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Pharmacokinetics of ingested xenobiotics in children: A comparison with adults

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Abstract

Both in the development of medicinal products and in risk assessment of other xenobiotics there is an increasing awareness that children should be considered as a special group. Children are exposed to other doses than adults and the pharmacokinetics and pharmacodynamics can be very different in children and adults. In general it could be concluded from our investigation that the effects of age on pharmacokinetics are most pronounced during the first 6-12 months of life. Full adjustment of dosing or TDI's for pharmacokinetic differences can be applied relatively easily and should, in our opinion, be seen as a first step in considering risk for the paediatric population. For risk assessment related to drugs and other xenobiotics, it seems to be essential that young animal models be used for determining NOAELs relevant for the paediatric population, especially for children less than one year of age. The use of a paediatric PBPK model, possibly combined with pharmacodynamics (PBPK/PD model), may be a valuable aid in risk assessment.

Contents

Samenvatting

Zowel in de ontwikkeling van geneesmiddelen als bij de risicoschatting van andere xenobiotica neemt het besef toe dat kinderen beschouwd zouden moeten worden als een speciale groep. Een vraag die zich daarbij voordoet is de noodzaak voor het ontwikkelen van aanvullende (jonge) diermodellen specifiek voor kinderen. Kinderen worden blootgesteld aan andere doses dan volwassenen en de farmacokinetiek en farmacodynamie kan sterk verschillen in kinderen en volwassenen. Dit rapport richt zich op verbindingen die via ingestie in het lichaam komen omdat dit een belangrijke blootstellingsroute is in kinderen. Om meer inzicht te krijgen in de verschillen tussen volwassenen en kinderen in farmacokinetiek van oraal ingenomen verbindingen is informatie verzameld betreffende de fysiologie van het maagdarmkanaal en processen zoals intestinale absorptie, distributie, metabolisme en excretie in beide groepen. Het blijkt bijvoorbeeld dat de intestinale absorptie niet dramatisch verandert met de leeftijd. De ontwikkeling van het maagdarmkanaal vindt plaats in de eerste 6 maanden tot ongeveer 2 jaar. Er worden over het algemeen alleen verschillen in snelheid van absorptie gevonden en niet in hoeveelheid die wordt geabsorbeerd. Echter, xenobiotica die met behulp van transportprocessen, die ook gebruikt worden voor de absorptie van nutriënten of essentiële stoffen voor de groei van kinderen, geabsorbeerd worden, zouden mogelijk beter opgenomen kunnen worden in kinderen dan in volwassenen. Daarnaast is ook de lichaamssamenstelling van kinderen verschillend van die van een volwassene. De relatieve hoeveelheid water in het lichaam is groter in een peuter en de hoeveelheid vet is kleiner. Dit kan leiden tot verschillen in het distributievolume van stoffen in volwassenen en kinderen, afhankelijk van de fysisch-chemische eigenschappen van een stof. Een ander belangrijk proces dat grote invloed kan hebben op de interne blootstelling is het metabolisme van een stof. Metabolisme kan resulteren in detoxificatie van stoffen, maar kan ook leiden tot de vorming van metabolieten die juist toxischer zijn. Voor de risicoschatting van een stof is het erg belangrijk of deze zal worden geactiveerd of gedeactiveerd. Over het algemeen is de enzymactiviteit na 6 tot 12 maanden op het niveau van een volwassene. Bij kinderen in de leeftijd van 6 maanden tot 12 jaar is de metabole activiteit hoger als gevolg van een hoger basaal metabolisme, maar in neonaten is het metabolisme onderontwikkeld ten opzichte van de volwassene. Ook de renale uitscheiding is van invloed op de klaring van een stof en ook dit proces lijkt leeftijdsafhankelijk te zijn. De renale bloedflow en de glomerulaire filtratiesnelheid bereiken het volwassen niveau op een leeftijd van 6 maanden. Samengevat kan voor al deze processen worden geconcludeerd dat de leeftijdseffecten op de kinetiek het grootst zijn gedurende het eerste jaar en dat dit kan leiden tot een onderschatting van de blootstelling in deze leeftijdsgroep. Hiermee moet rekening gehouden worden bij de risicoschatting van een stof.
Risicoschatting van geneesmiddelen is gebaseerd op een andere aanpak dan risicoschatting van andere xenobiotica. Voor medicijnen wordt een ‘No Observed Adverse Effect Level’ (NOAEL) bepaald uit dierstudies en worden farmacokinetische parameters als AUC en C_{max} bepaald bij een blootstelling aan de NOAEL. Vervolgens wordt een zogenoemde veiligheidsmarge berekend door de farmacokinetische parameters bij een klinische dosering in mensen te vergelijken met dezelfde parameters bij een blootstelling aan de NOAEL in proefdieren. Om een dosis in volwassenen te schalen naar een dosis in een kind wordt in het algemeen een schalingsmodel gebruikt gebaseerd op lichaamsgewicht of lichaams-oppervlakte. Het hangt af van de karakteristieken van de stof en van de leeftijd van het kind of dit zal leiden tot een vergelijkbare interne blootstelling en een vergelijkbaar farmaco-dynamisch effect in het kind. Het lijkt echter op basis van de kennis van de verschillen in fysiologie tussen een kind bij een bepaalde leeftijd en een volwassene, en de effecten die dit

kan hebben op de farmacokinetische parameters, goed mogelijk om de dosering vast te stellen die leidt tot een vergelijkbare interne blootstelling in het kind bij een bepaalde leeftijd. Dit betekent dat het voor geneesmiddelenonderzoek niet noodzakelijk is om jonge proefdiermodellen te ontwikkelen om te compenseren voor farmacokinetische verschillen. Dergelijke modellen zijn waarschijnlijk wel nodig om met name de effecten op ontwikkelende systemen in het kind vast te stellen. Voor de risicoschatting van andere xenobiotica wordt eveneens de NOAEL vastgesteld uit toxiciteitsstudies in proefdieren of, als die aanwezig zijn, wordt de NOAEL vastgesteld op basis van doses die leiden tot effecten in de mens. Voor dergelijke verbindingen is het in het algemeen noodzakelijk om een 'Tolerable Daily Intake' (TDI) te bepalen. In de standaardprocedure wordt de TDI afgeleid door de NOAEL te delen door onzekerheidsfactoren (10 x 10) voor intra- en interspeciesverschillen. Het blijft hierbij echter de vraag of de onzekerheidsfactor voor intraspeciesverschillen voldoende is om ook de verschillen tussen kinderen en volwassenen te bestrijken. Met name in neonaten (< 1 maand) kunnen deze verschillen groter zijn. Het ontwikkelen en gebruiken van jonge proefdiermodellen voor de verschillen in interne blootstelling tussen kinderen en volwassenen lijkt niet noodzakelijk. Met behulp van de fysiologische gegevens in kinderen en volwassenen kunnen farmacokinetische modellen gemaakt worden (PBPK-modellen) waarmee de interne blootstelling in volwassenen en kinderen gemodelleerd kan worden. Ook hier geldt dat het voor de effecten op ontwikkelende systemen wel belangrijk kan zijn om een NOAEL vast te stellen in jonge proefdieren. Op basis van alle informatie die nu beschikbaar is lijkt het goed mogelijk om doseringen of TDI's, voor wat betreft de farmacokinetieke verschillen, aan te passen voor kinderen. Deze aanpassing voor kinetische verschillen zou, naar onze mening, gezien moeten worden als een eerste stap in het bepalen van het risico van een stof voor kinderen. Het gebruik van een PBPK-model voor kinderen, mogelijk gecombineerd met de farmacodynamie (PBPK/PD-model) zou een waardevol hulpmiddel kunnen zijn in de risicoschatting en kunnen leiden tot een afname van het gebruik van diermodellen.

Summary

Both in the development of medicinal products as well as in risk assessment of other xenobiotics there is an increasing awareness that children should be considered as a special group. The question is whether it is necessary to develop complementary (young) animal models specific for children. Children are exposed to other doses than adults and the pharmacokinetics and pharmacodynamics can be very different in children and adults. This report is focussed on substances, which are ingested by mouth as this is an important route of exposure in children. In order to gain insight in differences in pharmacokinetic handling of oral compounds in adults and children information concerning the physiology of the digestive tract and processes like intestinal absorption, distribution, metabolism and excretion in both adults and children was collected. It appears that the intestinal absorption does not change dramatically with age. The maturation of the gastrointestinal tract occurs within 6 months and by late infancy. Generally, changes in rate rather than in extent of absorption of compounds are found. However, xenobiotics that are absorbed by transport processes used for absorption of nutrients/compounds essential for growth of children, may be better absorbed in children than in adults. Furthermore, the body composition of children is different from that in adults in that the relative amount of body water is higher in infants and the fat content is lower. This may result in differences in volume of distribution of compounds in adults and children, depending on the physico-chemical properties of the compound. Another important process that can have great impact on the internal exposure of a compound is the metabolism. Metabolism can result in detoxication of a compound, but can also result in more toxic metabolites. Whether or not a compound is deactivated is a very important issue in risk assessment. In general all enzyme activity is at adult level within 6-12 months of age. In children from 6 months until 12 years of age metabolic activity may even be higher due to a higher metabolic rate, but in neonates metabolism is generally impaired in comparison to adults. Finally, the renal excretion appears also to be age dependent. Renal blood flow and glomerular filtration rate reach adult levels in the first 6 months of age. In summary for all these processes it can be concluded that the effects of age on pharmacokinetics are most pronounced during the first year of life. This should be taken into account in the risk assessment of a compound.

Risk assessment for drugs is based on another approach than risk assessment for other xenobiotics. For drugs a No Observed Adverse Effect Level (NOAEL) is obtained in animals. Moreover pharmacokinetic parameters like AUC and C_{max} are determined at the level of NOAEL exposure. Subsequently a so-called safety margin can be calculated by comparing the pharmacokinetic parameters at the level of clinical dosing in humans with the same parameters at the level of NOAEL in animals. In order to scale a dose down to a child a scaling model usually based on bodyweight or body surface area, is applied. Whether this will result in similar internal exposure and similar pharmacodynamic effects is dependent on the characteristics of the compound and the age of the child. However, based on the knowledge of the physiology of a child at a certain age and the effects this may have on the pharmacokinetic parameters and based on the pharmacokinetic parameters determined in adults it should be possible to determine the dosage that will lead to a similar internal exposure in the child. Therefore in this type of risk assessment it will not be necessary to develop young animal models for pharmacokinetic differences in children and adults. However, such models may be necessary to study the effects on developing systems in children. In risk assessment of other xenobiotics the NOAEL estimated from toxicology studies in animals is used as well or, if available, a NOAEL is estimated from doses that show adverse effects in man. For this kind of substances a Tolerable Daily Intake (TDI) is

generally required. Commonly a TDI is derived by dividing the NOAEL by uncertainty factors (10 x 10) for intra- and interspecies differences. It remains however questionable whether the uncertainty factor for intraspecies differences will also cover the differences between children and adults. Especially in neonates (< 1 month of age) these differences may be greater. Development or use of young animal models to assess the differences in internal exposure in children and adults will not be necessary. With the aid of the physiological data in children and adults 'Physiologically Based PharmacoKinetic' (PBPK) models can be developed that can model the internal exposure in adults and children. However, also for this type of risk assessment it will be important to determine a NOAEL in young animals to compensate for possible effects on developing systems in children. Overall it can be concluded that full adjustment of dosing or TDI's for pharmacokinetic differences can relatively easily be applied and should, to our opinion be seen as a first step in considering risk for the paediatric population. The use of a paediatric PBPK model possibly combined with pharmacodynamics (PBPK/PD model) may be a valuable aid in risk assessment and may reduce the need for animal models.

1. Introduction

Both in the development of medinical products [EFPIA document] as well as in risk assessment of other xenobiotics [Ginsberg *et al.*, 2002] there is an increasing awareness that children should be considered as a special group. Regardless the compound it can be stated that 1) children are exposed to other doses than adults, 2) once exposure has occurred, the pharmacokinetic handling of xenobiotics is likely to differ from that in adults [Besunder *et al*., 1988; Morselli, 1989; Kearns and Reed, 1989], 3) pharmacodynamic differences are to be expected in which the sensitivity of rapidly developing tissues/systems in neonates and young children may differ from that in adults [Faustman *et al*., 2000; Pope *et al.,* 1991; Vesselinovitch *et al*., 1979]. It is to be expected that differences in pharmacokinetics of compounds between adults and children can, to a large extent, be predicted by careful consideration and characterisation of normal developmental physiology as it affects the processes governing xenobiotic disposition. While studies into the physiological development of children started in the 1930s and 1940s, the area of paediatric pharmacokinetics has blossomed since the 1970s when advances in the development of sensitive and specific drug assays began [Rane and Wilson, 1976; Kearns and Reed, 1998].

1.1 Medicinal products

In case of drug research the problem is that most products have not been developed and assessed specifically for paediatric use, and are prescribed to children outside the terms of their product license. Children are therefore often exposed to the risk of adverse drug reactions or to lack of efficacy, and are thus unable to benefit from many of the therapeutic advances offered to adults (Figure 1-1). Development costs, the difficulty of conducting research in children, and liability considerations discourage the conduct of the necessary research programs in paediatric indications. This situation led to the EU Health Council to adopt, in December 2000, a Resolution calling on the Commission to develop incentives and other measures to ensure that new and existing medicines be adapted for paediatric use. The U.S. FDA has the longest experience in adopting successive types of measures to promote paediatric research. [EPFIA document].

The type of studies which have to be performed for proper risk assessment of paediatric drugs are determined by the application of the drug. Medicinal products for diseases predominantly affecting paediatric patients require a development program which will be conducted in the paediatric population. For medicinal products, intended to treat diseases or conditions which occur also in adults, it may even be possible to wait until substantial postmarketing experience in adults is gathered [ICH guidance document]. Initial safety and tolerability data will always be obtained in adults, which implicates that in every development program there is a phase in which extrapolation from the adult situation to the paediatric situation has to be performed. In order to encourage and facilitate timely paediatric medicinal product development internationally, a guidance document was developed by the European Agency for the Evaluation of Medicinal Products (EMEA). This guidance document [EMEA Guidance, 2001] includes considerations when initiating a paediatric program for a medicinal product, types of studies (pharmacokinetic, efficacy, safety, pharmacokinetic/ pharmaco-dynamic), age categories, etc.

In Figure 1.1 the role of pharmaco- and toxicokinetics in the development of medicinal products for children is schematically depicted. From preclinical studies (a.o. toxicology studies in rodents and non-rodents) data on the No Observed Adverse Effect Level (NOAEL) are obtained. Since a few years attention is also paid to the accompanying kinetics of the compound in these studies. In that way data on internal exposure, expressed as AUC_{NOAEL} (mg/L*h), are available. The ratio of this exposure to exposure in man following the highest clinically relevant dosing scheme represents the so-called safety margin. It is important to realise that this safety margin is only valid for adults.

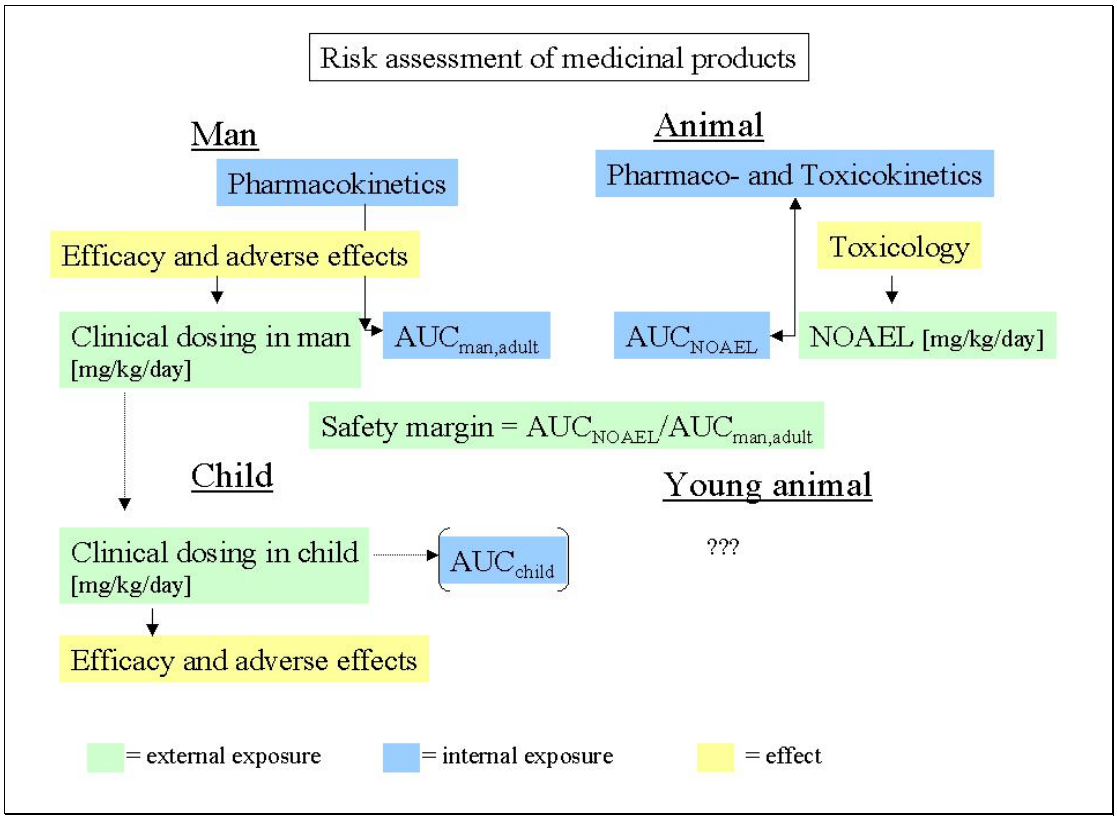

Figure 1.1 Role of kinetics in risk assessment of drugs for children

The clinical dosing in children is deduced from dosing regimen in adults (Figure 1.1), but is established on the basis of on weighing the pros (efficacy) and cons (acute adverse effects) of the proposed dosing regimen in children. On the basis of the FDA and ICH guidance documents for developing medicinal products for the paediatric population, information on the accompanying internal exposure in children (AUC_{child}) should be gathered. To our opinion, the acquired information on internal exposure at the NOAEL in experimental animals and at the level of the clinical dosing regimen in children will not always be sufficient for assessing a safety margin for the paediatric population. In most cases a NOAEL is assessed in (young) adult animals and focusses on endpoints in non-developing tissues/organs. This implies that NOAELs for products which are administered during critical windows of sensitivity in the developing child need special attention. Firstly, developing systems may respond differently from matured adult organs. Secondly, some adverse events and drug interactions that occur in paediatric patients may not be identified in adult studies. In addition, the dynamic processes of growth and development may not manifest an adverse event acutely, by at a later stage of growth and maturation [EMEA Guidance, 2001]. From

our pharmacokinetic point of view we want to stress that both the maximum concentration (C_{max}) as well as overall internal exposure (AUC) are parameters that should be considered in relation to adverse effects.

