a compound exceed a certain level. The process has a maximum transport value,  $T_m$ , which is characterized as being very high but limited. For chemicals that are 80% eliminated by tubular secretion rather than simple glomerular filtration, saturation of the tubular secretion process can easily be reached.<sup>3</sup> The capacity of tubular secretion is dependent on the mass and length of tubular cells, blood flow to the peritubular area, and the functioning of the necessary energy-producing process.<sup>3</sup> Tubular reabsorption is a passive process of diffusion that is selective for nonionic compounds, and therefore is dependent on urine pH.<sup>3</sup> Renal blood flow, kidney size, GFR, active tubular secretion, and reabsorption decrease with age, with the attenuation beginning between the second and fourth.<sup>130</sup> Renal clearance of drugs that are cleared predominantly by the kidney system is expected to parallel the maturation<sup>13</sup> and decline of the renal system.

### **2. Influence of Age and Gender on Processes That Influence Renal Elimination**

The processes governing renal function, GFR, tubular secretion, and tubular absorption, which are themselves a function of kidney size, the number and size of nephrons, and renal blood flow, among others, are immature at birth. The absolute weight of the kidneys in newborns is 21.5 g (female) to 24.1 g (male). The ratio of kidney weight to body mass in newborns is two-fold that of adults.<sup>26</sup> Kidney size rises rapidly after birth, increasing approximately three-fold by 9 to 12 months of age, and then enters a long phase of slower growth in proportion to body weight, which slows between the ages of 20 and 40 years<sup>1</sup> (Figure 4). From birth through adolescence, kidney weight as a fraction of body weight drops (Figure 10). At birth, kidney weight is 1% of body weight. The comparable adult level is 0.5%.<sup>24</sup> The drop in fractional kidney weight is expected; kidney weight scales with body weight to the  $\alpha$  power (Figure 11), and so growth occurs in proportion to body weight to the  $3/4$  power.



**FIGURE 10.** Increase in renal function during the early postnatal period: (a) glomerular filtration rate, and (b) tubular secretion. Hatched area represents adult values. (Adapted directly from Braunlich.<sup>3</sup>)



**FIGURE 11.** Total kidney weight scales with body weight to the 0.75 power ( $BW^{0.75}$ ) from birth to old age. (Adapted from Ogiu *et al.*<sup>4</sup>)

Renal blood flow is very low, and vascular resistance is high at birth.<sup>26</sup> Increases in cardiac output and reductions in vascular resistance result in increases in renal blood flow.<sup>26</sup> After reaching adult levels, renal blood flow decreases 1.1 to 1.9% per year after age 25<sup>46</sup> (Figure 12).

The reduced GFR in newborns is the result of the relatively small size and number of glomeruli, immature conditions of the nephrons, low renal blood flow, and the reduced rate of functioning glomeruli.<sup>3</sup> GFR increases rapidly after birth in response to increases in renal blood flow<sup>26</sup> and is more advanced than tubular absorption at birth (Figure 13).<sup>13,26</sup> This difference in maturation between the two processes disappears after 6 months. GFR at birth is between 2 to 4 ml/min, rising up to four-fold in the first 72 h of life<sup>13</sup> to 8 to 20 ml/min.<sup>6</sup> When normalized to kidney mass (ml/min/kg cell mass), GFR reaches adult levels between 3 and 10 days of age.<sup>3</sup>

Glomerular filtration decreases approximately 0.66% per year after age 30, as measured by inulin clearance, almost identical in magnitude with the reduction of the maximum tubular secretion capacity—0.62%—supporting the hypothesis that the nephron loses its activity as a unit (Figure 12). Several equations relate age, body weight, and creatinine serum concentrations to creatinine clearance (a surrogate for GFR):

$$Cl_{Creat} = \frac{(140 - Age) * BW}{72 * S_{creat}} * GenderFactor$$

Equation 1

where  $Cl_{creat}$  is the creatinine clearance, age is in years, body weight (BW) is in kg, gender factor is 1 for males and 0.85 for females,<sup>131</sup> and  $S_{creat}$  is the serum creatinine concentration (mg/dL).<sup>46</sup> Recently, alternative measures of GFR using serum cystatin C concentrations, an endogenous compound that provides a measure of GFR, which is not confounded by differences in lean body mass, have been used.<sup>132</sup> Gender-related differences in GFR are not observed when serum cystatin C concentrations are used.<sup>132</sup>

Some authors report a modestly reduced GFR in women compared with men, when normalized by surface area.<sup>10</sup> Beierle et al. noted that attributing these differences solely to gender and not other factors, such as body weight, is not feasible.

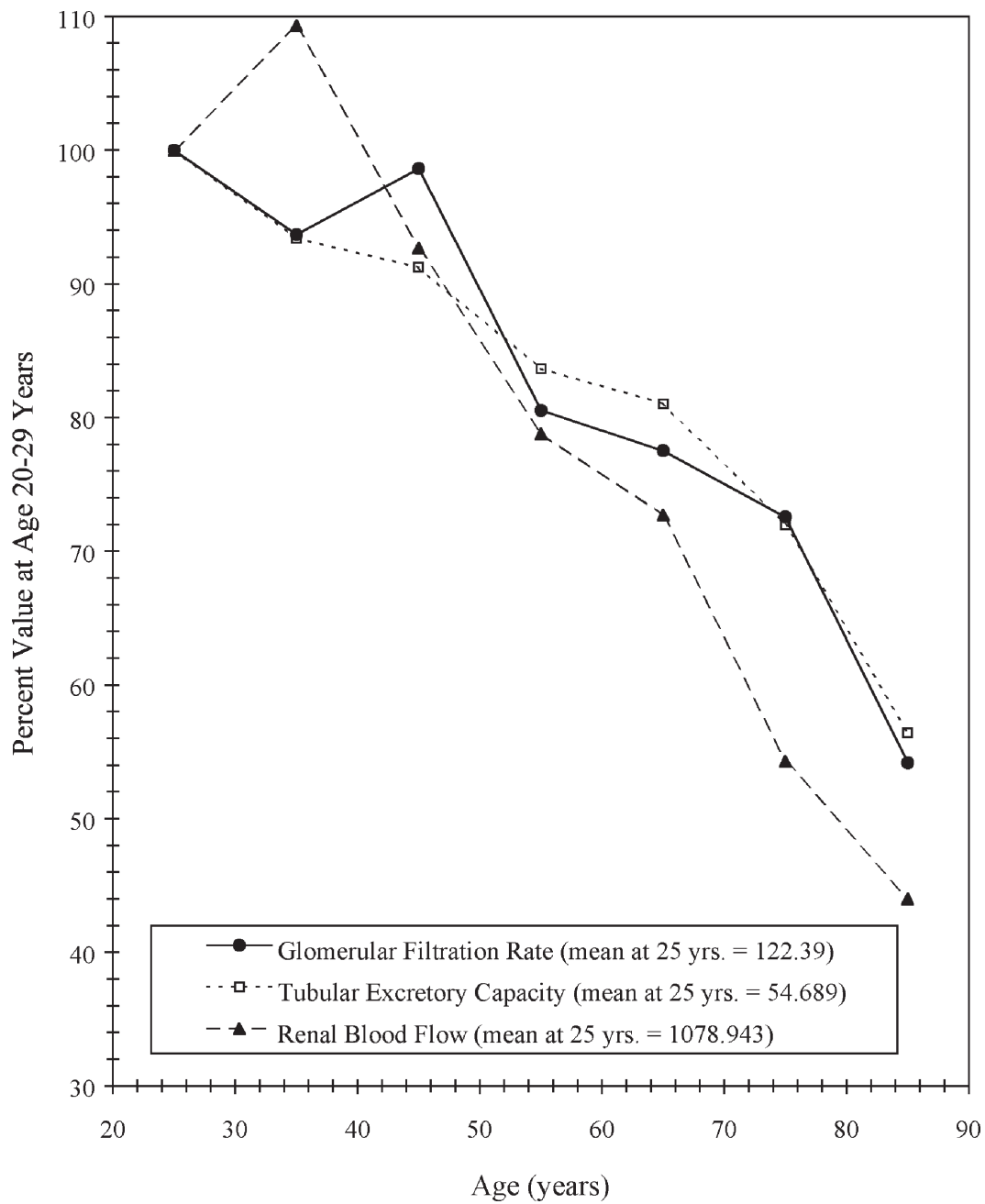
These other factors may also contribute to differences in GFR. Glomerular filtration may rise 50% during pregnancy.<sup>133</sup> and is reported to vary with menstrual cycle stage in one of two studies.<sup>133</sup>