Studies on the pharmacokinetics should generally be performed to support formulation development and determine pharmacokinetic parameters in different age groups to support dosing recommendations (see Figure 1.1). These studies are only conducted in patients with the disease and for obvious ethical reasons not in healthy paediatric volunteers. All approaches for studying kinetics in children are facilitated by knowledge of adult pharmacokinetic parameters. Knowing the pathways of clearance (renal and metabolic) of the medicinal product and understandig the age-related changes of those processes will often be helpful in planning paediatric studies. Where efficacy studies are needed, it may be necessary to develop, validate, and employ different endpoints for specific age and developmental subgroups. For example, measurement of subjective symptoms such as pain requires different assessment instruments for patients of different ages [EMEA Guidance, 2001].

1.2 Other xenobiotics

Risk assessment of exposure to other xenobiotics than medicinal products has its own features. Risk assessment for this group of compounds is mainly based on animal data, as a program of studies for obtaining information on pharmacokinetics/pharmacodynamics and safety in humans is lacking. Like in human drug research, a NOAEL is deduced on the basis of animal data. In order to come to an Acceptable Daily Intake (ADI) or a Tolerable Daily Intake (TDI), the NOAEL is divided by uncertainty factors. These factors are applied for correcting for extrapolation from animal data to human data and for intraspecies differences (Figure 1.2). It remains however difficult to estimate whether these uncertainty factors also sufficiently cover the differences between adults and the paediatric population.

*Intra*species differences concerning pharmacokinetics and pharmacodynamics are currently represented by a default (usually 10-fold) uncertainty factor (for noncarcinogens). Latest insights assume that half this factor accounts for racial, gender, genetic, and age differences, as well as intra- and interindividual differences due to disease states and intake of drugs [ICPS Guidance]. Although it implicates that this uncertainty factor thus accounts for child/adult differences, it is not clear whether this conclusion is valid under all circumstances. In case the database is incomplete or when there is a serious concern that children may be more susceptible to a certain substance, an additional assessment factor may be applied. In the US the opposite approach is taken. The Food Quality Protection Act of 1996 requires the application of an additional 10-fold margin to assure protection of infants and children for unacceptable pesticide exposure. This additional 10-fold FQPA factor can only be reduced or eliminated if reliable and complete data indicate that such a reduction is safe for infants and children.

*Inter*species differences concerning pharmacokinetics and pharmacodynamics are covered by a second default uncertainty factor (also usually 10-fold). In recent years physiologically based pharmacokinetic models (PBPK models) have been applied to adjust for pharmacokinetic differences between test animals and humans in order to come to more realistic risk assessment. It needs, however, to be stressed that application of these models is still not common practice. The models have mainly been build for environmental

contaminants [O'Flaherty, 1995; O'Flaherty *et al*., 1995; Polat *et al*., 1996]. Using PBPK-models acknowledges that the relationship between administered dose and effective internal dose can differ across species, with this difference having significant implications for risk assessment [Ginsberg *et al*., 2002]. While such refinements may have removed some of the uncertainty in *inter*species extrapolations, risk assessments have yet to account for child-adult differences in pharmacokinetics of xenobiotics.

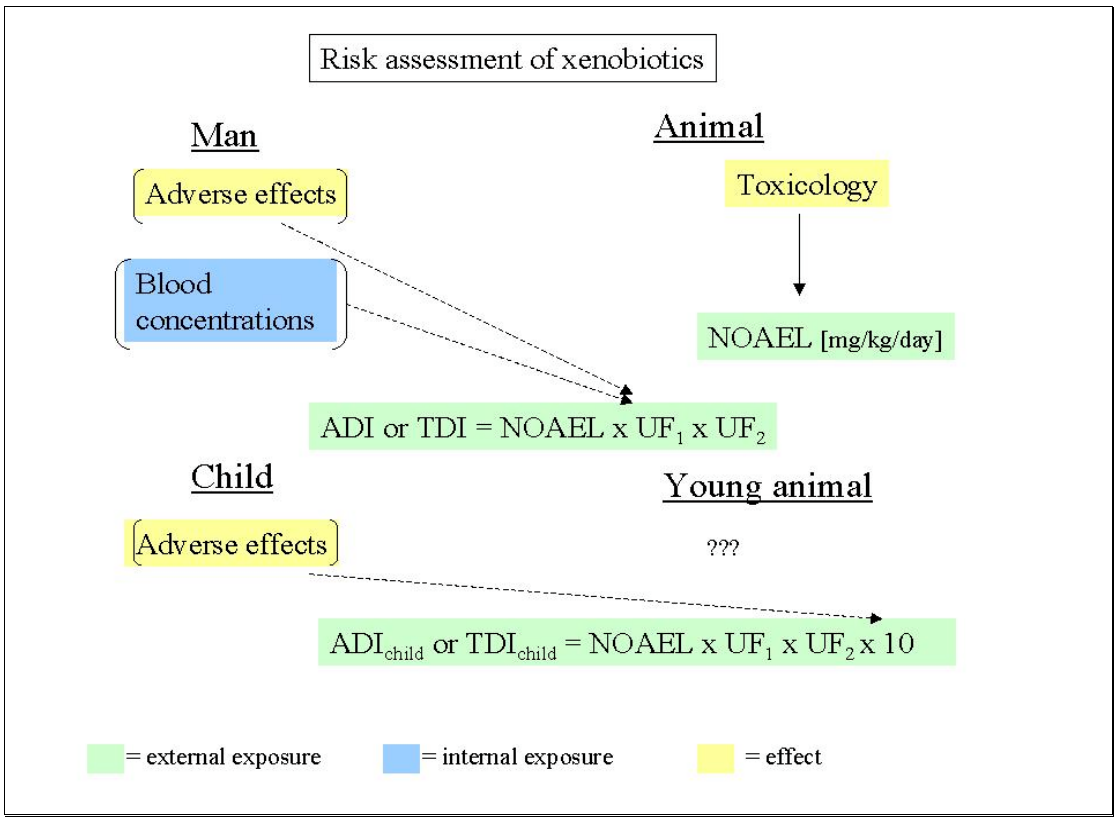

Figure 1.2 Role of kinetics in risk assessment of xenobiotics in children

1.3 Classification of childhood

During childhood many physiological changes take place which may have an impact on the kinetics and dynamics of a compound. For that reason childhood is divided into various classes of age. In literature a range of classifications can be found, but the differences between the various classification systems seem to be minor [Crom, 1994; EMEA Guidance, 2001; Ginsberg *et al*., 2002]. In this report the division of the paediatric population into subgroups is based on the Index Medicus [Crom, 1994]:

Neonates	< 1 month
Infants	1 to 23 months
Children	2 to 12 years
Adolescents	13 to 18 years

In the present report the terms 'child' and 'children' refer to the entire period from birth to adolescence, while the terms neonate and infant refer to the periods in life as described above.

1.4 Aim of this report

Due to developing physiological systems there may be differences in pharmacokinetic handling of compounds between adults and children. It is important to gain insight into these physiological differences in order to extrapolate existing data on toxicity or efficacy from adults. Otherwise, it may optimise risk assessment for any random compound in children. As oral intake of a compound is an important route of exposure in children, we have chosen to focus attention on the physiology of the digestive tract. For gaining insight into processes as intestinal absorption, distribution, metabolism and excretion, parameters as volume of distribution, protein binding, presystemic and liver metabolism and renal function were taken into account.

1.5 Relevance for policy makers

Insight into pharmacokinetic handling of compounds between children and adults may have a 'spin off' in various aspects of risk assessment. The information of this report may gain insight in which phases of childhood are specifically relevant for risk assessment, as the paediatric population covers a wide range of physiological conditions. In the second place this information will facilitate the estimation of pharmacokinetic differences between experimental animals and children. In other words, insight is gained into the usefullness of an animal model for the paediatric population. Moreover, a better quantification of absorption, distribution, metabolism and excretion in relation to adult data and in relation to animal data will be possible [EHC59]. It is therefore to be expected that this information will lead to refinement regarding the use of experimental animals.

1.6 Outline

Chapters 2 to 5 give information on the pharmacokinetic processes absorption, distribution, metabolism and excretion, respectively. In chapter 2 the development of the gastrointestinal tract and the sublingual, gastric and small intestinal absorption are described. Chapter 3 deals with volumes of distribution and plasma protein binding. Chapter 4, on metabolism, includes phase I and II liver metabolism, as well as intestinal metabolism. In chapter 5, renal excretion is described. The problems dealing with scaling between pharmacokinetic parameters in adults and children is discussed in chapter 6. Chapter 7 gives an overall discussion on the main differences between pharmacokinetics of the gastrointestinal tract in children and adults. It is also tried to draw conclusions from these differences for risk assessment in children.

2. Absorption

Being the first contact site of all orally ingested compounds, the epithelium of the gastrointestinal tract has an important absorptive function for nutrients but it also needs to present a barrier to absorption of potentially harmful compounds. An ingested compound has to cross the epithelial cell layer of the gastrointestinal tract in order to become taken up in the body. Absorption of compounds occurs mainly in the small intestine. Therefore, we have confined us in this chapter to the tract mouth to small intestine and not included the large intestine. First the differences in physiology between child and adult are described. Second the consequences for absorption of compounds in the different compartments are discussed.

2.1 Oropharyngal development

The anatomy of the pharynx and oesophagus in infants and children is similar to that of adults with obvious differences attributable to their smaller size [Anderson *et al.*, 1997]. The co-ordinated oral and pharyngeal movements necessary for swallowing solids develop within the first two months of life in term infants [Nelson *et al.*, 1996]. In neonates the flow rate of saliva is 10 times lower compared to young adults but increases rapidly within the first few months [Geigy 1968; Seidel *et al.*, 2001]. A maximal flow rate of saliva is reached at the age of 3-4 with an 8 times higher flow rate of saliva compared to adults basal flow rates [Radde, 1985]. Hereafter the flow rate of saliva declines and by the age of 6-12 the flow rate is only slightly higher than in (young) adults, 0.65 ml/min and 0.41ml/min, respectively [Gutman and Ben-Aryeh, 1974; Dawes, 1974; Navazesh *et al.*, 1992]. The composition of saliva of neonates is also different from adults. Alpha-amylase activity increases rapidly from low values at birth to approximately two-thirds of adult levels by 3 months [Sevenhuysen *et al.,* 1984]. As it is relatively easy to obtain a saliva sample, saliva composition is used as an indicator of systemic and metabolic changes. For example, the secretory IgA, undetectable at birth, increases rapidly during the next 6 months reaching adult values by 6-8 years in unstimulated saliva and already by 2-4 years in stimulated saliva [Gleeson *et al.*, 1982; Burgio *et al*., 1980].

The development of the oesophagus is of relatively little importance for absorption of compounds, except for the function of the gastro-oesophageal sphincter. Immaturity of the sphincter may lead to increased reflux of the stomach contents. This may subsequently lead to an altered absorption of compounds present in the stomach [Radde, 1985]. According to Boix-Ochoa and Canals [1976] maturation of an effective anti-reflux barrier is not achieved until about 3 months postnatal.

2.2 Gastric development

2.2.1 Ontogeny of the stomach

The major function of the stomach is to temporarily store food and release it slowly into the duodenum. In the stomach food, saliva, and gastric juices are mixed to form a semi-solid chyme. The hydrochloric acid secreted in the gastric juices kills bacteria and denaturates proteins. Enzymes in the gastric juices begin the digestion of proteins and facilitate the digestion of triglycerides.

The volume of an 'empty' (fasted conditions) stomach is 2.5 ml for neonates and young infants, 8.8 ml for children and 50 ml for adults [Geigy, 1968]. The volume can increase 50-fold after feeding. The parietal cell mass per unit area of the neonatal stomach at term is about two to three times that of the adult stomach, although the thickness of the mucosa and muscular wall are much thinner.

The secretion of pepsin increases 3-fold between week 35 of gestational age and term. The secretion further increases 4-fold during the first two days after birth. Thus, in preterm neonates the pepsin activity is relatively low, whereas the secretion of hydrochloric acid by the parietal cells is about the same concentration as in term neonates. The secretion of hydrochloric acid, intrinsic factor and pepsin gradually increases during the first months. At least by the age of 2 years, pepsin and acid output is comparable with that of adults when expressed on a body-weight basis [Dodge, 1987].

2.2.2 Gastric pH

In the neonate, maturational processes continuously modify gastric pH. At birth, gastric pH is neutral (pH 6-8) due to the presence of amniotic fluid in the stomach [Avery *et al*., 1966]). The subsequent pattern of gastric HCl secretion remains controversial. According to Nelson *et al*. [1996] acid secretion is low in the first 5 hours of life. Thereafter, gastric pH falls to a value of 1.5 - 3.0. From the available data, this fall in gastric pH is quite variable but appears to be independent of both birthweight and gestational age [Besunder *et al.*, 1988]. More likely, extrauterine factors are responsible for initiating acid production, since basal acidic output correlates with postnatal but not with postconceptual age. One such factor may be the influence of enteral feeding on the maintenance of gastric secretory function [Besunder *et al.*, 1988].

Due to immaturity of the gastric mucosa, which may persist for 10-15 days [Miller, 1941; Lebenthal *et al*., 1983], acid production may remain reduced comparable with a state of relative achlorhydria. This occurs more often in the preterm than term neonate. Investigations by Hyman *et al*. [1983] showed hypochlorhydria with gastric pH greater than 4 in 19 % of neonates at 1 week of age, 16 % at 2 weeks of age and 8 % at 3 weeks of age. No infant demonstrated a basal gastric pH greater than 4 after 6 weeks of age. Thereafter, gastric pH falls to a value of 1.5 - 3.0, which is comparable adult gastric pH (1.5-2.5). However, the greater secretion of saliva in the young child (Chapter 2.1) will cause dilution and a slight increase in pH of gastric contents even during fasting [Geigy, 1968].

2.2.3 Gastric motility and emptying

During the first days of life, gastric motility is very low. In addition, gastric contractions in neonates are less pronounced than in infants. As a result, the rate of gastric emptying is variable during the neonatal period and is affected by gestational maturity, postnatal age, the type of feeding and clinical disease states (see Table 2.1).

Table 2.1. Factors affecting gastric emptying rate [Besunder et al., 1988]

Increase	Decrease
Human milk	Prematurity
	Long chain fatty acids
	Gastro-oesophageal reflux
	Congenital heart disease
	Respiratory distress syndrome

An inverse relationship was found between gestational age and the amount of gastric retention 30 minutes after a 5 % glucose in water feeding [Gupta and Brans, 1978].

Stomach emptying is controlled to a great extent by feedback signals from the duodenum. These feedback inhibitory mechanisms slow down the rate of gastric emptying when 1) too much chyme is already in the small intestine or 2) the chyme is excessively acid (<pH 3.5), contains too much unprocessed protein or fat, is hypotonic or hypertonic, or is irritating. In this context, the following three factors of food ingestion have a major effect on the rate of gastric emptying; the volume of the meal, its osmotic pressure and its composition of macronutrients. This applies to infants as well as to adults, although Lebenthal and Siegel [1985] could not find a relationship between gastric emptying rate in infants and osmolality of the meal.

The type of feeding has been shown to influence the rate of gastric emptying. The rate of emptying of the three major foodstuffs (fat, carbohydrate and protein) from the stomach is regulated so that equal numbers of calories are delivered to the duodenum in the same time. As neonates and young infants receive food of high caloric density i.e. relatively high in fat content, one can expect the gastric emptying in this group to be relatively slow.

The following studies support a relative slow gastric emptying in young infants compared to adults and dependence on the type of feeding. Siegel *et al.* [1984a] reported that the type of fatty acid fed to infants affected the rate of gastric emptying. Slower emptying was seen in feedings with long-chain fatty acids than with medium-chain triglycerides (*e.g.* the commercial formula Similac® contains long-chain fatty acids). Furthermore, human milk emptied more rapidly in infants, following an exponential emptying pattern, whereas infant formula feeding showed a slower, linear emptying pattern [Cavell, 1981]. The fat in infant formula feeding may evoke a greater feedback response [Dodge, 1987]. In another study by Siegner and Fridrich [1975], an adapted cow's milk formula (composition: 1.7 g protein, 3.7 g fat and 7.2 g lactose per 100 ml) was emptied slower in infants aged 1 to 10 weeks compared to healthy adults, 87 minutes compared to 65 min, respectively. Since the volume of an empty stomach in neonates and young infants is small (2.5 ml), the food intake is less diluted initially compared to adults (empty stomach volume is 50 ml), this may contribute to a slower emptying rate of the gastric content in neonates and young infants. The age at which the gastric emptying time of infants approaches that of adults remains poorly defined, although some authors believe that this transition occurs within the first 6 to 8 months of life [Heimann, 1980; Besunder *et al.* 1988].

2.3 Fluids secreted by exocrine pancreas

The pancreas is a mixed exocrine-endocrine gland. The exocrine portion of the gland is 84 % by volume, ductular cells and blood form 4 %, while endocrine cells comprise only ~2 %. The remainder of the volume of the gland (10 %) is occupied by extracellular matrix. Most of the exocrine pancreas consists of acinar cells (>80 %) while the ducts comprise only 14 % by volume. The major function of the exocrine pancreatic secretion is to provide an optimal environment for efficient digestion and absorption of macronutrients. The secretions of bicarbonate neutralise the acid chyme emptied by the stomach into the duodenum providing a functional pH in the small intestine for the action of the digestive enzymes. Furthermore, the islets cells composed of two types of cells A and B secrete glucagon and insulin, respectively.

In the newborn the ratio of type A to B is approximately 1 whereas in adults the ratio of A to B is 4.

The amount of fluids secreted by the exocrine pancreas into the intestine increases with maturation. In the preterm neonate 34-36 weeks, lipase activity was only half that in term neonates. Between birth and 9 months of age there is a further 10-fold increase of lipase activity. Amylase activity is very low at birth and increases a 200-fold by the age of 9 months resulting in a less adequate utilisation of starches by young infants compared to older infants and children [Hadorn and Munch, 1987]. The activity of the pancreatic enzymes appear to adapt to changes in the type of macronutrients in the diet. A diet containing starches or a high protein diet in preterm neonates increased the production of amylase and trypsin, respectively, but a high fat diet had no effect on lipase output [Hadorn and Munch, 1987]. Overall there seems to be a functional immaturity of the pancreatic exocrine secretion rate even in the full-term neonate [Radde, 1985].

2.4 Bile secretion

Bile serves two important functions: first, it plays a very important role in fat digestion and absorption and second, bile serves as a means for excretion of several important waste products from the blood. These include especially bilirubin, an end product of haemoglobin destruction, and excesses of cholesterol synthesised by the liver cells.

In the liver two bile salts, the so-called primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA) are formed from cholesterol. Prior to being secreted into bile, the newly synthesised bile acids are conjugated to glycine or taurine. Bacterial, but not mammalian enzyme systems, can dehydroxylate the two bile acids to yield deoxycholic (DCA) and lithocholic acid, respectively. They are called the secondary bile acids. Near term, bile flow is low compared to adult levels and the bile is composed of only primary bile acids preferably conjugated to taurine (see Table 2.2) [Nelson *et al.*, 1996, Geigy, 1968].

Table 2.2 Biliary bile salt composition in neonates, infants and adults [Geigy, 1968].

	Ratio of glycine: taurine	**Choline : chenodeoxycholine:deoxycholine**
Neonate		
1-4 days	0.47	2.5 : 1 : 0
5-7 days	0.95	2.5 : 1 : 0
Infant		
7-12 months	2.4	1.1 : 1 : 0
Adult	3.1	1.2 : 1 : 0.6

Boehm *et al.* [1997] measured preprandial bile concentrations in aspirated duodenal juice during the first 60 days of life in 41 healthy breast-fed preterm neonates (gestational age 27-34 weeks). The total bile concentration was below 4 mM the first two weeks and increased continuously in the duodenal aspirates up to the end of the observations at 60 days. The CA/CDCA ratio was high at birth and decreased with increasing postnatal age. During the first weeks of life the bile acids were preferentially conjugated with taurine but the taurine/glycine ratio decreased also with postnatal age. Hardly any secondary bile acid was recovered in the duodenal juice.

Bile acids secreted into the duodenum are absorbed in the intestine and subsequently recaptured by the liver. This is called the enterohepatic circulation of bile acids.The process accelerates during meals and slows during fasting. Approximately 94% of the bile salts are reabsorbed in the small intestine. Most of the bile acids are absorbed by an active transport process in the distal ileum although glycine-conjugated dihydroxy bile acids and free unconjugated bile acids can be absorbed by passive diffusion in upper small intestine and the colon, respectively. However, in young infants the active absorption of taurocholic acid was deficient, commencing by 8 months of postnatal age [De Belle *et al.*, 1979]. Therefore, as much as 50-60 % of the bile acids may be absorbed in the jejunum and colon of young infants instead of absorption in the distal ileum [Watkins, 1987].

These data indicate that the establishment of an intestinal microbial flora necessary for intestinal bile acid transformation and the development of the enterohepatic bile acid circulation lasts some months of postnatal life.

2.5 Small intestine

The major functions of the small intestine are to digest food, to absorb major nutrients such as sugars and aminoacids, but also to serve as a barrier to digestive enzymes, ingested (potentially harmful) compounds, bacteria, and finally to remove undigested and unabsorbed (food) compounds into the large intestine. This multifunctional characteristic of the small intestine makes the epithelium structurally complex. In the last trimester of gestation, a complex villus-crypt structure of multiple cell lineages commences and digestive enzymes such as lactase and sucrase appear in the microvillus membrane of the enterocytes. Ontogeny of the digestive enzymes is reviewed by Henning *et al.* [1994]. The rate of cell maturation from crypt to villus type was approximately 2-fold lower in neonates compared to adults [Seidman and Walker, 1987]. The small intestinal function further matures throughout prenatal and postnatal life.

In children the villi of the small intestine tend to be broad leaf-shaped rather than finger-shaped projections as in adults, implying a relative smaller functional surface area of the small intestine in neonates [Nelson *et al.*, 1996]. Since the length and diameter of the small intestine increases continuously from birth till adulthood, the functional surface area of the small intestine increases more than 40-fold [ICRP publication 23, 1992]

So far, there have been relatively few studies done on pre- and postnatal maturation of intestinal motility [Heimann, 1980]. In infants, intestinal motor activity occurs less frequently than in adults, with a different pattern of rhythmic peristaltic activity [Radde, 1985]. In general, intestinal peristalsis is irregular and partially dependent on feeding and feeding habits [Heimann, 1980].

Another important difference between neonates and older individuals is the type and degree of bacterial colonisation of the gut. At birth the intestine is virtually sterile and a rapid colonisation occurs with a flora that is different in breast-fed and formula-fed infants [Raddle, 1985]. A further change in bacterial flora occurs at the time of weaning (4-6 months) which is important for the hydrolysis of compounds that are conjugated and secreted in the bile so that unconjugated compound can be absorbed by the intestinal epithelium (e.g. conjugated bile acids). On the other hand, the neonatal gut is capable of converting glucuronides, excreted

into the gastrointestinal tract from the bile, to their unconjugated and hence enterohepatic reabsorbable form by the presence of β-glucuronidase. This enzyme is absent in the adult gut.

2.6 Transporters

Evidence is increasing that active drug transport across cellular membranes is an important process involved in absorption, disposition and excretion of compounds. Active drug transport is essentially important in liver, gut, kidney and the blood brain barrier. Some of these transport proteins are expressed in one or several tissues responsible for absorption, distribution and elimination. Many of these transporters have been discovered in the last decade and not much information is available concerning the ontogeny of these compounds.

Inorganic sulfate (S_i) is an important anion for normal body function. It is involved in many physiological and pharmacological processes, including activation and detoxification of many endogenous and exogenous compounds. It has been proposed that steady-state serum S_i levels in humans vary during development: neonates and infants were shown to have an elevated mean serum S_i concentration of 0.47 mM, compared with adult levels of 0.33 mM [Cole and Scriver, 1980]. The higher serum S_i levels in the neonates may in part be attributed to a difference in amino acid and protein intake and the fact that the glomerular filtration rate is lower in young infants than adults [Edelman, 1978]. It is expected that neonates and infants have a reduced number of S_i transporters. Since the total renal tubular mass and brush border and basolateral surface areas of the proximal tubule are much smaller at birth than in adulthood and the proximal tubular segment where the majority of S_i reabsorption occurs is poorly developed in neonates and infants compared to the adult. Until now this has only been studied in rats and these results suggest that there are age-dependent increases in mRNA expression of two proximal tubular S_i transporters, NaS_i-1 and sat-1 [Markovich and Fogelis, 1999]. These transporters probably do not play an important role in transcellular transport of exogenous compounds.

Potassium regulation and homeostasis during infancy are, owing to growth and development, different in later life: infants need to retain more K^+ than adults, to avoid growth retardation. The positive K^+ balance in infancy is characterized by higher K^+ absorption in gut, lower K^+ secretion/excretion in kidney and immaturity of the mechanisms regulating intra/extracellular K^+ distribution. Several factors maintain the positive K^+ balance. They include higher expression of absorptive transporters in colon and probably in kidney, lower expression of secretive transporters in colon and kidney, lower renal K^+ excretion following K^+ loading, immaturity of hepato-renal K^+ reflex mechanisms, immaturity of tissue K^+ binding/releasing capacity and immaturity of the neuro-hormonal control of K^+ transport in several organs [reviewed in Aizman *et al*., 1998].

The above mentioned transporters appear to have mainly a physiological function for specific endogenous molecules and it is difficult to speculate on the effect of immaturity of such transporters on the absorption, elimination or distribution of exogenous compounds. A group of transporters that are much more important for the transcellular transport of exogenous molecules are the multidrug resistance protein (P-glycoprotein) and related transporters. These are members of the ATP binding cassette (ABC) superfamily of transport proteins which is among the largest and most widespread protein superfamilies known [Leslie *et al.*, 2001]. Its members are responsible for the active transport of a wide variety of compounds across biological membranes including phospolipids, ions, peptides, steroids, polysaccharide,

amino acids, organic anions, drugs and other xenobiotics. One of the best studies members of the ABC superfamily of transport proteins is P-glycoprotein. Human P-glycoprotein has been detected in the apical surface of epithelial cells from excretory organs, such as the bile canalicular membrane of hepatocytes in the liver, the proximal tubules in the kidney and in enterocytes lining the wall of the intestines [Thiebaut *et al.*, 1987]. These locations are indicative of a role of P-glycoproteins in the protection the host against xenotoxins, either by accelerating their excretion or by preventing their uptake from the gastrointestinal tract following oral ingestion. P-glycoprotein is also present in the capillary endothelial cells in the brain and the testis, pointing to a protective role at the blood-brain and blood-testis barrier [Cordon-Cardo *et al*., 1990; reviewed in Van Tellingen, 2001]. The absence of P-glycoprotein (studied in knockout mice) results in a decreased body clearance of many intravenously administered drugs. This is most probably due to a decreased excretion of unchanged drug from the systemic circulation. Three important excretion routes are of potential interest, namely: renal excretion, hepatobiliary excretion and excretion by the (small) intestines. The presence of P-glycoprotein in the intestine is an important factor in the handling of many substances as P-glycoprotein at this site either mediates direct efflux through the intestinal wall and/or limits the re-uptake after hepatobiliary excretion. The presence of P-glycoprotein in this tissue can also reduce the absorption of compounds following oral administration, thus protecting the host against orally ingested toxins [reviewed in Van Tellingen, 2001].
Little is known regarding the ontogeny of P-glycoprotein expression in the tissues. Van Kalken *et al*. (1992) investigated the expression of mdr1/P-glycoprotein in human fetal tissues and showed that expression of mdr1-mRNA could already be demonstrated in the embryonic phase of human development, after 7 weeks of gestation. However, some differences were found between fetal and adult human tissue distribution. Prenatal intestine did not show staining of the epithelium, although definite mdr1-mRNA expression was observed in late specimens. In kidney and liver, mdr1-mRNA expression and staining for P-glycoprotein were detected in early fetal life (11-14 weeks). Mahmood *et al.* (2001) studied the ontogeny of P-glycoprotein in mouse intestine, liver, and kidney. They found that P-glycoprotein expression in mice was limited at birth and increased significantly with maturation in intestine. In contrast, hepatic and renal P-glycoprotein expression was at adult levels at birth. Both studies might be an indication that P-glycoprotein expression is not at adult levels in the intestine at birth in human. This might be an indication that at young age the protection against orally ingested toxins is less than in adults. An interesting detail is that in cancer research it is found that agents that are substrates for P-glycoprotein induce expression of P-glycoprotein in cell lines upon exposure to these agents. We may speculate that the expression of P-glycoprotein in the intestine is only induced after challenge by toxins that are a substrate for P-glycoprotein and that for that reason the expression at birth is low.

The organic anion transporter (OAT) family handles a wide variety of clinically important compounds (antibiotics, nonsteriodal anti-inflammatory drugs, etc.) and toxins (e.g. a herbicide: 2.4-dichlorophenoxyacetic acid). This system plays a critical role in protecting against the toxic effects of anionic substances, whether of endogenous or environmental origin, by removing such substances from the blood via a transport mechanism found in the basolateral membrane of renal epithelial cells [Sweet *et al*., 2001].

The ontogeny of renal OA transport maturity has been studied indirectly through physiology (see paragraph 5.2.3). It was demonstrated that OA secretion is low at birth and increases over the first few weeks of neonatal life and then declines to adult levels. This increase in OA secretion was disproportionate to the increase in renal mass and was thought to reflect the

specific maturation of the organic anion transport system. Expression of OAT-gene has been studied in adults, but not in infants and children. Studies in rats and mice showed mRNA expression increased through birth, with the highest levels detectable at 1 day postpartum, followed be a decrease to adult levels [Lopez-Nieto *et al*., 1996; Nakajima *et al*., 2000]. Interestingly, this same pattern of expression was found for OCT1 (organic cation transporter) [Pavlova *et al*., 2000] [reviewed in Sweet *et al*., 2001].

2.7 Absorption

For the oral route, the extent of absorption of a compound in the gastrointestinal tract depends on both physiological factors and on the physicochemical properties of the compound. Physiological factors that will determine the extent of absorption of a compound include the gastrointestinal pH, gastric emptying, gastrointestinal transit, the composition of the intestinal lumen (e.g., pH, enzymes, food), and the intestinal epithelium. The physical properties (e.g. molecular weight, molecular size, lipophilicity, hydrogen bonding potential, pKa), chemical properties (e.g. chemical and enzymatic stability, interactions with other compounds or with food) but also solubility, and hence the importance of the matrix, influence the absorption of a compound. Dissolution of a compound is crucial, as whatever the absorption route across the gastrointestinal epithelium may be, a compound has to be dissolved in order to be accessible for absorption.

For the following reasons the major part of absorption of compounds takes place in the small intestine: 1) this segment of the gut possesses a much larger total surface area than either the upper or the lower gastrointestinal tract (200-500 m^2), 2) the residence time is several hours, 3) the low pH in the stomach and the digestive enzymes in the stomach and small intestine facilitate the dissolution of compounds, 4) the high blood flow in the small intestine (1 liter/min), and 5) the small intestine contains specialised absorptive cells, which facilitate absorption of intraluminal components.

When a compound is accessible for gastrointestinal absorption, the rate of absorption, i.e. the passage of the gastrointestinal epithelium, is the determining step in absorption of the compound. Various absorption mechanisms can be distinguished. Basically, a compound can be absorbed either by transport through the epithelial cell (transcellular pathway) or by transport along the cells (paracellular pathway). Since the transcellular pathway occupies more than 99.9 % of the total surface area (absorptive cells with microvilli), most compounds are absorbed by the transcellular route.

In order to be well absorbed in the gastrointestinal tract, compounds must meet various conditions. The ‘rule of 5’, small (M_r < 500 D), lipophilic (0<logD oct,7.4<4.15), and without too many H-donor and H-acceptor sites, will indicate whether a compound is likely to be transported via the passive transcellular route. When a compound is too hydrophilic (or too large or too many H-donor and H-acceptor sites) to be transported via the transcellular route and a high and fast absorption is required, such as is the case for nutrients, the compound should be transported across the intestinal epithelium by specific carrier-mediated mechanisms. For example, monosaccharides, di-tripeptides, folate are absorbed preferentially in the upper small intestine and vitamin B12 uptake in the terminal ileum. For compounds that are transported via the transcellular route, the amount of compound reaching the portal vein can be reduced by efflux transporters or by metabolism in the intestinal cells.

To access the diffusive paracellular pathway the compound must be small (Mr < 400 D) and hydrophilic. In general the absorption of paracellularly transported compounds is slow.

2.7.1 Oral (sublingual) absorption

Absorption of compounds in the mouth is generally of little importance because firstly the compound should be dissolved in the saliva before absorption can occur and secondly the short residence time of the compound in the mouth before swallowing. For these reasons, absorption in the oral cavity is confined to dissolved compounds with high permeability properties (in general small, reasonably lipophilic compounds). Nevertheless, the oral and pharyngeal mucosa are being used in adults as a route for drug administration because drugs so absorbed enter the systemic circulation directly without having to pass through the liver. Examples of these drugs are some hormones such as oligo- or polypeptides, which are destroyed by gastric HCl or by intestinal peptidases. Sublingual administration of a drug leads to rapid entry into the systemic circulation because of the thin epithelium, a rich blood supply, and a slight acidic condition. For a drug such as nitroglycerin that must act rapidly to relief of symptoms, the sublingual route is very useful since absorption of the drug by this route is extremely rapid (within 1 min). However, such administration requires the co-operation of the patient to keep the drug under the tongue for a prolonged period. The need for co-operation precludes administration of drugs sublingually in infants and small children [Radde, 1985].

2.7.2 Gastric Absorption

Due to the higher gastric pH in neonates compared to later in life, acid-labile compounds and weak bases such as atropine, caffeine as well as other methylxanthines may be absorbed more readily from the stomach in this stage of life [Radde, 1985]. Indeed, toxic effects of atropine and methylxanthines have been observed when this mechanism was not considered in the calculation of drug dosage in the young infant [McCracken *et al.*, 1978]. The relatively high pH retards the absorption of acidic compounds e.g. rifampicin [Morselli *et al.*, 1976] and will enhance the translocation of basic compounds. This may contribute to higher serum concentrations of basic compounds in neonates relative to older children and adults as shown for drugs such as ampicillin and penicillin [Silverio and Poole, 1973].

The prolonged residence time of a compound in the stomach when ingested together with food in neonates and infants, can lead to an increase in the absorption of compounds from the stomach, which require an acidic environment for dissolution. However, the feeding pattern in neonates and infants is frequent and with each feeding the gastric pH will temporarily raise. Furthermore, the almost continuous presence of milk in the stomach in neonates and infants may limit the absorption of compounds from the stomach, which are highly protein bound or lipid soluble. Some of these effects are counteracting and it is, therefore, difficult to predict what the effects on absorption of compounds in the stomach is. Furthermore, it should be kept in mind that in general the absorption of compounds in the stomach is much less than the absorption in the small intestine.