Tubular secretion of common blood solutes—glucose, phosphate, and bicarbonate—as well as PAH (para amino hippuric acid) is reduced in neonates, but reaches childhood levels at 30 to 40 weeks of age<sup>26</sup> (Figure 13). Factors contributing to reduced tubular secretion include low blood flow to the peritubular region, immaturity of the active transport system, small mass of tubular working cells, and smaller size of the proximal tubules.<sup>26</sup> Passive reabsorption may also be reduced in the newborn.<sup>26</sup> Maximum tubular secretion capacity decreases approximately 0.62% per year after age 30 (Figure 12).

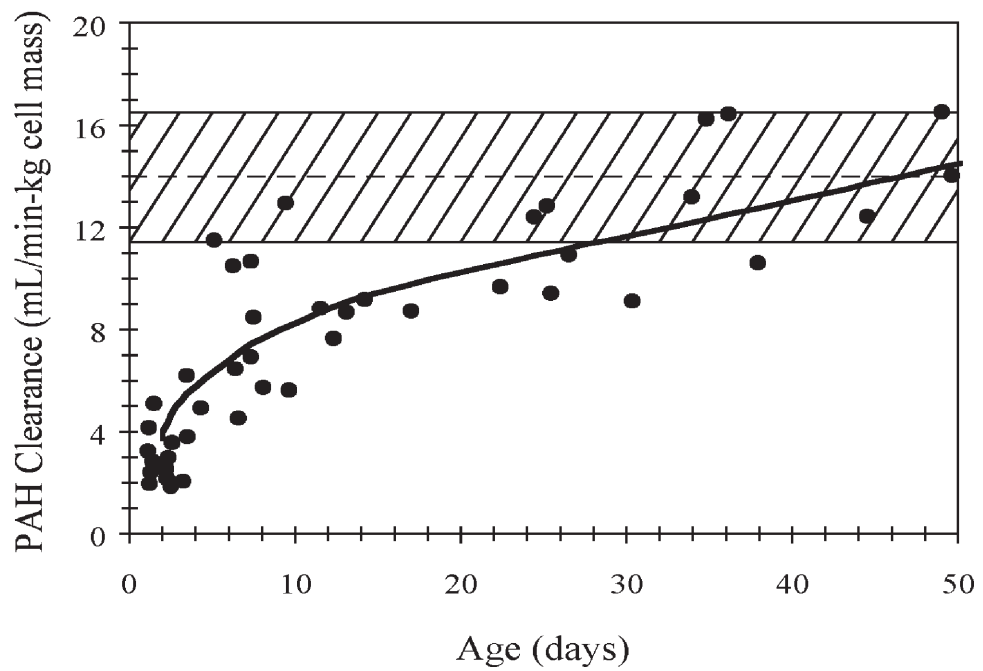
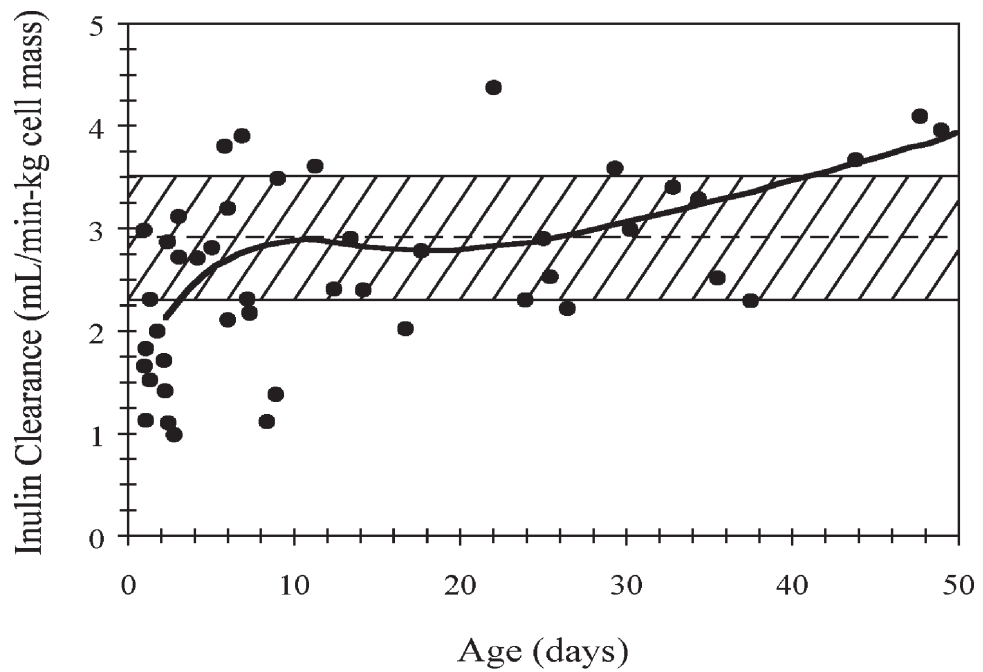
Urine flow rate can impact excretion of chemicals by the renal system. The urine flow rate affects the urine concentration, increasing or decreasing the concentration gradient for excretion processes must overcome, and may affect the diffusion time for compounds diffusing out of the urine. This has been demonstrated for several compounds, including chroamphenicol, ephedrine, phenobarbital, and theophylline.<sup>129</sup>