2.7.3 Small intestinal absorption

2.7.3.1 Role of gastric emptying, intestinal motility and absorptive surface area.

The rate of gastric emptying is an important determinant of the rate and extent of absorption of compounds. If the rate of gastric emptying is slowed as was observed in neonates, small portions of a compound are delivered to the small intestine for a prolonged period of time. This will, in turn, delay and reduce the peak serum concentration of the compound, without necessarily affecting the extent of absorption of the compound. Furthermore, the relative smaller absorptive surface area in infants and neonates, and thus less receptors and transport proteins per square unit intestine, will probably also result in a slower absorption of compounds. If a compound is only absorbed at a distinct area of the intestine, the extent of absorption of the compound may be lower as result of the relative smaller surface area. Both of these effects presuppose that intestinal motility remains constant. The following studies indicate that reduced gastric emptying time, absorptive surface area and/or intestinal motility reduce the absorption rate of compounds.

Firstly, riboflavin absorption was studied in 5-day old infants and in adults [Jusko *et al.*, 1970]. When saturation doses were given, lower urinary excretion rates of the vitamin were observed in neonates, although the total amount recovered from the urine was the same as that in adults. These investigators attributed their findings to slow intestinal motility and to a prolonged transit time through the gut in infants.

Secondly, Heimann [1980] studied the bioavailability of a diverse group of pharmaceuticals – sulphonamides, digoxin, β-methyldigoxin and the test substances D(+)xylose and L(+)arabinose in 580 hospitalised infants and children encompassing a wide distribution. The drugs were given as solution via a feeding tube. Despite the very different physicochemical properties of the drugs studied, a similar bioavailability pattern was observed. Although the total amount of drug absorbed varied greatly between the individuals, a finding that is also observed in adults, the *amount* absorbed was not correlated with age. In contrast, the *rate* of absorption was directly related to age, being much slower in neonates and young infants than in older infants and children. Anyhow, the results by Heimann [1980] suggest that in older infants and children orally administered drugs will be absorbed into systemic circulation at a rate and extent similar to that observed in healthy adults.

These studies point towards a general slower absorption of compounds (with different physicochemical properties and mechanisms of absorption) in neonates and young infants compared to older infants and adults. It needs to be stressed, however, that the total amount of drug absorbed might not differ between that absorbed in the immature compared to the mature organism.

2.7.3.2 Role of pancreatic and bile secretions.

In breast-fed neonates, lingual lipase and the lipase in breast milk contribute to 60-70 % of hydrolysis of ingested fat. Therefore, despite the physiological pancreatic deficiencies, the term neonate absorbs over 85 % of lipids in the maternal milk. Probably due to the absence of lipase in breast milk, the formula-fed neonate absorbs less (~70 %) of lipids in the formula [Hadorn and Munch, 1987]. In addition to inadequate lipolysis, the secretion of bile acids by the liver in neonates is deficient resulting in intraluminal concentration of bile acids so low [Boehm *et al*., 1997; Watkins, 1987] that it compromises the formation of mixed micelles. The possible consequence of deficient bile excretion is inefficient intestinal fat digestion. Indeed, long-chain fatty acids, a constituent of mixed micelles, are poorly absorbed in

neonates in contrast to medium-chain fatty acids, which are absorbed by passive diffusion in the proximal small intestine. Furthermore, the absorption of fat-soluble vitamins, vitamin D and vitamin E is reduced in neonates probably because of the inadequate bile salt pool in the ileum [Raddle, 1985]. Impaired fat digestion could be of toxicological importance when lipophilic compounds such as DDT and structurally similar compounds, and polychlorinated biphenyls are ingested. After a few months, the infant is capable of efficiently absorbing fat soluble compounds because of a co-ordinated postnatal maturation of bile salt metabolism [Boehm *et al*., 1997; Heubi *et al*., 1982].

2.7.3.3 Intestinal epithelium - mucosal barrier function

At birth, the neonate must be prepared to protect the body from orally ingested toxic xenobiotics, bacteria and other potentially harmful viruses, and antigens. Penetration of the mucosal barrier by these agents may lead to systemic toxic effects but also to inflammatory reactions.

Several studies in human neonates have shown that the developing mucosal barrier is more permeable to macromolecules [Seidman and Walker, 1987]. Essential macromolecules like epidermal growth factor and IgG from maternal breast milk are transported via specific receptor-mediated endocytosis. Non-essential and potentially antigenic large molecules appear to be taken up from the lumen in very small amounts and only via non-specific endocytotic activity. Most of the macromolecules are degraded in lysosomal compartments after uptake in the cell but a very small amount can be transported in an intact form. This is most clearly seen in preterm neonates. Here lactoferrin from the breast milk was demonstrated in intact form in the urine of the neonate [Henning *et al.*, 1994]. The ability of the neonate's gastrointestinal tract to exclude antigenic proteins increase with gestational age as well as postnatal age. The difference in β-lactoglobulin concentrations in serum between milk fed preterm and term neonates, disappeared after 10 days of life [Henning *et al.*, 1994]. In neonates, a more (paracellular) permeable intestinal epithelium, reduced endocytotic activity, a higher concentration of intact proteins in the intestinal lumen due to higher gastric pH and reduced digestive enzymes by the pancreas may all contribute to the higher absorption of macromolecules/proteins in neonates. The level of ontogenic concordance in gut maturation between humans and animals in the neonate and suckling period is not high, inasmuch as the human neonate starts life with a more mature gastrointestinal tract than the neonate rat [Henning *et al.*, 1994].

A good absorption of macronutrients (carbohydrates, fat and proteins) as well as a good absorption of ions and trace elements is essential for the growth of children. Nutrients and ions are generally absorbed by active transport processes in the intestine. Expression of these transport processes is in line with the needs of the growing child. For example, calcium and iron absorption is higher in infants and children than in adults, commensurate with the needs of the growing child. Therefore, compounds that are absorbed by transport processes that are involved in the growth of children are likely to be absorbed better in children than in adults. An example is the absorption of lead. Young children (2 months to 8 years), have been shown to absorb more ingested lead than adults, 40-50 % vs 10-15 %, respectively [Mushak, 1991]. Binding of lead to receptors in the enterocyte that serve for active transport of iron and calcium may account for active transport of lead. But similar to the passive diffusion of calcium at higher (>2 mM) intraluminal calcium concentrations, lead can be absorbed by means of passive diffusion. It has been suggested that the higher absorption of lead in children compared to adults involves also enhanced pinocytotic activity in early life [Mushak, 1991].

2.8 Conclusions

Absorption of compounds does not appear to change dramatically with age. The maturation of the gastrointestinal tract occurs within about 6 months and by late infancy, most of the processes are comparable to that of the adult. Nonetheless, the higher gastric pH, prolonged gastric emptying, irregular motility, relative smaller intestinal surface area during the early months of life may affect the absorption of compounds. Generally, changes in rate rather than in extent of absorption of compounds are found. However, the absorption of fat-soluble vitamins, and fat-soluble compounds is impaired, whereas absorption of macromolecules such as IgG from mother milk is increased during the first half year. Xenobiotics that are absorbed by transport processes that handle the absorption of nutrients/compounds essential for growth of children may be significantly better absorbed in children than in adults.

3. Distribution

The time to onset of action of xenobiotics and the intensity and duration of their effects depend not only on the rates of absorption and elimination, but also on their distribution in various tissues and body fluids [Morselli, 1989]. The rate and the amount of xenobiotic distribution depend on several factors which will be depicted below.

3.1 Factors influencing the distribution

The distribution of xenobiotics within the body is influenced most notably by the relative size of body water, fat and tissue compartments in the body and the amount and character of plasma proteins [Morselli, 1989; Milsap and Jusko, 1994].

3.1.1 Body composition

The maturational changes in the compartmentalisation and amount of body water and fat have been well characterised by Friis-Hansen [1971] and are reproduced in Figure 3.1. Total body water, expressed as a percentage of total body weight, is the resultant of the relative amounts of intracellular and extracellular water. It is as much as 85 % in preterm and 78 % in full-term neonates and it decreases from approximately 63 % in 2-year old infants to adult values of about 55 % by 12 years of age.

At birth, the fat content increases from 18 % until approximately 30 % at 12 months of age followed by a decrease in 15-year old boys to approximately 17 %. In girls, there is no sharp decrease in fat content as seen in boys at puberty. Instead, the fat content remains fairly stable at puberty and gradually increases with age. Females have approximately 1.5 times greater percentage body fat compared to boys. Adult fat content is approximately 35 % in females and 30 % in males.

Drugs are distributed between extracellular water and body fat according to their lipid:water partition coefficient. Because the relative amount of body water is higher in infants, drugs that distribute in parallel with body water content have higher volumes of distribution (V_D) values in infants than in adults. As the reverse is true for lipophilic compounds, a lipophilic drug such as diazepam would have a smaller V_D in infants. The information concerning the V_D of diazepam are somewhat contradictory as Rowland and Tozer conclude in their textbook that the V_D in infants and adults does not change (both 1.2 L/kg). Diazepam is a drug of low extraction and large V_D, and both its clearance and V_D are dependent on protein binding and Rowland and Tozer probably calculated the V_D for unbound drug. It is important to realise that the composition of adipose tissue is not constant but is subject to maturational changes also. Adipose tissue in neonates may contain as much as 57% water and 35% lipids, whereas values in the adult approach 26% and 71%, respectively [Friis-Hansen, 1971]. Some differences in distribution characteristics of several drugs are depicted in the table below.

In neonates, either premature or full term, the organs have a relative and absolute size which is different from those of older children and adults [Friis-Hansen, 1961]. For example, the liver is much larger in neonates in relation to their body weight. Neonates have a relatively underdeveloped muscular system and a high proportion of the body weight is formed by the head. In the foetus and neonate, the blood brain barrier is underdeveloped, the myelin content

of the brain is lower and the cerebral blood flow is relatively larger than in adults [Widdowson, 1981]. Therefore, higher exposure of the brain to small hydrophilic xenobiotics is expected in, especially, neonates.

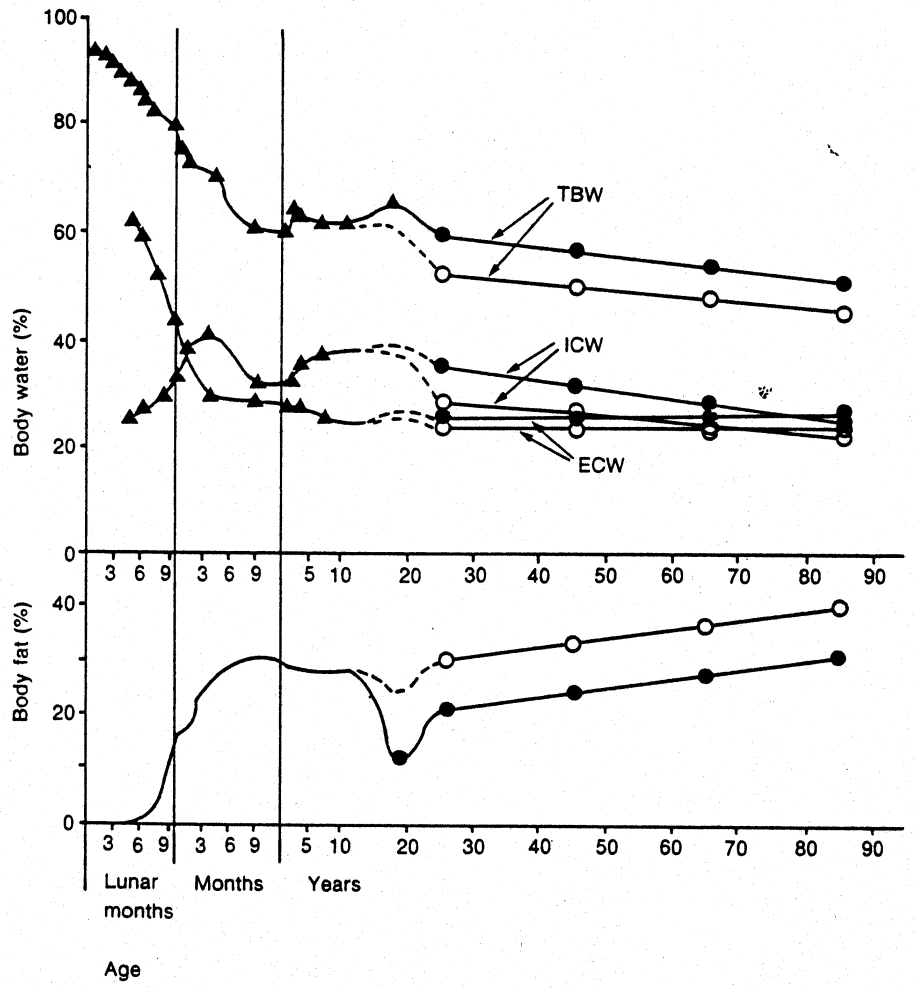

Figure 3.1. Developmental changes in total body water (TBW), intracellular water (ICW), extracellular water (ECW) and body fat content relative to age and sex (▲ = both sexes, ● = males, ○ = females) [from Friis-Hansen, 1971].

Table 3.1 Comparative distribution of drugs in neonates and adults (from Milsap et al. 1992)

	Volume of distribution (l/kg)		
Drug	**Neonates**	**Adults**	**Ratio Adult/Neonate**
Gentamicin	0.77-1.62	0.30-0.57	2.5
Theophyllin	0.20-2.80	0.44-0.50	3
Diazepam	1.40-1.82	2.20-2.60	0.7
Phenytoin	1.20-1.40	0.60-0.67	2

As a consequence of higher volumes of distribution of water-soluble drugs in infants higher doses per kilogram bodyweight must be given to infants compared to adults to achieve comparable plasma and tissue concentrations [Don Brown and Campoli-Richards, 1989].

The differences in body composition between children and adults may result in differences in distribution of compounds. The distribution of metals deviates from organic compounds (both medicinal products as well as other xenobiotics). For example, lead, cadmium and methyl-

mercury accumulate in various tissues including brain, bone, liver and kidney. With respect to the uptake of lead into human brain, there is little direct evidence. By analogy with animals, it seems likely that the concentration of lead in the brain of infants exposed to lead would rise more rapidly than the concentration in the brain of older children or adults exposed to a similar dose [http://www.inchem.org/documents /ehc/ehc/ehc59.htm]. Methylmercury is known to accumulate in brain tissues, predominantly in grey matter, but whether the brain of infants and young children accumulate more than those of adults is not known. Other tissues take up metals more readily in the early period of life, when the animal is growing rapidly, than when growth has slowed down or ceased; this particularly applies to the bones. The concentration of lead in human bones doubles between infancy and the late teen years [Barry, 1975]. The liver and kidney also accumulate metals. At birth, the concentrations of cadmium and mercury are low. Henke *et al*. (1970) determined the cadmium concentrations in the liver and kidneys of 41 children and adults, using neutron activation analysis, and found that they increased 200-fold during the first 3 years after birth. The total increase in cadmium in the liver and kidney was greater than appears from the concentration, because both organs increased considerably in weight [http://www.inchem.org/documents/ehc/ehc/ehc59.htm]

3.1.2 Protein binding

Plasma protein binding of compounds is dependent upon the amount of available binding proteins, the affinity constant of the compound for the protein(s), the number of available binding sites, and the presence of pathophysiological conditions or endogenous compounds which may alter the drug-protein binding interaction [Piafsky, 1980]. The characteristics of protein binding during different stages of childhood are depicted in the table below.

Table 3.2. Physiological variables influencing protein binding in infancy and childhood [adapted from Radde 1985] relative to adult values.

Parameter	**Patient age group**		
	Neonate	**Infant**	**Child**
Total protein	Decreased	Decreased	Equivalent
Plasma albumin	Decreased	Equivalent	Equivalent
Fetal albumin	Present	Absent	Absent
Plasma globulin	Decreased	Decreased	Equivalent
Free fatty acids	Increased	Equivalent	Equivalent
Unconjugated bilirubin	Increased	Equivalent	Equivalent
Blood pH	Low	Equivalent	Equivalent
α1-acid glycoprotein	Decreased	Data not available	Equivalent

In neonates, total plasma protein concentrations are approximately 6 g/l whereas in adults they amount 7 g/l. At first sight this difference is not large but it appears to be substantial because neonatal plasma contains foetal albumin which has lower binding capacity compared to normal plasma albumin. As acidic compounds generally bind to albumin, plasma protein binding of these compounds is reduced in neonates and infants. In addition, in these age groups, reduced levels of plasma globulin and α1-acid glycoprotein are responsible for the reduced binding of basic compounds like alprenolol [Thiessen *et al.,* 1972; Herngren *et al.,* 1983]. As plasma protein levels approach adult levels from about one year of age [Gitlin and Boesman, 1966; Herngren *et al*., 1983], the largest differences in protein binding are to be expected in neonates and infants [Windorfer *et al.,* 1974; Morselli *et al.*, 1980].

As a consequence of the reduced plasma protein binding, relatively high levels of circulating free pharmacologically active drug may be found particularly in neonates and infants [Don Brown and Campoli-Richards, 1989]. This can be illustrated by data from Wood and Wood [1981] who demonstrated a 3-fold reduction in α1-acid glycoprotein plasma levels in healthy, term neonates compared with maternal plasma. This reduction was held responsible for the larger free fractions of lignocaine and propanolol found in neonatal blood compared to maternal blood. However, the following example illustrates that a simple reduction in serum protein levels does not always entirely account for the reduced protein binding of drugs. Herngren *et al.* [1983] observed a significant correlation between albumin levels and binding of cloxacillin, an acidic drug. However, a decreased serum albumin concentration alone could not explain the reduced protein binding of cloxacillin in the neonate. The authors speculated that other factors, such as decreased albumin binding affinity, also influences the binding of cloxacillin to albumin. In addition, one needs to be aware of confounding factors like a reduced clearance which may mask the role of protein binding on free drug concentrations [Andersen *et al.*, 1997]. In general terms, one can assume that the influence of protein binding on free plasma-drug concentrations is limited to drugs which have a high degree of protein binding (>95 %).

3.1.3 Free fatty acids and unconjugated bilirubin

Concentrations of free fatty acids and unconjugated bilirubin are increased in the neonate and these plasma constituents have the capacity to displace acidic drugs from their binding sites. Although there is a direct relationship between the in vitro binding of compounds by protein and the concentration of free fatty acids (FFA), the clinical importance of drug displacement by FFA has often been overestimated. If the drug is distributed in the total body water space, as is the case with phenytoin, for example, then the drug binding has to be 95 % or greater before a clinically important increase in the unbound fraction occurs with an increased free fatty acid concentration [Fredholm *et al*., 1975]. Rudman *et al*. [1971] demonstrated significant reductions in albumin binding of salicylic acid, phenylbutazone, dicoumarol and phenytoin at high serum levels of free fatty acids approximating 2000 μEq/l or an FFA/albumin molar ratio of 3.5. Although these values are rarely attained, they have been observed under certain pathophysiological conditions such as Gram negative septicaemia [Gallin *et al*., 1969]. A number of drugs may compete with and displace bilirubin from binding sites on the albumin molecule, thus increasing the risk of the infant's developing kernicterus [Brodersen, 1980].

3.1.4 Blood pH

Neonates have a slightly lower pH (7.26-7.29) during the first few days of life compared to adults (7.35-7.45). Any decrease in blood pH will render weak acids more dissociated from their binding sites, and thus the ratio of unbound to bound drug changes in favour of the unbound moiety. The reverse is true with drugs that are weak bases or with an increase in pH. Thus, the frequently observed metabolic or respiratory acidosis of the neonate and, especially, of the premature infant may be associated with decreases in the binding of weak acid compounds to their plasma proteins [McLeod *et al.,* 1992; and Radde, 1985].

3.2 Conclusions

From a recent publication which compares the pharmacokinetic parameters of 45 drugs between children and adults, it can be concluded that there is a tendency towards larger

volumes of distribution of these compounds in children of all age groups (from neonates up to adolescents) [Ginsberg *et al.,* 2002]. This observation may be not surprising taking into account the reduced protein binding, increased permeability of the blood-brain barrier and the greater amount of water per body weight in children. However, differences in distribution must be balanced against diminished hepatic function and renal elimination before arriving at a final dosage recommendation [Don-Brown and Campoli-Richards, 1989]. The influence of protein binding on free plasma concentrations is only noticable for compounds with more than 95 % protein binding.

4. Metabolism

4.1 Introduction

An important determinant of drug clearance is metabolism, something that is not only determined by ontogenic regulation but also by genetic processes which add to the variability of drug metabolism during different stages of childhood. Therefore, an understanding of the developmental regulation of different metabolic pathways will increase the knowledge of inter- and intraindividual variability in drug disposition during childhood. The most important organ for the elimination of a wide range of xenobiotics is the liver, but metabolism is also present in other organs, such as small intestine, kidney etc., as well.

The liver constitutes 5 % of the body weight at birth but only 2 % in the adult. At birth, hepatocytes between the portal triads and central veins in the human liver are arranged in plates at least three cells thick. By five months after birth the sheets have thinned to two cells thick. These extra cell layers limit entry and exit of substances from the sinusoids compared with the adult liver. This may have influence on compounds which clearance is diffusion limited. The typical adult pattern of predominantly one cell thickness is not established in the human until about five years of age in the child [Macsween, 1994; Gow *et al*., 2001]. During the postnatal period, there are also increases in hepatocyte size, average diameter hepatic lobules and quantity of endoplasmic reticulum [Kanamura *et al*., 1990; Macsween, 1994].

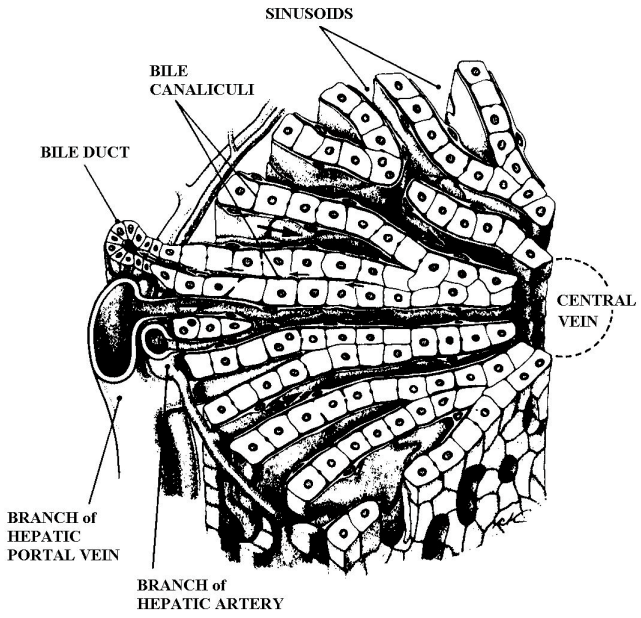

Figure 4.1 Schematic view of part of a liver lobe. Livercells are radially situated with sinosoïds in between, which transport blood in the direction of the central vein. Bile flows in the opposite direction through the bilecanaliculi [Henderson et al., 1992].

In general, the activity of drug metabolising enzymes increases from birth and approaches adults values by 1 to 3 years of age [McLeod *et al*., 1992]. Developmental differences in drug metabolism largely result from the impact of ontogeny on the activity of drug-metabolising enzymes [Kearns, 1998].

4.2 Phase 1 metabolism

4.2.1 Cytochrome P450

The cytochrome P450 system constitute the majority of the phase I enzymes and are mostly expressed in the liver but are also expressed to a lesser extent in the intestine, kidneys, and lungs. In the adult human liver, more than 15 isoforms of P450 are expressed, with a wide variability for a given isoform.

To understand the ontogeny of P450 isoforms, the liver content of individual P450 proteins and RNA and the capacities of these proteins to metabolise endobiotic and xenobiotic compounds were investigated by several investigators using post-mortem liver samples from newborns and children between the ages of 1 hour and 10 years who had died from various pathologies, such as infection, hypotrophy, malformation, respiratory distress, or sudden death.

The total P450 content was shown to remain stable from the first trimester of gestation to 1 year of age and account for about 30-50 percent of the adult level. Adult levels were 0.3 ± 0.037 nmol/mg protein whereas levels in neonates and infants ranged from 0.08 – 0.13 nmol/mg protein [Treluyer *et al.*, 1997]. The different isoforms of P450 show different patterns of development.

CYP1A

The developmental changes of CYP1A1 from the foetal stage to adulthood have not been investigated. CYP1A2 is absent in the human foetal liver, but appears gradually after birth and reaches adult levels only after several months or even later [Hakkola *et al.*, 1998]. The protein develops very late during the postnatal period: the first rise in the protein level is observed only during the 3 months after birth, progressively increasing into adulthood. The evolution of the protein is correlated with the increase in the associated enzymatic activities, such as the dealkylation of methoxy- and ethoxyresorufin [Sonnier and Cresteil, 1998]. Caffeine and theophylline are two compounds that are widely used in infants and children. For both compounds, CYP1A2 is the primary route of metabolic clearance. For theophylline, clearance is considerably lower in neonates but increases over time. Furthermore, infants have a very limited capacity to metabolise the drug, and the majority is excreted unchanged in the urine. CYP1A2-mediated metabolism becomes quantitatively more important with increasing age, a process that appears to be completed at about 5 to 6 months of age [Kraus *et al.*, 1993]. These changes in theophylline biotransformation are accompanied by increased dosage requirements over the first year of life [Nassif *et al.*, 1981]. Elimination of caffeine is also dependent upon CYP1A2 activity, and its developmental profile is similar to that of theophylline [Aranda *et al.*, 1979]. Consistent with in vitro data, functional CYP1A2 activity is among the last of the P450 activities to be acquired by the newborn and appears to be further delayed in breast-fed infants [Leeder, 1997].

CYP1B1

CYP1B1 is expressed both in adults and during the foetal period, however, mainly extrahepatically and very little expression is found in liver tissue [Hakkola *et al.*, 1998].

CYP2A
The CYP2A subfamily consists of three isoforms, CYP2A6, CYP2A7 and CYP2A13. There is not much known about the developmental activation of CYP2A subfamily. CYP2A6 is not expressed in foetal liver. The expression of CYP2A7 in human adult liver is lower compared to that of CYP2A6 and CYP2A13 appears not to be expressed at any significant level in liver. CYP2A6 catalyses coumarin 7-hydroxylation relatively specifically [Hakkola *et al*., 1998]. It has been shown that the urinary excretion of 7-hydroxycoumarin is similar to the adult level in children of 6-13 years of age [Pelkonen *et al.*, 1997].

CYP2B
The CYP2B subfamily consists of two isoforms, CYP2B6 and CYP2B7. They are expressed at low variable levels in human adult liver and have not been detected in foetal liver [Hakkola *et al*., 1998]. In one sample from an infant liver a high level of CYP2B6 was detected compared with adult livers [Shimada *et al*., 1994].

CYP2C
The existence of four functional forms of CYP2C has been demonstrated, i.e. CYP2C8, CYP2C9, CYP2C18 and CYP2C19 [Goldstein and de Morais, 1994]. The CYP2C subfamily as a whole is abundantly expressed in human adult liver and constitutes approximately 20 % of the total liver P450 [Shimada *et al*., 1994]. CYP2C proteins were confirmed to be absent from the foetal liver but to rise during the first week after birth, regardless of gestational age at birth. The level of CYP2C rises in the first week to 30 % of the adult level and than the level remains fairly stable up to the age of 1 year. It is likely that the activation of CYP2C genes occurs during the first week after birth, promoting the synthesis of CYP2C proteins and related activities. The early rise in the levels of CYP proteins in the liver was also confirmed with in vivo data. Urine samples from infants given diazepam for sedative purposes were collected and analysed. The production of metabolites was very low in neonates aged 1 to 2 days, was notably higher after 1 week of age, and then remained stable up to age 5 years [Treluyer *et al*., 1997]. Phenytoin is widely used for the treatment of seizure disorders in children and adults. Biotransformation of phenytoin to (S)-5-(4-hydroxyphenyl)-5-phenylhydantoin (S-HPPH) by CYP2C9 is the principal P450-related metabolic route. Phenytoin can also be metabolised by CYP2C19 to yield R-HPPH. Under normal conditions 95% of the HPPH recovered in urine is S-form of the CYP2C9 route. Phenytoin pharmacokinetic data reported by Chiba *et al*. [1980] demonstrated an age dependency in Vmax, which declined from approximately 14.0 mg/kg/day at 6 months of age to 8 mg/kg/day at 16 years of age. These changes were not associated with age-associated differences in the urinary excretion of HPPH. This fact would then account for the higher phenytoin dosage requirements (on the basis of body weight) in children compared with those in adults [Leeder, 1997]. Furthermore, recent pharmacokinetic data for the CYP2C9 substrate ibuprofen collected from 26 patients with cystic fibrosis ranging in age from 5.5 to 29.6 years demonstrated an inverse linear correlation between age and the apparent oral clearance of the drug [Kearns *et al.,* 1999].

CYP2D6
CYP2D6 is a polymorphic enzyme which is very important in the metabolism of many drugs, although it actually represents a minor form in adult liver constituting only about 2 % of the total liver P450 [Shimada *et al*., 1994]. A clear increase in the CYP2D6 *protein expression* was found during the first postnatal week. In a group of samples involving infants and children up to 5 years of age, the level had reached about two thirds of the average adult

levels. However, a fairly high level of CYP2D6 *mRNA* was detected already at the time of birth, and during the neonatal period the level was raised up to two to three times that in adult liver. This shows that the CYP2D6 mRNA level does not correlate with protein in the neonates and infants [Treluyer *et al*., 1991]. The mechanism of the developmental regulation of CYP2D6 expression appears to be quite complex.

CYP2E1
CYP2E1 is expressed quite abundantly in human adult liver, where it constitutes approximately 7 % of the total liver P450 [Shimida *et al*., 1994]. In the neonatal period, CYP2E1 protein level, combined with chlorzoxazone hydroxylation activity, is rapidly increased during the first 24 hr after birth. In contrast, the corresponding mRNA level was not significantly elevated at this period, which could suggest that a post-transcriptional mechanism, possibly stabilisation of small amounts of existing protein, could be responsible for the observed rise in CYP2E1 protein. CYP2E1 protein, metabolic activity and mRNA level were all gradually elevated during the next few months and correlation between CYP2E1 protein and mRNA levels were found at the age of one month. CYP2E1 protein level and chlorzoxazone hydroxylation activity comparable to adults were observed at the group of children between 1 and 10 years of age [Vieira *et al*., 1996].

CYP3A
The major P450 subfamily expressed in the human liver is the CYP3A subfamily, which comprises three isoforms: CYP3A4, CYP3A5 and CYP3A7. Although highly homologous in terms of protein sequence these proteins display different substrate specificities and different patterns of expression. CYP3A4 is the major isoform expressed in adult liver [Schuetz *et al*., 1994] and intestine [Kolars *et al.,* 1994] and is known to metabolise more than 50 different drugs of diverse chemical structure. CYP3A7 is predominantly expressed in foetal liver [Komori *et al*., 1990], whereas CYP3A5 is the major isoform expressed in human kidney [Schuetz *et al*., 1992].

Foetal CYP3A content (immunoquantitated from liver microsomes) ranges from 30-100 % of the adult CYP3A content [Shimida *et al*., 1996]. The CYP3A7 is mostly expressed in the foetal liver, whereas CYP3A4 is the major P450 isoform present in the adult liver, accounting for 40 percent of the total P450 content [Cresteil *et al*., 1985]. Ratanasavanh *et al*. (1991) studied CYP3A levels in human livers from foetuses, neonates, children and adults with immunohistochemistry, immunoblotting and RNA blotting. Large intraspecies differences were found in the CYP3A expression, but the levels were similar in all age groups. In subsequent studies, CYP3A4, CYP3A5, and CYP3A7 were recognised by antiserum against CYP3A4: the amount of reacting material was similar in all samples, whatever the age of the child, indicating that the overall level of the three isoforms was nearly constant from the three months of gestation to adulthood [Lacroix *et al*., 1997]. CYP3A4 mRNA was detected in foetal liver microsomes at 10 % of adult levels, increasing immediately after birth and reaching approximately 50 % of adult levels between 6 and 12 months of age [Lacroix *et al*., 1997, Hakkola *et al*., 1998]. The levels of the other isoenzyme, CYP3A7, are higher in foetal liver and decrease immediately after birth, resulting in similar overall levels of CYP3A. Both isoenzymes, CYP3A4 and CYP3A7, have similar substrate specificities.

CYP3A5 is consistently demonstrated in embryonic liver [Schuetz *et al*., 1994]. CYP3A7 constitutes about 32 % of the total CYP content in the human foetal liver [Shimidat *et al*., 1996] and is not detected in other organs during embryogenisis [Yang *et al*., 1994]. The hydroxylation of DHEA-S (dehydroepiandrosterone 3-sulphate) is mainly catalysed by

CYP3A7 and to a much lesser extent by CYP3A4. Immediately after birth, DHEA-S hydroxylation was more than doubled, with the highest activity being reached between postnatal days 1 and 7. The activity decreased dramatically after the first week to 12 months of age [Lacroix *et al*., 1997; De Wildt *et al.*, 1999a].

Midazolam is only slightly metabolised by CYP3A7 and the reduced clearance of midazolam in the neonate may be explained by developmentally low CYP3A4 activity following birth [de Wildt *et al*., 1999a]. This appears to be true for many therapeutic drugs. Ginsberg *et al.* (2002) concluded from his database of drugs that most drugs were nog metabolized as efficiently by CYP3A7 as by CYP3A4 and required maturation of CYP3A4 function to be eliminated efficiently.

In vitro and in vivo data clearly support a marked reduction in the activity of CYP3A4/5 in neonates and infants up to 2 months of postnatal life. However, from the second month until the first 2 to 3 years of life, CYP3A4 activity appears to exceed adult values, as reflected by the clearance of midazolam, cyclosporin and tacrolimus. When these data are converted using an allometric model, the age-dependent changes in clearance persist, which is suggestive of elevated CYP3A activity in this age group (see also Chapter 6) [De Wildt *et al*., 1999a].

4.2.2 Microsomal epoxide hydrolase

Microsomal epoxide hydrolase is a critical biotransformation enzyme that catalyses the hydrolysis of a large number of epoxide intermediates, which arise frequently from the oxidation of pharmaceutical and environmental compounds by the cytochrome P450 mixed function oxygenase system. Analysis included enzymatic activity determinations, immunochemical quantitation of protein levels and RNA hybridisation assays in foetal and adult tissues. The hepatic protein levels and enzyme activity were strongly conjugated with increasing gestational age. Foetal liver activities measured after approximately 4 months of gestation approached levels of approximately one-half of those in adult livers surveyed [Omiecinski *et al*., 1994]. No information about newborn children is available.

4.2.3 Alcohol dehydrogenase

Alcohol dehydrogenase (ADH) is the rate-limiting enzyme responsible for the biotransformation of ethanol. The effect of development on the isoenzyme kinetic constants has not been characterised. However, early pharmacokinetic data for ethanol in neonates clearly support reduced clearance of ethanol and its accumulation in plasma, findings that corroborate the previously reported developmental immaturity in ADH activity [Pikkarainen and Raiha, 1967; Idanpaan-Heikkila *et al.,* 1972]. However, in a case report of acute alcohol intoxication in a 30 month-old child, the plasma clearance of ethanol was found to be more rapid than previously reported in adults and also to proceed by a first-order process [Lopez *et al.*, 1989]. These data might imply either a much lower K_m or higher V_{max} value for ADH in young children or, alternatively, suggest a quantitative importance for other enzymes (e.g. aldehyde dehydrogenase) that contribute to ethanol metabolism. These data were supported by a previous study of chloral hydrate disposition in paediatric patients that reported an elimination half-life for trichloroethanol in children between 1 and 13 years of age that was significantly lower than that for preterm and term neonates [Mayers *et al*., 1991]. In view of the aforementioned data, it would appear that by 12 to 30 months of postnatal age, ADH activity equal to or greater than that observed in adults is reached [Kearns, 1995].