### 3. Physiological Determinants of Lactational Clearance

Lactational elimination occurs by passive diffusion of nonionized or lipophilic compounds. Elimination of ionizable compounds will be a function of  $Pk_a$  and milk pH, which is acidic (pH = 6.5).<sup>44</sup> The dependence of pH results implies that concentrations of acidic compounds are likely to be lower in milk than plasma, and concentrations of basic compounds are likely higher in milk than plasma. The high fat content, particularly immediately following childbirth, makes milk a good storage depot for lipophilic compounds. Lactational excretion of lipophilic compounds, such as DDT, polychlorinated and polybrominated biphenyls, dibenzo-*p*-dioxins and furans, have been demonstrated, and may be a major source of elimination. Lipophilic compounds diffuse with dietary fats into milk, where the excreted compound becomes a source of exposure for the neonate.<sup>44</sup>



**FIGURE 12.** Age-related decrease in renal function parameters. Numbers are reported as fraction of the value at maturity (20 to 29 years). (Adapted from Mayersohn.<sup>2</sup>)



**FIGURE 13.** Increase in renal function during the early postnatal period: (a) glomerular filtration rate, and (b) tubular secretion. Hatched area represents adult values. (Adapted directly from Braunlich.<sup>3</sup>)

#### 4. Observed Differences in Renal or Lactational Clearance

*Neonates, Infants, and Children.* The renal clearance rates of compounds that are GFR dependent and those that are tubular secretion dependent are both reduced in the neonate. Digoxin, which is modestly lipophilic, is cleared primarily by the renal system. Clearance is 35 to 75% lower in neonates, increasing from 32.9 ml/min/1.73 m<sup>2</sup> in infants less than 1 week old, to 88.9 ml/min/1.73 m<sup>2</sup> at 3 months and 144.4 ml/min/1.73 m<sup>2</sup> at 18 months of age.<sup>129</sup> The clearance of pipercuronium, an aminosteroid neuromuscular blocking drug, which is cleared predominantly (43 to 56%) by the renal system, is 42% lower in infants than adults.<sup>134</sup> The hydrophilic antibiotic, isepamicin, is cleared almost exclusively (97%) by the renal system and also shows significant age-related differences in  $Cl_{ren}$ .  $Cl_{ren}$  in L/h/kg is 0.066 1 to 7 days after birth, rising to 0.128 to 0.157 between 2 weeks and ~14 years, after which it declines steadily to 0.055 by ages 71 to 80 years.<sup>135</sup> Similarly, clearance of hydrophilic aminoglycoside antibiotics (gentamicin, amikacin, tobramycin, kanamycin), netilmicin, and indomethacin occurs through glomerular filtration and is reduced in the neonate. The serum half-lives of these compounds correlate with GFR and neonatal age.<sup>129</sup> Clearance (total) of amikacin is lower in newborns (36 ml/min/1.73 m<sup>2</sup>) than adult (100 ml/min/1.73 m<sup>2</sup>). Clearance of kanamycin is lower in newborns (7 to 9 ml/min/1.73 m<sup>2</sup>) than adults (95 to 99 ml/min/1.73 m<sup>2</sup>). Renal clearance of vancomycin is lower in premature neonates than adults, with rates that are 50% and 23% (premature neonates) lower than adults values, respectively.<sup>25</sup>

The newborn kidney has a reduced rate of transport (tubular secretion) of organic anions, such as furosemide, chlorothiazide, ethacrynic acid, acetazolamide and aldactone, as well as weak organic acids, such as penicillins, sulfonamides, cephalosporins, and phenolsulfonphthalein.<sup>129</sup> This group of compounds comprises both water-soluble and modestly lipophilic characteristics; the important characteristic appears to be a functional group targeting them for active tubular secretion. Renal clearance of furosemide is lower in neonates than adults, with neonatal rates that are 50% of adult levels.<sup>25</sup> Renal clearance of penicillins and sulfonamides is tubular secretion dependent

and is similarly reduced in the neonate in some cases. Ampicillin clearance is much lower in newborns (41.5 to 63 ml/min/1.73m<sup>2</sup>) than adults (260 to 420 ml/min/1.73m<sup>2</sup>).<sup>26</sup> Penicillin is cleared primarily by the kidney, and by both GFR and tubular secretion. Clearance is reduced in the newborn compared with older children and adults. Renal excretion of semisynthetic penicillins is also lower in the newborn, increasing markedly by 3 weeks of age. Resulting serum half-lives are similar to those in adults.<sup>129</sup>

The extent of binding of compounds to serum proteins (see Section II.B) can influence renal elimination and the resulting free serum concentrations.<sup>3</sup> Renal clearance of free ceftriaxone is reduced in the infant, but it is partially compensated by a parallel ~2-fold decrease in plasma protein binding.<sup>129</sup> The resulting fraction of the dose excreted unchanged in the urine is lower in adults (46%) than newborn infants (70%).

Differences are not observed for nafcillin, which is cleared primarily (90%) by biliary excretion. The renal clearance of digoxin is 35 to 75% lower in neonates (32 to 56 ml/min/1.7 m<sup>2</sup>) when compared with adults (130 to 150 mg/min/1.7 m<sup>2</sup>).<sup>26</sup>

*Elderly.* Lithium, a hydrophilic metal ion, is almost exclusively cleared by the kidney by glomerular filtration, which is limited by the high—80%—reabsorption in the proximal tubule.<sup>136</sup> Consistent with the decline in GFR with age, unadjusted lithium clearance values are lower in the elderly (0.83 to 94 L/h) compared with adults (2.49 L/h). The hydrophilic antibiotics—gentamicin and isepamicin—are cleared almost exclusively (90 to 97%) by the renal system and show significant age-related differences in  $Cl_{ren}$ . Isepamicin  $Cl_{ren}$  in L/h/kg is 0.066, 1 to 7 days after birth, rising to 0.128 to 0.157 between 2 weeks and ~14 years, after which it declines steadily to 0.055 by ages 71 to 80 years.<sup>135</sup>  $Cl_{ren}$  of gentamicin declines with age, particularly between 60 and 70 years.

*Gender.* The renal clearance of two modestly lipophilic compounds, digoxin and amantadine, as well as azimilide and pramipexole, is lower in women than men. The renal clearance of digoxin is 12 to 14% less in women than men.<sup>10</sup> Renal clearance ( $Cl_{ren}$ ) of amantadine, when corrected for body weight, mass index, and surface area, is

1.5-fold greater in males than females.<sup>10</sup>  $Cl_{ren}$  of azimilide is 19% higher in women when compared with men.<sup>10</sup> Pramipexole, a dopamine agonist, excreted predominantly by the kidney (80%), has a  $Cl_{ren}$ , which is 24 to 29% lower in women compared with men, similar to the calculated difference in creatinine clearance (GFR) (27.8%), but lower than other measures of GFR in women, which indicate no difference.<sup>132</sup>

*Physiological Determinants of Lactational Clearance.* Numerous chemicals have been found in human breast milk<sup>137,138</sup> and represent a potential source of exposure to the nursing infant. Human breast milk is a complex mixture of milk proteins, lactose, and triglycerides synthesized by mammary tissue and vitamins, fatty acids, and minerals added from the blood supply to the mammary tissue.<sup>138</sup> Exogenous chemicals may also be transferred from the blood supply across mammary epithelial cells into the mammary duct lumen. The most important determinants for this transfer are (1) degree of ionization, (2) lipid solubility, and (3) molecular weight.<sup>138</sup> The concentration of chemical in milk has been estimated for some volatile organic chemicals using a PBPK model that considers blood flows, tissue volumes, and chemical partitioning from blood to milk in the mammary gland.<sup>139,140</sup> In such models, more highly lipophilic compounds will preferentially partition from maternal blood into milk, and increases in blood flow will increase the rate at which such equilibration into milk occurs.