4.2.4 Other phase 1 enzymes

Other enzymes that are considered to be phase 1 enzymes are carboxyester hydrolases, amidases, cholinesterases, arylesterases, azoreductases, nitroreductases, N-oxide reductases, monoamine oxidases, aldehyde oxidase, aldehyde dehydrogenase, S-oxidase and flavoprotein monooxygenases. At the moment no information concerning the ontogeny and further development of these enzymes in children was available.

4.3 Phase 2 metabolism

The phase 2 enzymes include the glucuronosyl transferases, sulfotransferases, arylamine N-acetyl transferases (NAT1 and NAT2), glutathione S-transferases, and methyl transferases. As a group, the ontogeny of phase 2 enzymes has not been well studied; however, the limited data available indicate that, as for the cytochromes P450, important differences exist between children and adults and that the phase 2 enzymes do not all follow the same developmental patterns. For example, 'gray baby' syndrome in neonates receiving chloramphenicol has been associated with immaturity of chloramphenicol glucuronosyl transferase activity [Weiss *et al*., 1960]. In addition, glucuronidation of acetaminophen is impaired in neonates and infants relative to adolescents and adults, but is compensated for, to some extent, by increased sulfation [Miller *et al.*, 1976].

4.3.1 Uridine 5'-diphosphate glucuronosyltransferases (UGTs)

Mammalian UGTs are part of a gene superfamily consisting of enzymes that catalyse the addition of the glycosyl group from a nucleotide sugar to a small hydrophobic molecule (aglycone) [Mackenzie *et al.,* 1997]. The mammalian UGTs are responsible for the glucuronidation of hundreds of hydrophobic endogenous and xenobiotic compounds. Endogenous substrates are bilirubin, bile acids, thyroxine and steroids [Burchell *et al*., 1995]. Although UGTs do enable drug detoxification by enhancing renal excretion of hydrophilic intermediates, in some instances the glucuronide metabolites may be pharmacologically active or toxicologically reactive. For example, morphine-6-glucuronide is approximately 100 times more potent as an analgesic than morphine. Reduced clearance of this metabolite may therefore lead to a prolonged analgesic effect, with an increased risk of adverse effects [Paul *et al*., 1989].

At least 18 different human UGT isoforms have been identified by gene sequencing and cDNA cloning. The determination of substrate specificity for the different UGTs is complicated by overlapping substrate activities (i.e. one substrate is metabolised by more than one isoform) and broad substrate specificity (i.e. one isoform glucuronidates a wide range of substrate) [Mackenzie *et al.,* 1997; Burchell *et al*., 1997].

Bilirubin is a substrate for the isoform UGT1A1. Immunoreactivity and catalytic UGT activity towards bilirubin are nearly undetectable in foetal liver, and the activity increases immediately after birth, reaching adult levels around 3 to 6 months of age. In addition, enzyme activity develops in parallel with immunodetectable protein levels. This finding suggests that the decreased activity of UGT1A1 in the foetus and neonate is related to regulation at the level of transcription or translation and not to the existence of a 'foetal' or otherwise inactive UGT1A1 isoform. Finally, the increase in catalytic activity directly after birth is not dependent upon gestational age, suggesting that birth-related events play a role in the expression and activation of the UGT1A1 gene [Onishi *et al.* 1979; Burchell *et al.* 1989].

Esterone represents an endogenous substrate for UGT1A3. This hormone is glucuronidated by foetal and neonatal human liver microsomes at a level of approximately 30 % of adult activity [Burchell *et al*., 1989]. The ontogeny of UGT1A3 remains to be characterised.

Paracetamol is mainly metabolised by UGT1A6, and to a much lesser extent by UGT1A9 [Bock *et al*., 1993]. The rate of formation of paracetamol glucuronide is rather low after birth not reaching adult values before 10 years of age [Levy *et al*.; 1975, Alam *et al*., 1979]. This apparent lack of UGT activity is, however, compensated for by higher sulfotransferase activity in infants and young children [Levy *et al*., 1975]. Therefore, although the percentage of acetaminophen dose excreted unchanged in urine is similar in neonates, infants and adults, the ratio of glucuronide to sulphate metabolite increased from 0.34 in neonates to 1.8 in adults [Miller *et al.*, 1976].1-Naphtol has been proposed as a probe substrate for UGT1A6. UGT activity towards 1 naphtol increases slowly after birth, with approximately 50 % of the adult activity being reached by 6 months of age [De Wildt *et al*., 1999].

Propofol is mainly glucuronidated by UGT-1A9 [Sutherland *et al*., 1992; Le Guellec *et al*., 1995]. The clearance of this anaesthetic agent, corrected for bodyweight, is 20 to 55 % higher in children aged 1 to 11 years than in adults [Murat *et al*., 1996; Kataria *et al*., 1994]. However, other investigators failed to detect a correlation between the pharmacokinetic parameters of propofol and age [Reed *et al*., 1996]. Given that propofol is a high-extraction drug, which makes its clearance primarily dependent upon liver blood flow and to a lesser extent on enzyme activity, it may not represent a suitable in vivo probe for UGT1A9 [Murat *et al*., 1996; Gray *et al.*, 1992]. Thus, the pharmacokinetic data of propofol cannot be used to predict UGT activity [De Wildt *et al*., 1999].

UGT2B17 plays an important role in the metabolism of androgenic steroids. The catalytic activity of foetal and neonatal liver microsomes for formation of testosterone glucuronides is 3 % and 13 % of adult levels, respectively [Leakey *et al.*, 1987].

Morphine is largely metabolised by the isoform UGT2B7 [Coffman *et al*., 1997]. Plasma morphine clearance values from studies of continuous intravenous infusion increase slowly after birth, reaching adult values between 6 months and 30 months of age when using the per kg size model [Choonara *et al*., 1989]. However, when available clearance data from different age groups were corrected using the ¾ power model, the adult levels were reached at an earlier age, between 2 and 6 months (see also Chapter 6) [Anderson *et al*., 1997]. Naloxone, an opiate antagonist, is also largely metabolised by UGT2B7. The elimination half-life ($t_{1/2\beta}$) of naloxone is approximately 3 to 4 times longer in neonates than in adults [Moreland *et al*., 1980]. The benzodiazepines and NSAIDs are, at least partially, glucuronidated by UGT2B7. In children aged 2.3 to 17.8 years, lorazepam plasma clearance values normalised to bodyweight are similar to those reported for adults. However, when lorazepam clearance was normalised for body surface area, it was lower in children [Crom *et al*, 1991]. Summarising, data pertaining to the disposition of UGT2B7 substrates suggest that activity around birth is approximately 10 % of the adult activity.

The UGT isoform responsible for the glucuronidation of serotonin has not been determined to date, but the glucuronidation by foetal liver microsomes is at the same level as in the adult [Leakey *et al.*, 1987].

Other factors that should be considered before considering plasma clearance or drug:metabolite ratios as surrogate markers for UGT activity, are the overlapping isoform

specificity, the availability of alternative metabolic pathways or differences in factors, like renal function [De Wildt *et al.*, 1999].

4.3.2 N-Acetyl Transferase 2

One of the earliest-discovered and most widely recognised genetic polymorphisms is represented by NAT2 activity. Approximately 50 % of the white and black populations residing in North America are phenotypically slow metabolisers. The impact of development on NAT2 activity was examined by Pariente-Khayat *et al.* [1991] in 54 infants aged approximately 1 week to 15 months using oral caffeine administration and quantitation of the 1-methyl xanthine ratio in urine. All infants between 0 and 2 months of age were phenotypically slow acetylators in contrast with infants aged from 4-7 and 7-12 months, 50 % and 62 % of whom were characterised as fast acetylators. These data demonstrated that before 15 months of age, developmental regulation of the NAT2 gene product had profound effects on the expression of the pharmacologic phenotype. By 3 to 4 years of age, European children and white and black children in the United States have NAT2 phenotypes that correspond to the frequency distributions reported for adults of the same races. Accordingly, NAT2 activity seems to be fully expressed by 3 years of age, with possible competence (i.e. compared with adults values) reached as early as 10 to 12 months of age [Leeder and Kearns, 1997].

4.3.3 Sulfotransferases

Catalytic studies with human foetal liver cytosolic fractions have demonstrated that there is significant sulfotransferase activity toward numerous substances present from mid-gestation [Barker *et al*., 1994]. From mid-gestation the development of sulfotransferase activity toward 2-naphtol seems to be more advanced than that of UGT with the foetal to adult ratio of sulfotransferase activity far greater than the foetal to adult ratio for UGT activity [Pacifici *et al.*, 1990]. The sulphate conjugate production in vivo of salicylamide, morphine and paracetamol are reported to be similar in neonate and adult in human [Alam *et al*., 1977; Choonara *et al*., 1990]. In neonates, infants and children up to the age of 9 years, paracetamol sulphate is the major metabolite, whereas above this age paracetamol glucuronide is the major metabolite. The rate constant for paracetamol sulphate formation is the same in the neonate as in the adult, whereas the rate of constant for glucuronide formation undergoes substantial change over this period (see paragraph 4.3.1) [Gow *et al*., 2001].

4.3.4 Thiopurine *S*-methyltransferase

Thiopurine *S*-methyltransferase (TPMT) is a cytosolic enzyme that catalyses the S-methylation of aromatic and heterocyclic sulfur-containing compounds, such as mercaptopurine, azathiprine, and thioguanine. In neonates, peripheral blood TPMT activity is reported to be 50 % greater than in race-matched adults and demonstrates a distribution of activity that is consistent with the polymorphism characterised in adults. The higher TPMT activity observed in neonates may have therapeutic implications, but no data to date indicate how long this higher activity is maintained or how rapidly adult levels of activity are acquired [Leeder and Kearns, 1997].

4.3.5 Glutathione *S*-transferase

Glutathione conjugation plays an important role in the detoxification of potential alkylating agents. Glutathione S-transferases (GSTs) are a family of enzymes found at high levels in the liver and play an important role in the detoxification of electrophilic alkylating agents. The

developmental expression of the GSTA-class, M-class and P-class has been defined in human lung and kidney using radioimmunoassay, immunohistochemistry and column chromatography. Expression of GSTA-class isoenzymes increased significantly at postnatal age (between term and 110 week postnatal age) in kidney but not in lung. Expression of GSTM-class isoenzymes was continuous throughout development in both tissues. Expression of the P-class isoenzymes fell during in utero ontogeny in lung, the pattern of down-regulation being similar to that previously observed in liver. There was no change in the expression of this isoenzyme in kidney [Beckett *et al*., 1990].

4.3.6 Other phase 2 enzymes

Other enzymes that are considered to be phase 2 enzymes are O-methyl transferases and N-methyl transferases. At the moment no information concerning the ontogeny and further development in children of these enzymes was available.

4.4 Other enzymes

4.4.1 Hepatic microsomal Glucose-6-Phosphatase

Hepatic microsomal glucose-6-phosphatase plays an important role in the homeostatic regulation of blood glucose concentrations. The complete absence of the hepatic glucose-6-phosphatase enzyme is a severe metabolic disorder (Von Gierke's disease or type 1a glycogen storage disease) that usually manifests itself early in infancy. Human hepatic glucose-6-phosphatase enzyme activity develops at 11 weeks' gestation and slowly increases to ± 10 % of adult activity at term. In the first week after birth, activity rises to adult values. Increases in enzyme activity coincide with increasing concentrations of the glucose-6-phosphatase enzyme protein [Burchell *et al*., 1990].

4.5 Intestinal metabolism

4.5.1 Phase 1 metabolism

The ontogeny of hepatic CYP3A4 has been studied, but relatively little is known about the development of the enzyme in the human intestine. However, it has been found that mRNA for CYP3A7, the main foetal hepatic form of cytochrome P450 [Raucy and Carpenter, 1993]], has been detected in low abundance relative to CYP3A4 in adult human liver [Lacroix *et al*., 1997], but has not been found in adult small intestine [Kolars *et al*., 1994; Kivisto *et al*., 1996]. In a recent study duodenal biopsies and surgical sections were collected from 104 paediatric patients (age range 2 weeks to 17 years) and from 11 foetuses. An S9 fraction was prepared in each case and CYP3A4 expression was assessed by Western blotting and by immunohistochemistry; activity was measured by the rate of formation of 6β-hydroxytestosterone from testosterone. Villin expression was used as a marker of enterocyte harvest to normalise CYP3A4 expression and activity data. CYP3A4 expression in the S9 fraction, normalised to villin, increased significantly with age. The increase in CYP3A4 expression was mirrored by a corresponding change in enzyme activity [Johnson *et al*., 2001].

4.5.2 Phase 2 metabolism

Busulfan is an alkylator commonly used to prepare recipients for transplantation of hematopoietic stem cells. Busulfan AUC, not its dose, is a critical determinant of potentially fatal toxicity, engraftment, and relapse of chronic myelogenous leukemia. When given an oral dose equivalent to adults on a mg/kg or mg/m^2 basis, most but not all children < 4 years of age exhibit an AUC busulfan substantially lower than individuals > 8 years of age. Apparent oral clearance (Cl/F) in young children thus is about 50 % higher than in older individuals. The conjugation of busulfan with glutathione to form a thiophenium ion is catalyzed by GSTA1-1 and M1-1. The relative abundance of GSTA1-1 makes it the determinant of AUC. Busulfan conjugase activity in children < 4 years is greater than that of older individuals, apparently at least in part from enhanced intestinal GSTA1-1 expression in young children [Gibbs *et al*., 1999; Slattery, 2000].

4.5.3 Drug metabolism in the lumen of the gastrointestinal tract

Neonates and infants acquire their intestinal flora rapidly after birth. This delicate process of intestinal colonisation is influenced by many factors [Grönlund *et al*., 1999]. At birth, the enterobacteria and enterococci are the predominant microflora of the human gastrointestinal tract, whereas bifidobacteria are absent but appear after 7 days and become the predominant flora after 13 days. Fresh human milk promotes the growth of bifidobacteria. Hence, the intestinal microflora in breast-fed infants is composed almost exclusively of bifidobacteria with few coliforms, while at weaning there is a significant decrease in lactobacilli and an increase in putrefactive bacteria. In some instances, the lack of xenobiotic metabolising ability observed in infants is not due to absence of certain microflora but rather to immaturity of the bacterial enzyme systems in the gut lumen. For example, the extent of metabolism of several compounds such as digoxin, cholesterol, and methane increases with age and this reflects the developmental processing in bacterial enzymes such as β-glucosidase and reductases [Ilett *et al*., 1990].

Table 4.1. Ontogeny of faecal bacterial enzymes in healthy infants aged 0-6 months

	0 months (n = 29)		1-2 months (n = 27)		6 months (n = 26)	
	Mean	Range	Mean	Range	Mean	Range
Urease [a]	0.9	0-12.4	0.4	0-4.6	10.7	0-48.1
β-glucosidase [a]	1.1	0-4.1	2.1	0-9.9	7.9	0-82.8
β-glucuronidase [a]	0.7	0-5.4	0.3	0-1.5	0.9	0.04-8.0

[a] Values are given as nmoles/mg protein x min.

The appearance of bacterial enzymes in faeces after birth was slow for urease but more rapid for β-glucosidase and β-glucuronidase. At 6 months of age, nearly all infants harboured β-glucosidase and β-glucuronidase, while urease was detected in only 67 % of the infants. Urease and β-glucosidase activities increased significantly during the 6-months follow-up period, while the β-glucuronidase activity did not change during the study period.

Mode of delivery did not influence the faecal enzyme activities. In contrast, type of feeding was shown to affect the faecal enzyme activities. Infants who received formula feeds before 2 months of age were more often urease positive at 1-2 months of age than exclusively breast-fed infants. In additions, they had constantly higher median enzyme activities than breast-fed infants. Also at 6 months of age, formula-fed infants had higher urease, β-glucosidase and β-glucuronidase activity than those that were breast-fed. These results partly disagree with another study from Finland in which the faecal enzyme activities of 3 to 24 month-old infants were studied [Mykkänen *et al*., 1997]. In this study no differences were found in faecal

enzyme activities between formula-fed and breast-fed infants at 6 months of age. According to Grönlund *et al*. [1999] a possible explanation could be that the solid foods that the infants consumed at 6 months of age might have affected their results. Mykkänen *et al.* noticed that consuming large amounts of solid foods increased the faecal β-glucosidase and β-glucuronidase at 6 and 12 months of age [Mykkänen *et al*., 1997].

4.6 Conclusions

The development of P450 isoenzymes can be described with three major groups:

- A foetal group that includes CYP3A7 and CYP4A1, mostly active on endogenous molecules, but also on some exogenous chemicals
- An early neonatal group, which includes CYP2D6 and CYP2E1, that develop quickly during the hours or days after birth
- The late neonatal P450s, which develop later. CYP3A4 and CYP2C rose during the first weeks after birth and CYP1A2 being the last isoform to be expressed in the human liver.

Table 4.2 Summary of the ontogeny of Phase 1 enzymes.