The transfer and accumulation of chemicals in breast milk is complex. It is not only a function of the physicochemical properties of the exogenous chemical and blood flow to the mammary tissue but also a function of the protein and fat composition of the milk. Changes in protein levels may result in increases in mammary excretion of protein-bound chemicals, such as heavy metals (lead, cadmium, mercury).<sup>138</sup> A 10-fold difference in protein concentrations have been noted between milk released immediately after birth (up to 1 week, post-partum) and mature milk.<sup>138</sup> Consequently, exposure in the first week of life from compounds that tend to be protein bound may be higher than at a few weeks of age.

Milk fat is excreted as a fat globule with a lipoprotein membrane.<sup>138</sup> Highly lipophilic chemicals, such as DDT, polychlorinated and polybrominated biphenyls, and dibenzo-*p*-dioxins/furans,

may partition into milk and bind to or partition into the lipid core of the fat globule.<sup>138</sup> Fat content can change over the time course of nursing with higher fat content, and, consequently, higher chemical excretion the longer the infant nurses. Moreover, fat content varies among species; milk from mice contains three times more fat than human milk.<sup>141</sup>

The concentration of an exogenous chemical in milk is only one determinant of exposure to the nursing infant. The other is the amount of milk ingested daily and the month/years over which nursing occurs. According to Byczkowski et al.,<sup>138</sup> milk intake increases from approximately 100 ml/day on post-partum day 2, rising to 500 ml/day by the second week and up to 700 to 800 ml/day in subsequent months with variations reported between 600 to 1000 ml/day at that time. Body burden will then change both with the increased milk intake but also with increased body weight as the infant grows.

*Physiological Determinants of Placental Transfer.* The placenta is a complex, multicompartmented membrane that functions in maternal-fetal exchange of nutrients (from the maternal circulation) and excretory products (from the fetal circulation). The movement of endogenous and exogenous chemicals is a function of (1) molecular weight, (2) lipid solubility, (3) degree of ionization, (4) binding to tissue or plasma components, (5) degree of biotransformation by placental enzyme systems, and (6) the rate of placental clearance.<sup>142</sup>

Placental transfer occurs at the interface of maternal sinuses into which blood flows from maternal arteries, and the placental villi into which fetal capillaries, both arteries and veins, grow.<sup>143</sup> The villi carrying fetal blood are surrounded by sinuses that contain maternal blood, both of which, villi and sinus, are contained in the area between the placental stratum spongiosum and the chorion-amnionic membrane.<sup>143</sup>

In general, transfer from maternal blood to fetal blood is inversely proportional to molecular weight.<sup>142</sup> With the exception of gamma globulins, compounds with molecular weights greater than 1000 (units not given) are poorly transmitted, if at all. In general, as with other membranes and tissues, more highly lipid-soluble compounds and/or unionized compounds will be more readily

transported across the villi to fetal circulation. However, not all lipid-soluble compounds are transported across the placenta at the same rate as across other tissues.<sup>142</sup>

Binding to tissue or plasma proteins and placental biotransformation significantly influence placental transfer.<sup>142</sup> Differential affinities of proteins in maternal and fetal plasma and tissues will alter placental transfer and fetal distribution. Preferential binding to maternal plasma relative to binding to fetal plasma will likely favor decreased distribution to the fetus. In contrast, preferential binding in fetal tissues, such as the liver, muscle, may create a fetal "sink" and enhance placental transfer.

The placenta is often thought of as an inert switching station in which nutrients and waste products are exchanged. However, the placenta is metabolically active. The placenta contains aryl hydrocarbon hydroxylase (AHH), aromatases, other MFOs, glucuronidase, and sulfatase.<sup>142</sup>

#### IV. CONCLUSIONS

For several of the parameters summarized in Table 2, there were insufficient data to make conclusions, particularly in the elderly. However, these should not necessarily be considered data gaps. Much of the data provided in this report were obtained from studies with pharmaceutical agents. The pharmacokinetics of pharmaceutical agents have been studied extensively. Consequently, the absence of published data in a particular area is likely an indication that no differences have been observed that would justify further study.

As discussed previously, the pharmacokinetics of a chemical is determined by the interaction of complex biological systems. For some of the parameters, inconsistent findings (e.g., increases and decreases) have been reported, which is not surprising for such a wide array of chemicals. However, it is also possible that the investigators were misled by the unstated assumption that they were actually measuring a single pharmacokinetic parameter, such as absorption. Although it is easier to describe and to think of absorption, distribution, metabolism, and excretion as isolated events, in fact, they are interrelated and should be evaluated collectively. In order to do this, PBPK modeling is an essential tool. Moreover, the application of a PBPK model allows for a more accurate estimation of the quantitative factors determining the dose to a particular tissue. Consequently, data-derived adjustments could be made to risk estimates. The next phase of this work will consist of using PBPK models to develop examples of approaches through the development of specific case studies to investigate quantitatively incorporating information on age- and gender-specific pharmacokinetic differences in risk assessments for chemicals.

#### ACKNOWLEDGEMENT

We gratefully acknowledge the Risk Assessment Methods Technical Implementation Panel (RAM TIP) of the American Chemistry Council for providing the funding and support needed to conduct this project.