Enzyme		Neonate	Infant	Child	Adult	Adult level	Effect on clearance
CYP1A1	activity	?	?	?	-	?	
	mRNA	?	?	?	+		
	protein	+	?	?	?		
CYP1A2	activity	+/-	+	+++	+++	at 5-6 months	Decreased clearance of drugs like theophylline and caffeine in the first 6 months
	mRNA	-	+	+++	+++		
	protein	-	+	+++	+++		
CYP1B1	activity	+	+	+	+	?	Nd. Expression mainly extrahepatically
	mRNA	?	?	?	?		
	protein	?	?	?	?		
CYP2A6	activity	?	+	++	++	6-13 years	unknown
	mRNA	?	+	++	++		
	protein	?	+	++	++		
CYP2A7	activity	?	?	?	?	?	?
	mRNA	?	?	?	+		
	protein	?	?	?	?		
CYP2A13	activity	?	?	?	- (liver)	?	?
	mRNA	?	?	?	- (liver)		
	protein	?	?	?	-		
CYP2B (2B6, 2B7)	activity	?	++	?	+	Higher levels found in infant than adult (n=1)	
	mRNA	?	++	?	+		
	protein	?	++	?	+		
CYP2C	mRNA	++	++	+++	+++	30 % of adult level from 1st week until 1 year	
	protein	+	++	+++	+++		
CYP2C8	activity	?	?	?	?		
CYP2C9	activity	+	+	++	++	Increasing in 1st week to 50 % and adult levels not before 1 year	
CYP2C18	activity	?	?	?	?		
CYP2C19	activity	+	+	++	++	9 months	
CYP2D6	activity	+/-	+	+	+		
	mRNA	++	+	+	+		
	protein	+/-	+	+	+		

CYP2E1	activity	+	+	++	++	Protein levels after 9 months	Chlorzoxazone hydroxylation in children (1-10 yrs) was comparable to adults
	mRNA	+/-	+	++	++		
	protein	+	+	++	++		
CYP3A4	activity	+/-	++	+++	+++	In the first year/replacing 3A7	Midozalam clearance is reduced in neonates.
	mRNA	?	++	+++	+++		
	protein	?	++	+++	+++		
CYP3A5	activity	?	?	?	?	?	?
	mRNA	+	+	++	++		
	protein	+	+	++	++		
CYP3A7	activity	++	+	-	-	Activity is high in foetus, with a peak in first week after birth and decrease in first year	
	mRNA	?	?	?	?		
	protein	?	?	?	?		
ADH	activity	+/-	+/-	+	+	Between 12-30 months	
	mRNA	?	?	?	?		
	protein	?	?	?	?		

Table 4.3 Summary of the ontogeny of Phase 2 enzymes.

Enzyme		**Neonate**	**Infant**	**Child**	**Adult**	**Adult level**	**Effect on clearance**
UGT1A1	activity	+/-	+	+	+	3-6 months	
	mRNA	?	?	?	?		
	protein	+/-	+	+	+		
UGT1A3	activity	?	?	?	-	?	
	mRNA	?	?	?	+		
	protein	+	?	?	?		
NAT2	activity	+/-	+/-	+	+	10-12 months	
	mRNA	?	?	?	?		
	protein	?	?	?	?		
Sulfotransferase	activity	+	+	+	+	from birth	
	mRNA	?	?	?	?		
	protein	?	?	?	?		
TPMT		++	?	?	+	50% higher in neonates than adults	
		?	?	?	?		
		?	?	?	?		

In general all metabolism is at adult level within 6-12 months of age. Due to a higher basal metabolic rate in children, the metabolic activity may be even higher in children than in adults. However, whether this results in more, equal or even less sensitivity to xenobiotics in the older infant and child depends on the nature of the compound. It is difficult to generalise about age-dependent deficiencies in the metabolism of xenobiotics because the various enzyme systems mature at different time points. The age at which metabolism is similar to the adult value, may be different for each compound. Knowledge of the biotransformation pathway of a compound and the ontogenic profile of metabolic enzymes involved in its biotransformation and knowledge on the potentially activating or deactivating capacity of the biotransformation pathway, may make it possible to predict the metabolism in neonates, infants and children. This will help to potentially estimate the risk of exposure in these age groups.

5. Elimination

Xenobiotics are eliminated from the body via excretion and metabolism. Elimination via metabolism has been described in chapter 4. Some drugs are excreted via the bile into the faeces. Others, particularly volatile substances are excreted in the breath. For most drugs, however, excretion occurs predominantly via the kidneys. The elimination of a drug from the body is expressed as the half-life ($t_{1/2\beta}$) of a drug. The $t_{1/2\beta}$ is defined as the time that is required to reduce the amount of drug in the body (or the plasma concentration) to one half of the original value. The $t_{1/2\beta}$ of a drug is a parameter that may be employed clinically to devise initial drug dosing guidelines. The following equation can be used to describe $t_{1/2\beta}$:

$$t_{1/2\beta} = 0.693\ V_D / CL$$

In this equation V_D denotes the body's volume of distribution (in units of volume) and CL denotes the clearance, which is the volume of drug that is eliminated from the body per time unit [Rowland and Tozer, 1995].

5.1 Renal excretion

Most drugs and/or their metabolites are excreted from the body by the kidneys. Renal excretion is dependent upon glomerular filtration, tubular reabsorption and tubular secretion. The amount of a drug that is filtered per unit time is influenced by the extent of protein binding and renal plasma flow [Whelton, 1982]. If the latter is constant, the greater the extent of protein binding, the smaller will be the fraction of circulating drug that is filtered. The developmental aspects of renal function and its influence on renal drug excretion will be discussed.

5.1.1 Renal blood flow

Renal blood flow and renal plasma flow increase with age as a result of an increase in cardiac output and a reduction in peripheral vascular resistance [Hook and Bailie, 1979]. The kidneys of a neonate only receive 5 to 6 % of total cardiac output compared with 15 to 25 % for adults [Hook and Bailie, 1979]. Renal plasma flow averages 12 ml/min (0.72 L/h) at birth and increases to 140 ml/min (8.4 L/h) by 1 year of age [West *et al*., 1948]. If renal plasma flow is corrected for body surface area, adult values are reached before 30 weeks of extrauterine life [West *et al*., 1948]. Calcagno and Rubin [1963], using clearance of para-aminohippurate to estimate renal plasma flow, demonstrated adult rates by 5 months of age.

Renal blood flow appears to increase in proportion to the development of the renal tubules [West *et al*., 1948]. In animals, this increase in renal blood flow is associated with intrarenal redistribution of blood flow, resulting in increased flow to outer cortex with increasing postnatal age [Aschinberg *et al*., 1975]. The clinical implications for these developmental changes in renal blood flow are unclear, but suggest that as tubular mass and function increases so does blood supply to the important outer cortical nephrons.

5.1.2 Glomerular filtration rate

At birth, glomerular filtration rate (GFR) is directly proportional to gestational age [Arant, 1978]. However, this linear relationship is not evident prior to 34 weeks gestation. In contrast, GFR, as measured by creatinine clearance or clearance of inulin remains relatively constant at low rates of 1 ml/min prior to 34 weeks of gestation [Arant, 1978]. The reason for this is unknown but appears to correlate with the ontogeny and functional organisation of the glomerulus. The GFR for full-term neonates at birth ranges from 2 to 4 ml/min [Arant, 1978]. In the first 2 to 3 days of postnatal life there is a marked increase in GFR in full term babies to rates between 8 and 20 ml/min. The increase in GFR after birth has been shown to be dependent on postconceptual age, and not postnatal age [Arant, 1978; Leake *et al.*, 1976]. Adult values for GFR are reached by 1.5 to 6 months of age. The postnatal increase in GFR is most likely due to the combined effects of an increase in cardiac output, a decrease in peripheral vascular resistance, an increase in mean arterial blood pressure, an increased surface area available for filtration and an increase in membrane pore size [Morselli *et al.*, 1980].

The clinical implications for the maturation of GFR become apparent when one considers drugs that are primarily eliminated by glomerular filtration. Several studies have investigated the pharmacokinetics of aminoglycosides in preterm and term infants [Arbeter *et al.*, 1983]. Szefler *et al*. (1980) demonstrated a decreasing $t_{1/2\beta}$ for gentamicin with increasing gestational age neonates less than 7 days of age (see table 5.1). Kasik *et al.* [1985] studied pre term children and found a much stronger correlation between gentamicin $t_{1/2\beta}$ and postconceptual age, compared with postnatal age. Similar results have been obtained with tobramycin [Arbeter *et al*., 1983]. Since the $t_{1/2\beta}$ for aminoglycosides is prolonged in newborns of postconceptual age less than 34 weeks, these patients should have their dosing interval lengthened or individual dose decreased, compared with full term infants.

Table 5.1: Mean gentamicin half-life (± SD) and gestational age in neonates less than 7 days of age [Modified from Szefler et al., 1980]

Gestational age (wks)	No. in study	$T_{1/2\beta}$ (h)
26-34	34	7.96 ± 2.07
35-37	22	6.68 ± 1.95
> 37	18	4.95 ± 1.5

Abbreviation: $t_{1/2\beta}$ = elimination half life

5.1.3 Development of tubular function

Proximal convoluted tubules in the normal kidney of a full term infant are small in relation to their corresponding glomeruli. This glomerulotubular imbalance in size is reflected by functional differences in the transport capacity (secretion) of the proximal tubular cells [Hook and Bailie, 1979]. Using the tubular transport maximum for para-amino hippurate (T_mPAH), a compound secreted by the proximal tubules, as an indicator of tubular function, West *et al.* [1948] found a 10-fold increase in T_mPAH in the first year of life, with adult values (corrected for body surface area) being reached by 30 weeks of life. Therefore, tubular function matures at a slower rate than glomerular function. Reasons for this reduced functional capacity include not only the small size of the tubules, but also a smaller mass of functioning tubular cells, reduced blood flow to the outer cortex, and immaturity of energy-supplying processes [Morselli *et al*., 1980].

Many drugs rely on either the organic anion or cation transport systems present in the proximal tubules for renal excretion. Penicillin is actively secreted by the para-amino

hippurate pathway [Radde, 1985]. Results of pharmacokinetic studies with ampicillin, ticarcillin, penicillin G (benzylpenicillin) and meticillin show that the $t_{1/2\beta}$ for the penicillins varies inversely with gestational and postnatal age [reviewed in Besunder *et al.*, 1988]. For meticillin, the $t_{1/2\beta}$ at birth was 4.3 hours in pre term neonates less than 33 weeks gestation, compared with 2 hours in full term neonates [Sarff *et al*., 1977]. McCracken *et al*. [1973] studied full term infants only, and their data revealed elimination half-lives of 3.2, 1.7 and 1.4 hours for postnatal ages 0-6, 7-13 and greater than 14 days, respectively. A higher pH in the stomach, resulting in enhanced absorption of basic compounds may also contribute to this effect (see paragraph 2.7.2).

In all the studies cited above, the $t_{1/2\beta}$ for penicillins was highly variable but generally decreased to 1 to 2 hours by 2 weeks postnatal age. These observations may be partially explained by the findings in animals that the capacity of the secretory pathways responsible for penicillin secretion may undergo substrate stimulation. Hook and Hewitt [1977] administered procaine benzylpenicillin for 2 days to newborn rabbits and measured the secretory capacity for several organic anions. Transport capacity for para-amino hippurate was more than doubled by the administration of benzylpenicillin, whereas there was no effect on the transport of urate and acetylsalicylate, compounds known to be secreted by a different pathway. The same authors also demonstrated that after 2 weeks of life benzylpenicillin administration had no effect on para-amino hippurate secretion, indicating that substrate stimulation occurs prior to maturation of the secretory pathways. This age-dependent substrate stimulation has also been shown in other animals [reviewed in Besunder *et al.*, 1988]. Although substrate stimulation has not been formally studied in human neonates, there is evidence that it does occur. Kaplan *et al*. [1974] showed a reduction in $t_{1/2\beta}$ for ampicillin in both pre term and term infants following multiple doses compared with a single dose. Schwartz *et al*. [1976] reported a case of a human neonate in which they were unable to maintain therapeutic serum concentrations for dicloxacillin following parental penicillin therapy. Peak serum concentrations were normal, indicating normal absorption kinetics. However, the clearance was reported to be higher than that reported in older children, suggesting enhanced tubular secretion.

Frusemide (furosemide) is another drug secreted by the para-amino hippurate pathway in the proximal tubules. In addition to being filtered, evidence for tubular secretion is inferred from adult data describing reduced rates of plasma clearance and urinary excretion following probenecid administration [Odlind and Beerman, 1980]. Aranda *et al.* [1978] found an 8-fold prolongation in $t_{1/2\beta}$ and an 8-fold reduction in the elimination rate constant for frusemide in fluid-overloaded term and preterm neonates with normal renal function, compared with adults.

The tubular reabsorption of chemicals from the lumen into the tubular cells also varies with age and depends, in part, on the pH of the urine. Weak organic acids are reabsorbed more readily by the infant, but, more important, the low capacity for biotransformation by the infant liver results in organic chemicals reaching the kidneys in their original lipophilic form, and these are not excreted, but reabsorbed into the circulation [for reviews: Braunlich, 1981].

6. Normalisation of pharmacokinetic parameters

Pharmacokinetic parameters like volume of distribution and renal clearance are generally normalised to size (body weight, body surface area) for two reasons. Firstly, in paediatric pharmacology it is important to calculate appropriate drug dosages for infants and children. Secondly, in the field of paediatric risk analysis it is important to estimate an tolerable daily intake (TDI) for xenobiotics in children.

Regarding paediatric pharmacology insight in scaling doses in order to obtain similar exposure in children is necessary. Regarding paediatric risk assessment it is important to assess if the pharmacokinetic uncertainty factor, often attributed to intraspecies variability, used for deriving an TDI is sufficient for all age-groups. Pharmacokinetic interspecies differences are currently represented in the risk assessment process for noncarcinogens as part of the default (generally 10-fold) intraspecies uncertainty factor. This 10x factor can be seen as comprising equally a half-log (3.16x) pharmacokinetic component and a similar half-log pharmacodynamic component [Renwick, 1998].

The so called 'size models' are the most common methods applied to normalise pharmacokinetic parameters to size. In these models the size parameter is based on total body weight, ideal or lean body weight or body surface area (Table 6.1).

Table 6.1. Relationship between body weight and surface area [Renwick , 1998]

Age (years)	Weight[a] (kg)	Height[a] (cm)	Surface area[b] (m^2)
0	3.4	50.4	0.207
0.25	5.7	60.0	0.293
0.5	7.4	65.8	0.350
0.75	8.9	70.6	0.398
1.0	9.9	74.7	0.434
1.5	11.3	81.4	0.489
2.0	12.4	87.1	0.534
3	14.5	96.0	0.613
4	16.5	103.3	0.682
5	19.1	110.5	0.763
6	21.5	116.8	0.835
8	26.8	129.0	0.985
10	32.3	139.5	1.129
12	39.0	150.7	1.293
Adult male	72.1	175.3	1.874
Adult female	60.3	167.6	1.681

a) Average of boys and girls (from Document Geigy) for 0-12 years (adult 25-29 years)
b) Calculated by Dubois-Dubois equation.

The simplest model is the allometric model, which is formulated as:

$$Y = a \times W^b \qquad \text{(Eq. 1)}$$

in which Y is the biological characteristic to be predicted, W is the body mass or weight and *a* and *b* are empirically derived constants. Power relations like equation 1 have been used to describe size relations in diverse fields like palaeontology, animal morphology and physiology and interspecies comparison in risk analysis [reviewed in Anderson *et al.*, 1997].

It has been calculated that for functional properties of the body such as metabolic rate, a typical value for *b* is ¾.

Table 6.2 Body surface area equations

Dubois-Dubois[a]	(Surface area [SA] = $7.184 \cdot 10^{-3}$ x height [ht] $^{0.725}$ x weight [wt] $^{0.42}$)
Gehan-George[b]	(SA = 0.02350 x ht $^{0.2246}$ x wt $^{0.51456}$)
Haycock[c]	(SA = 0.024265 x ht $^{0.3964}$ x wt $^{0.5378}$)

Where SA is in square meters, height is in centimeters and weight is in kilograms.
[a] [DuBois and Dubois, 1916]
[b] [Gehan and George, 1970]
[c] [Haycock *et al*., 1978]

TDI and dosing of drugs are often expressed as mg/kg bw (per day for TDI). Normalisation to bodyweight is the easiest way to scale between individuals and age groups as only the body weight has to be measured. Furthermore the surface area model is widely used. It requires the measure of height as well as body weight to estimate size. There are several equations available for calculation of body surface area (see Table 6.2). All of them have the same general form but use different coefficients and exponents. Although the oldest of these equations by Dubois-Dubois is widely used in paediatrics, it yields biased results, particularly in very small or young children, probably because very few young individuals were used in the study population from which this equation was derived. The Haycock and Gehan-George equation yields very similar results. However, the latter equation is recommended as it is based on direct measurements in over 400 subjects, including both children and adults [Crom, 1994]. While dosing based on surface area might often be preferred, clinical experience indicates that errors inmeasuring height or length (particularly in smaller children and infants) and caluclation errors of body surface area from weight and height are common.

TDI values are expressed as mg/kg bw. As shown in Table 6.1, the ratio of adult male vs a child at 10 years, 1 year, 0.5 years or at birth for bodyweight is 2.2; 7.3; 9.7; 21.2, respectively and for surface area is 1.7; 4.3; 5.4; 9.1, respectively. Thus in scaling down from an adult to a child, the equivalent dose for an infant or child based on surface area, will be higher than that based on body weight. For example, a 10 mg dose in an adult man would be scaled down to 1.37 mg based on body weight and to 2.32 mg based on body surface area for a 1-year old infant [Renwick, 1998].

Predicting clearance by using the per kilogram model will underestimate clearance by more than 10 % at bodyweights less than 47 kg, increasing to an error of 200 % for a newborn of 3.4 kg. Using the allometric surface area model to predict clearance will give a better prediction, but leads to an overprediction of 10 % at bodyweights below 20 kg. [Anderson *et al*., 1997]. Dosing by surface area instead of body weight is generally stated to be preferable for prescribing drugs for therapeutic use. The rationale for using surface area is that it gives a better adjustment or scaling for parameters such as rates of intermediary metabolism, calorie intake and basal metabolic rate. This may only be valid for drugs which are distributed in extracellular body water [Snodgrass, 1992], because total body water and extracellular water are better paralleled by surface area than by body weight. Surface area-based dosing may avoid underdosing of water-soluble drugs.