## REFERENCES

1. **ICRP**, *Report of the Task Group on Reference Man*. International Commission on Radiological Protection, New York, 1972.
2. **Mayersohn, M.**, Pharmacokinetics in the elderly. *Environmental Health Perspectives*, 102(Suppl 11), 119–124, 1994.
3. **Braunlich, H.**, Kidney Development: Drug Elimination Mechanisms, in *Drug Disposition During Development*, Morselli, P., Ed. Spectrum Publications Inc., New York, 89–101, 1977.
4. **Ogiu, N., et al.**, A statistical analysis of the internal organ weights of normal Japanese people. *Health Phys*, 72(3), 368–83., 1997.
5. **Crystal, R.G., et al.**, Eds., *The Lung : Scientific Foundations*. Vol. 2. Raven Press: New York, 1991.
6. **Plunkett, L.M., Turnbull, D., and Rodricks, J.V.**, Differences Between Adults and Children Affecting Exposure Assessment, in *Similarities and Differences Between Children and Adults: Implications for Risk Assessment*, Guzelian, P., Henry, C., and Olin, S., Eds., ILSI Press, Washington, DC, 79–94, 1992.
7. **Bender, A.D.**, Effect of age on intestinal absorption: implications for drug absorption in the elderly. *Journal of the American Geriatric Society*, 16(12), 1331–1339, 1968.
8. **Eddington, N.D.**, Pharmacokinetics, in *Handbook of Pharmacology and Aging*, Roberts, J., Snyder, D.L., and Friedman, E., Eds., CRC Press, Boca Raton, 1–22, 1996.
9. **Klaassen, C.D.**, Distribution, Excretion, and Absorption of Toxicants, in *Toxicology: The Basic Science of Poisons*, Klaassen, C.D., Amdur, M.O., and Doull, J., Eds., Macmillan Publishing, New York, 33–63, 1980.
10. **Beierle, I., Meibohm, B., and Derendorf, H.**, Gender differences in pharmacokinetics and pharmacodynamics. *International Journal of Clinical Pharmacology and Therapeutics*, 37(11), 529–47, 1999.
11. **Bearer, C.F.**, How are children different from adults? *Environmental Health Perspectives*, 103(Suppl 6), 7–12, 1995.
12. **Cunico, R.L., et al.**, Skin barrier properties in the newborn. *Biol Neonate*, 32(177–182), 1977.
13. **Milsap, R.L. and Jusko, W.J.**, Pharmacokinetics in the infant. *Environmental Health Perspectives*, 102(Suppl 11), 107–110, 1994.
14. **Roskos, K.V., Maibach, H.I., and Guy, R.H.**, The effect of aging on percutaneous absorption in man. *Journal of Pharmacokinetics and Biopharmaceutics*, 17(6), 617–630, 1989.
15. **Reid, L.M.**, The pulmonary circulation: remodeling in growth and disease. The 1978 J. Burns Amberson lecture. *Am Rev Respir Dis*, 119(4), 531–46, 1979.
16. **Dunnill, M.S.**, The problem of lung growth [editorial]. *Thorax*, 37(8), 561–3, 1982.
17. **Musante, C.J. and Martonen, T.B.**, Computer simulations of particle deposition in the developing human lung. *Journal of the Air and Waste Management Association*, 50(8), 1426–1432, 2000.

18. **Snodgrass, W.**, Physiological and Biochemical Differences Between Children and Adults as Determinants of Toxic Response to Environmental Pollutants., in *Similarities and Differences Between Children and Adults: Implications for Risk Assessment*, Guzelian, P., Henry, C., and Olin, S., Eds., ILSI Press, Washington, DC, 35–42, 1992.
19. **Gillooly, M. and Lamb, D.**, Airspace size in lungs of lifelong non-smokers: effect of age and sex. *Thorax*, 48(1), 39–43, 1993.
20. **Mauderly, J.L.**, Effect of age on pulmonary structure and function of immature and adult animals and man. *Fed Proc*, 38(2), 173–7., 1979.
21. **Cook, D.R.**, Paediatric anaesthesia: Pharmacological considerations. *Drugs*, 12(3), 212–221, 1976.
22. **Roth, W.L., Freeman, R.A., and Wilson, A.G.**, A physiologically based model for gastrointestinal absorption and excretion of chemicals carried by lipids. *Risk Analysis*, 13(5), 531–543, 1993.
23. **Vost, A. and Maclean, N.**, Hydrocarbon transport in chylomicrons and high-density lipoproteins in rat. *Lipids*, 19(6), 423–435, 1984.
24. **Stewart, C.F. and Hampton, E.M.**, Effect of maturation on drug disposition in pediatric patients. *Clinical Pharmacology*, 6(7), 548–564, 1987.
25. **Hattis, D., et al.**, *Development of a Comparative Child/Adult Pharmacokinetic Database Based Upon the Therapeutic Drug Literature*. Clark University and the Connecticut Department of Public Health, 2000.
26. **Morselli, P.L., Franco-Morselli, R., and Bossi, L.**, Clinical pharmacokinetics in newborns and infants. Age-related differences and therapeutic implications. *Clinical Pharmacokinetics*, 5(6), 485–527, 1980.
27. **Burin, G.J. and Saunders, D.R.**, Addressing human variability in risk assessment—the robustness of the intraspecies uncertainty factor. *Regulatory Toxicology and Pharmacology*, 30(3), 209–216, 1999.
28. **Parkinson, A.**, Biotransformation of Xenobiotics, in *Casarett and Doull's Toxicology, The Basic Science of Poisons*, Klaasen, C., Ed. McGraw-Hill, New York, 113 - 186, 1996.
29. **Maurel, P.**, The CYP3 family, in *Cytochromes P450 Metabolic and Toxicological Aspects*, C, I., Ed. CRC Press, Boca Raton, FL, 241–270, 1996.
30. **de Wildt, S.N., et al.**, Cytochrome P450 3A: ontogeny and drug disposition. *Clinical Pharmacokinetics*, 37(6), 485–505, 1999.
31. **Guengerich, F.P. and Shimada, T.**, Oxidation of Toxic and Carcinogenic Chemicals by Human Cytochrome P-450 Enzymes. *Chemical Research in Toxicology*, 4(4), 391–407, 1991.
32. **Glue, P. and Clement, R.P.**, Cytochrome P450 enzymes and drug metabolism—basic concepts and methods of assessment. *Cellular and Molecular Neurobiology*, 19(3), 309–323, 1999.
33. **Kovarik, J.M. and Koelle, E.U.**, Cyclosporin pharmacokinetics in the elderly. *Drugs and Aging*, 15(3), 197–205, 1999.

34. **Calabrese, E.**, Xenobiotic Metabolism, in *Age and Susceptibility to Toxic Substances*, Calabrese, E., Ed. John Wiley, New York, 33–78, 1986.
35. **Kawajiri, K., Hayashi, S.I.**, The CYP1 family, in *Cytochromes P450 Metabolic and Toxicological Aspects*, C, I., Ed. CRC Press, Boca Raton, FL, 77–97, 1996.
36. **Shimada, T., et al.**, Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *Journal of Pharmacology and Experimental Therapeutics*, 270(1), 414–423, 1994.
37. **Tanaka, E.**, Gender-related differences in pharmacokinetics and their clinical significance. *Journal of Clinical Pharmacology and Therapeutics*, 24(5), 339–346, 1999.
38. **Sonawane, B.R. and Yaffe, S.J.**, Clinical implications of hepatic immaturity: bilirubin glucuronidation and induction by phenobarbital, in *Drug Metabolism in the Immature Human*, Soyka, L.F., Redmond, G.P., Ed. Raven Press, New York, 119–128, 1981.
39. **Pacifici, G.M., et al.**, Tissue and species differences in enzymes of epoxide metabolism. *Xenobiotica*, 11(2), 73–79, 1981.
40. **Mendrala, A.L., et al.**, In vitro kinetics of styrene and styrene oxide metabolism in rat, mouse, and human. *Archives of Toxicology*, 67(1), 18–27, 1993.
41. **Rane, A., Pacifici, G.M.**, Formation and metabolism of toxic metabolites in the human fetus, in *Drug Metabolism in the Immature Human*, Soyka, L.F., Redmond, G.P., Ed. Raven Press, New York, 29–35, 1981.
42. **Kauffman, R.E.**, Acute Acetaminophen Overdose: An Example of Reduced Toxicity Related to Developmental Differences in Drug Metabolism, in *Similarities and Differences Between Children and Adults: Implications for Risk Assessment*, Guzelian, P., Henry, C., and Olin, S., Eds., ILSI Press, Washington, DC, 97–103, 1992.
43. **Klaassen, C.D. and Rozman, K.**, Absorption, distribution, and excretion of toxicants, in *Casarett and Doull's Toxicology. The Basic Science of Poisons*, Amdur, M.O., Doull, J., and Klaassen, C.D., Eds., Pergamon Press, New York, 50–87, 1991.
44. **Rozman, K.K. and Klaassen, C.D.**, Absorption, distribution, and excretion of toxicants, in *Casarett and Doull's Toxicology. The Basic Science of Poisons*, Klaassen, C.D., Amdur, M.O., and Doull, J., Eds., McGraw-Hill, New York, 91–112, 1996.
45. **Fullmer, C.S.**, Intestinal interactions of lead and calcium. *NeuroToxicology*, 13(4), 799–807, 1992.
46. **Ritschel, W.A.**, Ed. *Gerontokinetics. Pharmacokinetics of Drugs in the Elderly*. The Telford Press, Inc.: Caldwell, NJ, 1988.
47. **Calabrese, E.**, Gastrointestinal/Dermal/Pulmonary Absorption or Xenobiotics: Effect of Age., in *Age and Susceptibility to Toxic Substances*, Calabrese, E., Ed. John Wiley, New York, 5–24, 1986.

48. **Widdicombe, J.**, Drug uptake from the airways and lungs. *Indian Journal of Physiology and Pharmacology*, 42(1), 3–14, 1998.
49. **Johanson, G.**, Modeling of Respiratory Exchange of Polar Solvents. *Annals of Occupational Hygiene*, 35(3), 323–339, 1991.
50. **Andersen, M.E.**, Saturable metabolism and its relation to toxicity. *Critical Reviews in Toxicology*, 9(105–150), 1981.
51. **Andersen, M.E.**, A physiologically based toxicokinetic description of the metabolism of inhaled gases and vapors: analysis at steady state. *Toxicology and Applied Pharmacology*, 60(509–526), 1981.
52. **Guilmette, R., Wicks, J., and Wolff, R.**, Morphometry of human nasal airways in vivo using magnetic resonance imaging. *J Aerosol Med*, 2(365–377), 1989.
53. **Sarangapani, R. and Wexler, A.S.**, Modeling particle deposition in extrathoracic airways. *Aerosol. Sci. Technol.*, 32(72–89), 2000.
54. **Schoenberg, J.B., Beck, G.J., and Bouhuys, A.**, Growth and decay of pulmonary function in healthy blacks and whites. *Respir Physiol*, 33(3), 367–93, 1978.
55. **Quanjer, P.H., et al.**, Compilation of reference values for lung function measurements in children. *Eur Respir J Suppl*, 4(184S–261S.), 1989.
56. **Sherrill, D.L., Camilli, A., and Lebowitz, M.D.**, On the temporal relationships between lung function and somatic growth. *Am Rev Respir Dis*, 140(3), 638–44, 1989.
57. **Hislop, A.A., et al.**, Postnatal growth and function of the pre-acinar airways. *Thorax*, 27(265–274), 1972.
58. **Krumpe, P.E., et al.**, The aging respiratory system. *Clin Geriatr Med*, 1(1), 143–75, 1985.
59. **Weibel, E.R.**, *Morphometry of the Human Lung*. Berlin, Springer, 151, 1963.
60. **Thurlbeck, W.M.**, Postnatal human lung growth. *Thorax*, 37(8), 564–71, 1982.
61. **Crapo, R.O.**, Pulmonary-function testing. *N Engl J Med*, 331(1), 25–30., 1994.
62. **Gibson, G.J., et al.**, Sex and age differences in pulmonary mechanics in normal nonsmoking subjects. *J Appl Physiol*, 41(1), 20–5., 1976.
63. **Leith, D.E. and Mead, J.**, Mechanisms determining residual volume of the lungs in normal subjects. *J Appl Physiol*, 23(2), 221–7., 1967.
64. **Mellemegaard, K.**, The alveolar-arterial oxygen difference: its size and components in normal man. *Acta Physiol Scand*, 67(1), 10–20., 1966.
65. **Hamer, N.**, Effect of membrane permeability on pulmonary as exchange efficiency. *Clin. Sci.*, 23(85), 1962.
66. **Krumholz, R.A.**, Pulmonary membrane diffusing capacity and pulmonary capillary blood volume: an appraisal of their clinical usefulness. *Am Rev Respir Dis*, 94(2), 195–200., 1966.
67. **Verbeken, E.K., et al.**, Anatomy of membranous bronchioles in normal, senile and emphysematous human lungs. *J Appl Physiol*, 77(4), 1875–84, 1994.

68. **Edge, J., Millard, F., and Reid, L.,** The radiographic appearance of the chest in persons of advanced age. *Br J Radiol*, 37(769–774), 1984.
69. **Petrini, M.F., Phillips, M.S., and Walsh, D.A.,** Pulmonary tissue volume and blood flow as functions of body surface area and age. *Lung*, 166(1), 47–63., 1988.
70. **Hofmann, W.,** Mathematical model for the postnatal growth of the human lung. *Respir Physiol*, 49(1), 115–29., 1982.
71. **Overton, J.H. and Graham, R.C.,** Predictions of ozone absorption in human lungs from newborn to adult. *Health Phys*, 57 Suppl 1(4), 29–36., 1989.
72. **Martonen, T.B., Graham, R.C., and Hofmann, W.,** Human subject age and activity level: factors addressed in a biomathematical deposition program for extrapolation modeling. *Health Phys*, 57 Suppl 1(8), 49–59., 1989.
73. **Agnew, J.E.,** Bronchiolar aerosol deposition and clearance. *Eur Respir J*, 9(6), 1118–22., 1996.
74. **Stanley, P.J., et al.,** Effect of betamethasone and betamethasone with neomycin nasal drops on human nasal mucociliary clearance and ciliary beat frequency. *Thorax*, 40(8), 607–12., 1985.
75. **Leikauf, G.D., et al.,** Dose-dependent effects of submicrometer sulfuric acid aerosol on particle clearance from ciliated human lung airways. *Am Ind Hyg Assoc J*, 45(5), 285–92., 1984.
76. **Davis, J. and Grant, L.,** The Sensitivity of Children to Lead, in *Similarities and Differences Between Children and Adults: Implications for Risk Assessment*, Guzelian, P., Henry, C., and Olin, S., Eds., ILSI Press, Washington, DC, 150–162, 1992.
77. **Swift, D.L.,** Age-related scaling for aerosol and vapor deposition in the upper airways of humans. *Health Phys*, 57 Suppl 1(293–7., 1989.
78. **Oldham, M.J., Mannix, R.C., and Phalen, R.F.,** Deposition of monodisperse particles in hollow models representing adult and child-size tracheobronchial airways. *Health Phys*, 72(6), 827–34., 1997.
79. **EPA, Air Quality Criteria Document for Particulate Matter**, 1996.
80. **Bennett, W.D., Zeman, K.L., and Kim, C.,** Variability of fine particle deposition in healthy adults: effect of age and gender. *Am J Respir Crit Care Med*, 153(5), 1641–7., 1996.
81. **Xu, G.B. and Yu, C.P.,** Effects of age on deposition of inhaled aerosols in the human lung. *Aero Sci Tech*, 5(359–357), 1986.
82. **Phalen, R.F., et al.,** Tracheobronchial deposition predictions for infants, children and adolescents. *Ann Occup Hyg*, 32(Suppl), 11–21, 1988.
83. **McCue, J.D.,** Antibiotic use in the elderly: issues and nonissues. *Clinical Infectious Diseases*, 28(4), 750–752, 1999.
84. **Corley, R.A., Gordon, S.M., and Wallace, L.A.,** Physiologically based pharmacokinetic modeling of the temperature-dependent dermal absorption of chloroform by humans following bath water exposures. *Toxicological Sciences*, 53(1), 13–23, 2000.

85. **Nomiyama, K. and Nomiyama, H.**, Metabolism of Trichloroethylene in Human. Sex Difference in Urinary Excretion of Trichloroacetic Acid and Trichloroethanol. *Internationales Archiv Fur Arbeitsmedizin*, 28(1), 37–48, 1971.
86. **Pritchard, J.N., Jefferies, S.J., and Black, A.**, Sex differences in the regional deposition of inhaled particles in the 2.5–7.5 um size range. *J Aero Sco*, 17(385–389), 1986.
87. **Kim, C.S. and Hu, S.C.**, Regional deposition of inhaled particles in human lungs: comparison between men and women. *J Appl Physiol*, 84(6), 1834–44., 1998.
88. **Jaques, P.A. and Kim, C.S.**, Measurement of total lung deposition of inhaled ultrafine particles in healthy men and women. *Inhal Toxicol*, 12(8), 715–31., 2000.
89. **Crom, W.R.**, Pharmacokinetics in the child. *Environmental Health Perspectives*, 102(Suppl 11), 111–117, 1994.
90. **Ulijaszek, S.J., Johnston, F.E., and Preece, M.A.**, Eds., *Human Growth and Development*. Cambridge University Press: New York. 497, 1998.
91. **DeVane, C.L.**, Metabolism and pharmacokinetics of selective serotonin reuptake inhibitors. *Cellular and Molecular Neurobiology*, 19(4), 443–466, 1999.
92. **Masseff, R., et al.**, Evolution of alpha-fetoprotein serum levels throughout life in humans and rats, and during pregnancy in the rat. *Annals of the New York Academy of Sciences*, 259(17–28), 1975.
93. **Dunn, J.**, Transport of Estrogens in Human Plasma, in *Catechol Estrogens*, Merriam, G. and Lipsett, M., Eds., Raven Press, New York, 167–176, 1983.
94. **Dunn, J.**, Computer Simulation of Steroid Transport in Human Plasma, in *Computers in Endocrinology*, Rodbard, D. and Forti, G., Eds., Raven Press, New York, 277–285, 1984.
95. **Zubenko, G.S. and Sunderland, T.**, Geriatric psychopharmacology: why does age matter? *Harvard Review of Psychiatry*, 7(6), 311–333, 2000.
96. **Schussler, G.C.**, The thyroxine-binding proteins [published erratum appears in *Thyroid* 2000 Apr;10(4):372]. *Thyroid*, 10(2), 141–149, 2000.
97. **Newcomer, M.E.**, Retinoid-binding proteins: structural determinants important for function. *FASEB Journal*, 9(2), 229–239, 1995.
98. **Kurz, H., Mauser-Ganshorn, A., and Stickel, H.H.**, Differences in the binding of drugs to plasma proteins from newborn and adult man. I. *European Journal of Clinical Pharmacology*, 11(6), 463–467, 1977.
99. **Grandison, M.K. and Boudinot, F.D.**, Age-related changes in protein binding of drugs: implications for therapy. *Clin Pharmacokinet*, 38(3), 271–90, 2000.
100. **Hay, W.W., Jr., et al.**, Eds., *Current Pediatric Diagnosis and Treatment*, 14th ed. Appleton & Lange: Stamford, CT, 1998.
101. **Morselli, P.L.**, Ed. *Drug Disposition During Development*. Spectrum Publications, Inc.: New York, 1979.
102. **Belgorosky, A. and Rivarola, M.A.**, Progressive decrease in serum sex hormone-binding globulin from infancy to late prepuberty in boys. *J Clin Endocrinol Metab*, 63(2), 510–512, 1986.

103. **Bedecarras, P., et al.**, Characterization of serum SHBG isoforms in prepubertal and pubertal girls. *Clin Endocrinol*, 49(5), 603–608, 1998.
104. **Hakkinen, K. and Pakarinen, A.**, Serum hormones and strength development during strength training in middle-aged and elderly males and females. *Acta Physiol Scand*, 150(2), 211–219, 1994.
105. **Kurz, H., Michels, H., and Stickel, H.H.**, Differences in the binding of drugs to plasma proteins from newborn and adult man. II. *European Journal of Clinical Pharmacology*, 11(6), 469–472, 1977.
106. **Ritschel, W., et al.**, Pharmacokinetics of coumarin and its 7-hydroxymetabolites upon intravenous and peroral administration of coumarin in man. *European Journal of Clinical Pharmacology*, 12(6), 457–461, 1977.
107. **Gilmore, D.A., et al.**, Age and gender influence the stereoselective pharmacokinetics of propranolol. *Journal of Pharmacology and Experimental Therapeutics*, 261(3), 1181–6, 1992.
108. **Cogliano, J.**, Trichloroethylene health risk assessment: Using mechanistic information to improve dose-response assessment. *The Toxicologist*, 48(1–S), Abstr # 387, 1999.
109. **Veering, B.T., et al.**, The effect of age on serum concentrations of albumin and alpha 1-acid glycoprotein. *British Journal of Clinical Pharmacology*, 29(2), 201–206, 1990.
110. **Piafsky, K.M., et al.**, Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma alpha 1 acid glycoprotein. *New England Journal of Medicine*, 299(26), 1435–1439, 1978.
111. **Triggs, E. and Charles, B.**, Pharmacokinetics and therapeutic drug monitoring of gentamicin in the elderly. *Clinical Pharmacokinetics*, 37(4), 331–341, 1999.
112. **Wilson, K.**, Sex-related differences in drug disposition in man. *Clin Pharmacokinetics*, 9(3), 189–202, 1984.
113. **Klinger, W.**, Development of Drug Metabolizing Enzymes, in *Drug Disposition During Development*, PL, M., Ed. Spectrum Publications, Inc., New York, 71–87, 1977.
114. **Birkett, D.J. and Grygiel, J.J.**, Age determinants of methylxanthine metabolism in man, in *Drug Metabolism in the Immature Human*, Soyka LF, R.G., Ed. Raven Press, New York, 229–239, 1981.
115. **Cresteil, T.**, Onset of xenobiotic metabolism in children: toxicological implications. *Food Additives and Contaminants*, 15(Suppl), 45–51, 1998.
116. **Lacroix, D., et al.**, Expression of CYP3A in the human liver—evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. *Eur J Biochem*, 247(2)(625–634), 1997.
117. **Jacqz-Aigrain, E. and Cresteil, T.**, Cytochrome P450-dependent metabolism of dextromethorphan: fetal and adult studies. *Dev Pharmacol Ther*, 18(3–4)(161–168), 1992.
118. **Treluyer, J., et al.**, Expression of CYP2D6 in developing human liver. *Eur J Biochem*, 202(2)(583–588), 1991.
119. **Treluyer, J., et al.**, Developmental expression of CYP2C and CYP2C-

- dependent activities in the human liver: in vivo/in vitro correlation and inducibility. *Pharmacogenetics*, 7(441–452), 1997.
120. **Sonnier, M. and Cresteil, T.**, Delayed ontogenesis of CYP1A2 in the human liver. *Eur J Biochem*, 251(3)(893–898, 1998.
  121. **Vieira, I., Sonnier, M., and Cresteil, T.**, Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. *Eur J Biochem*, 238(476–483, 1996.
  122. **Sipes, I.G., Gandolfi, A.J.**, Biotransformation of toxicants, in *Casarett and Doull's Toxicology*, C.D. Klaassen, J.O.A., J. Doull, Ed. Macmillan Publishing Company, New York, 64–98, 1986.
  123. **Landi, M.T., et al.**, Chapter 16. Human cytochrome P4501A2. *IARC Science Publications*, 148(173–195, 1999.
  124. **Ryan, D.E. and Levin, W.**, Age- and gender-related expression of rat liver cytochrome P450, in *Handbook of Experimental Pharmacology*, J. Schenkman, H.G., Ed. Springer-Verlag, New York, 461–476, 1993.
  125. **Richardson, T.H., Johnson, E.F.**, The CYP2C subfamily, in *Cytochromes P450 Metabolic and Toxicological Aspects*, C. I., Ed. CRC Press, Boca Raton, FL, 161–181, 1996.
  126. **Vahter, M.**, Methylation of inorganic arsenic in different mammalian species and population groups. *Science in Progress*, 82(Pt 1), 69–88, 1999.
  127. **Dutton, G.J.**, *Glucuronidation of Drugs and Other Compounds*. Boca Raton, FL, CRC Press, 1980.
  128. **Pastino, G.M., Yap, W.Y., and Carroquino, M.**, Human variability and susceptibility to trichloroethylene. *Environmental Health Perspectives*, 108(Suppl 2), 201–214, 2000.
  129. **John, E.G. and Guignard, J.-P.**, Development of Renal Excretion of Drugs During Ontogeny, in *Fetal and Neonatal Physiology*, Polin, R. and Fox, W., Eds., W.B. Saunders Company, Philadelphia, 188–193, 1998.
  130. **Podrazik, P.M. and Schwartz, J.B.**, Cardiovascular pharmacology of aging. *Cardiology Clinics*, 17(1), 17–34, 1999.
  131. **Stalam, M. and Kaye, D.**, Antibiotic agents in the elderly. *Infectious Disease Clinics of North America*, 14(2), 357–369, 2000.
  132. **Bokenkamp, A., et al.**, Cystatin C—a new marker of glomerular filtration rate in children independent of age and height. *Pediatrics*, 101(5), 875–881, 1998.
  133. **Fletcher, C.V., Acosta, E.P., and Strykowski, J.M.**, Gender differences in human pharmacokinetics and pharmacodynamics. *Journal of Adolescent Health*, 15(8), 619–629, 1994.
  134. **Atherton, D.P. and Hunter, J.M.**, Clinical pharmacokinetics of the newer neuromuscular blocking drugs. *Clinical Pharmacokinetics*, 36(3), 169–189, 1999.
  135. **Tod, M., Padoin, C., and Petitjean, O.**, Clinical pharmacokinetics and pharmacodynamics of isepamicin. *Clinical Pharmacokinetics*, 38(3), 205–223, 2000.
  136. **Sproule, B.A., Hardy, B.G., and Shulman, K.I.**, Differential pharmacokinetics of lithium in elderly pa-

- tients. *Drugs and Aging*, 16(3), 165–177, 2000.
137. **Giroux, D., Lapointe, G., and Baril, M.**, Toxicological index and the presence in the workplace of chemical hazards for workers who breast-feed infants. *Am Ind Hyg Assoc J*, 53(7), 471–474, 1992.
138. **Byczkowski, J.Z., Geahart, J.M., and Fisher, J.W.**, “Occupational” exposure of infants to toxic chemicals via breast milk. *Nutrition*, 10(1), 43–48, 1994.
139. **Shelley, M.L., Andersen, M.E., and Fisher, J.W.**, A risk assessment approach for nursing infants exposed to volatile organics through the mother’s occupational inhalation exposure. *Appl Ind Hyg*, 4(1), 21–26, 1989.
140. **Byczkowski, J.Z. and Fisher, J.W.**, Tetrachloroethylene exposure assessment of breast-fed infants. undated.
141. **Byczkowski, J.**, Lactational Transfer of Chemicals. Seminar presentation. February 5, 1992. 1992.
142. **Miller, R.K., Koszalka, T.R., and Brent, R.L.**, The transport of molecules across placental membranes, in *The Cell Surface in animal Embryogenesis and Development*, Poste, G. and Nicolson, G.L., Eds., Elsevier, New York, 145–223, 1976.
143. **Guyton, A.C. and Hall, J.E.**, *Textbook of Medical Physiology*. 9th ed. Philadelphia, W.B. Saunders Company, 1996.
144. **Widdowson, E.M.**, Changes in Body Proportions and Compositions During Growth, in *Scientific Foundations of Paediatrics*, Davis, J. and Dobbing, J., Eds., W. Heinemann Medical Books Ltd., London, 153–163, 1974.
145. **Pikkarainen, P.H. and Raiha, N.C.**, Development of alcohol dehydrogenase activity in the human liver. *Pediatric Research*, 1(3), 165–168, 1967.