Anderson *et al*. discussed that both the surface area model and the kilogram models cannot be reliably used to predict dose regimens for children from schedules established for adult patients. Developmental changes are predicted by age and are only indirectly dependent of size or bodyweight. Therefore dosage regimens are dependent on pharmacokinetic

parameters like, clearance and volume of distribution, and these parameters change with age. Ginsberg *et al*. (2002) has used published literature to compare pharmacokinetic parameters between children and adults for 45 drugs. Regression results show that age has a significant impact on drug half-life and clearance with immaturity of metabolic and clearance systems evident over the first weeks to months of life for all pathways analysed. For example, the mean child/adult ratio for CYP1A2 is 4 to 9 through 2 months of age, while the caffeine half-life ratio is 13 to 17 in these age groups. Both the mean ratio and the extreme ratio are above the pharmacokinetic uncertainty factor of 3.16. Beyond 6 months of age, most pharmacokinetic functions analysed were comparable to or faster than adult function.

Most drugs used in the study by Ginsberg *et al.* (2002) were metabolised to a great extent and the plasma elimination half-lives of all drugs in the database are less than 1 day. It should be kept in mind that changes in terminal half-lives are not always equivalent to changes in clearance. This is only the case if half-life is corrected for changes in volume of distribution. Ginsberg *et al.* (2002) concluded that volume of distribution was not a major factor in creating differences in plasma elimination half-life between children and adults for the drugs they studied. However, certain classes of environmental chemicals (e.g. PCBs, dioxins) express adult half-lives that are considerably longer. In such cases, partitioning into lipid or other tissue depots may occur to a much greater extent and parameters like volume of distribution, may than play a more significant role. Because of differences in percentage of body water between child and adult, a compound will have a higher volume of distribution in child if they are highly water-soluble and a lower volume of distribution if they are highly lipid-soluble, depending on the physico-chemical properties (see also Chapter 3).

The comparisons across age suggest that the size of neonate/adult differences in pharmacokinetic function can be larger than the 3.16-fold pharmacokinetic uncertainty factor often attributed to intraspecies variability. Scaling a dosage using the size models may result in underdosing or overdosing of the child, depending on the pharmacokinetic properties of the drug used and on the age-group. Scaling a dosage of a compound that is mainly cleared through the metabolic or renal pathway based on body weight will underestimate the exposure in neonates, and this effect will be even greater if scaling based on body surface area is used. For infants and children the opposite is true for metabolic cleared compounds, scaling based on body weight will overestimate the exposure and scaling based on surface area may be better. Underestimation of the exposure is worse than overestimation, especially for xenobiotics, but also for drugs this may lead to unexpected toxicity in the child. Compounds that are renally cleared show a shorter half-life in infants from 6 months to 2 years of age and are in this age group probably beter scaled by the surface area model. In general it can be concluded that, with respect to internal exposure of a compound, dosage should be based on their pharmacokinetic properties with respect to the childs state of development.

7. Discussion and conclusions

Risk assessment for the paediatric population would ideally incorporate paediatric studies on the pharmacokinetics of a compound. These data are nor for drugs neither for other xenobiotics easy to acquire for obvious ethical reasons. In this report we have tried to gain insight into physiological factors in the liver, kidneys and gastrointestinal tract which may affect the pharmacokinetics of a compound in comparison to adults. In general it can be concluded that the effects of age on pharmacokinetics are most pronounced during the first year of life.

For intestinal absorption it can be concluded that it does not change dramatically with age. The maturation of the gastrointestinal tract occurs within about 6 months and by late infancy. Nonetheless, the higher gastric pH, prolonged gastric emptying, irregular motility and relative smaller intestinal surface area during the early months of life may affect the absorption of compounds. Generally, changes in rate rather than in extent of absorption of compounds are found. However, the absorption of fat-soluble vitamins, and fat-soluble compounds is impaired, whereas absorption of macromolecules such as IgG from mother milk is increased during the first half year. Xenobiotics that are absorbed by transport processes that handle the absorption of nutrients/compounds essential for growth of children, will be significantly better absorbed in children than in adults.

From a recent publication which compares the pharmacokinetic parameters of 45 drugs between children and adults, it can be concluded that there is a tendency towards larger volumes of distribution of these compounds in children of all age groups (from neonates up to adolescents) [Ginsberg *et al.,* 2002]. This observation may be not surprising taking into account the reduced protein binding, increased permeability of the blood-brain barrier and the greater amount of water per body weight in children. However, differences in distribution must be balanced against diminished hepatic function and renal elimination before arriving at a final dosage recommendation [Don-Brown and Campoli-Richards, 1989]. The influence of protein binding on free plasma concentrations is only noticable for compounds with more than 95 % protein binding.

The major group of enzymes responsible for metabolism of xenobiotics are the cytochrome P450 enzymes. The development of P450 isoenzymes can be described with three major groups:

- A fetal group that includes CYP3A7 and CYP4A1, mostly active on endogenous molecules, but also on some exogenous chemicals;
- An early neonatal group, which includes CYP2D6 and CYP2E1, that develop quickly during the hours or days after birth;
- The late neonatal P450s, which develop later. CYP3A4 and CYP2C rose during the first weeks after birth and CYP1A2 being the last isoform to be expressed in the human liver.

In general all metabolism is at adult level within 6-12 months of age. Due to a higher basal metabolic rate in children, the metabolic activity may be even higher in children than in adults. However, whether this results in more, equal or even less internal exposure to xenobiotics in the older infant and child depends on the nature of the compound. It is difficult to generalise about age-dependent deficiencies in the metabolism of xenobiotics because the various enzyme systems mature at different time points. The age at which metabolism is similar to the adult value, may be different for each compound. Knowledge of the

biotransformation pathway of a compound and the ontogenic profile of metabolic enzymes involved in its biotransformation, makes it possible to predict the metabolism in neonates, infants and children. This will help to estimate the risk of exposure to potentially toxic xenobiotics in these age groups.

Renal excretion also appears to be age dependent. Renal blood flow increases with age and reaches adult values at approximately 6 months of age. In addition, glomerular filtration rate is directly proportional to gestational age, which may result in larger elimination half lives for xenobiotics in pre-term neonates. Adult values for glomerular filtration rate are reached at 1.5 to 6 months of age. It has been shown that proximal convoluted tubules in the normal kidney of a full term infant are small in relation to their corresponding glomeruli, resulting in a decreased tubular transport for actively secreted compounds. Probably as a result of this, tubular function matures at a slower rate than glomerular function reaching adult values at 30 weeks of life.

Scaling a dosage using the size models may result in underdosing or overdosing of the child, depending on the pharmacokinetic properties of the drug used and on the age-group. Scaling a dosage of a compound that is mainly cleared through the metabolic or renal pathway on the basis of body weight will underestimate the exposure in neonates, and this underestimation will be even greater if scaling based on body surface area is used. For infants and children the opposite is true for metabolic cleared compounds. Scaling based on body weight will overestimate the exposure and scaling based on surface area may result in less overestimation and thus be better. Underestimation of the exposure is worse than overestimation, especially for xenobiotics, but also for drugs this may lead to unexpected toxicity in the child. Compounds that are renally cleared show a shorter half-life in infants from 6 months to 2 years of age and are in this age group probably better scaled by the surface area model. In general it can be concluded that, with respect to internal exposure of a compound, dosage should be based on their pharmacokinetic properties with respect to the childs state of development.

Recently, Ginsberg *et al*. (2002) reported on the impact of child/adult differences towards clearance via CYPs, glucuronidation, and renal elimination. They concluded that the uncertainty factor describing intraspecies variation will not always cover pharmacokinetic differences between the paediatric population and adults. The data of this report subscribe this conclusion. However, in the publication of Ginsberg *et al.* processes like intestinal absorption, presystemic metabolism and protein binding are not considered and no account is made for compounds with long half-life and low metabolism.

As stated in the introduction of this report, risk assessment for medicinal products is based on a different approach than risk assessment for other xenobiotics. However, for both types of assessment it seems to be essential that young animal models will be applied for determining NOAELs that are relevant for the paediatric population (Figure 7.1 and 7.2). Also, pharmacokinetic differences with adults need to be taken into account.

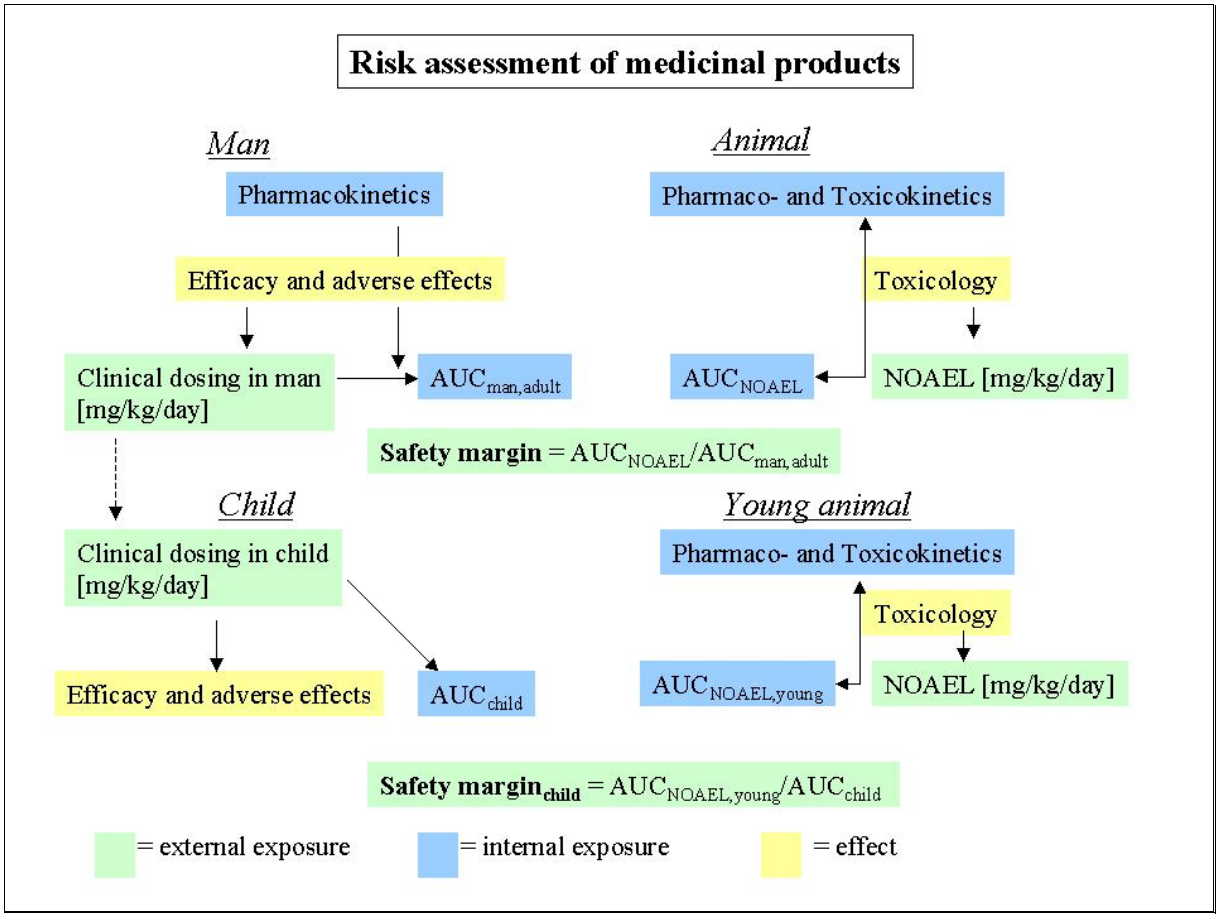

Figure 7.1. The ideal role of kinetics in risk assessment of medicinal products for children.

In development programmes for medicinal products it should be a prerequisite that a safety margin specifically for children is deduced. This implies that the dynamics of a compound are studied in chronic exposure studies in animals in which certain tissues and systems are, like in children, still under development. On the basis of these studies a NOAEL for young animals and the related exposure parameters AUC or C_{max} can be obtained. The pharmacokinetics of the suggested clinical dosing in children should subsequently be compared to the parameters at the level of the NOAEL for young animals. First then it will be possible to assess a reliable safety margin for children. The suggested clinical dosing in children will be based on physiological differences between adults and children. These physiological differences are assumed to result in pharmacokinetic differences between adults and children. To our opinion, the dosing in this stadium of the development programme will be mainly guided by acute effects in children. Following evaluation of the safety margin between young animals and children, it will be possible to calculate the maximum acceptable level of the AUC or C_{max} in children in order to deduce then a related clinical dosing in this population. This latter dosing will thus be based on adverse effects on the longer term. In this approach it is assumed that the relationship between pharmacokinetic parameters and pharmacodynamics/adverse effects is identical in all species and moreover is similar for children and adults.

In Figure 7.2 the ideal role of kinetics in the risk assessment of other xenobiotics is described. For these substances, the use of a paediatric PBPK-model possibly combined with pharmacodynamics (PBPK/PD model) may be valuable.

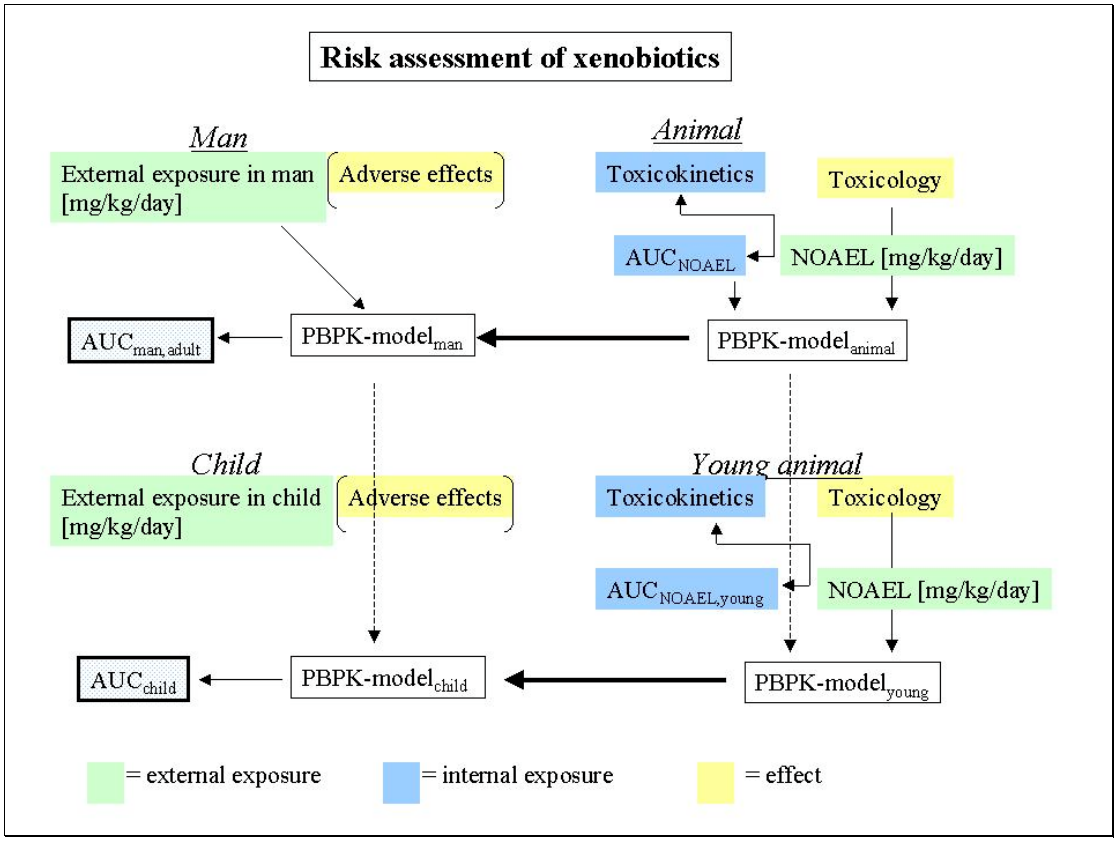

Figure 7.2. The ideal role of pharmacokinetics in risk assessment of other xenobiotics.

Normally, uncertainty factors for interspecies (factor 10) and intraspecies (factor 10) differences are taken into account in risk assessment of xenobiotics. PBPK-models give however the opportunity to scale the pharmacokinetics of a substance from an animal to man, but also from an adult to a child. These models can refine the pharmacokinetic component in this uncertainty factor, which is assumed to be 3.16 on a log scale [ICPS-document]. Parallel to this an adult animal model could thus also be scaled to a young animal model. All these options implicate that different routes will be possible to come to a PBPK-model for children. The role of PBPK-models in risk assessment can also vary. On one hand it is possible to apply the PBPK-model for calculating a safety margin, given a certain external exposure. In this case the AUC (or C_{max}) will be calculated for a fixed external exposure. This AUC (or $C_{max)}$ will be compared to the AUC_{NOAEL} (or C_{max}) which will lead to a certain safety margin. For policy makers this safety margin might be of less relevance, as it is a margin and not a standard. However, a standard can be deduced by choosing for a certain safety margin. In this case an acceptable intake can be calculated by means of a PBPK-model. PBPK-models are then used for well-founding a standard. Otherwise, it is possible to calculate, also by means of a PBPK-model, which external exposure is equivalent to a safe AUC. In that case the PBPK-model is a tool to set a standard.

To our opinion information on exposure parameters at the level of NOAEL in young animals will be essential for good risk assessment in children. It is however more difficult to decide whether it is better to scale a PBPK-model for human adults to a PBPK-model in children than scaling a PBPK-model for young animals to a PBPK-model for children. Probably experience with this way of risk assessment will gain more insight in this question. From a pharmacokinetic point of view it will be essential that exposure to active substances is representative for the paediatric situation. From a pharmacodynamic point of view it will be

essential that animals are tested during the same critical windows of sensitivity as in which children are exposed to a compound.

Based on the present knowledge we expect that it will be easier to perform interspecies extrapolations for pharmacokinetics than for pharmacodynamics, implying that the choice of a young animal model should mainly be guided by the developmental stages of target systems/organs. Due to the variety of physiologic stages that children pass through over the course of the first months and years of life, it is unlikely that a single extrapolation or PBPK-model will suffice to describe the range of children's pharmacokinetic functions. Chemical dosimetry should therefore be considered in the various developmental stages of childhood in relation to adults.

Overall, it can be concluded that developing physiological processes have significant impact on risk assessment of compounds. In the first place it is possible that in comparison to adults internal exposure in children will exceed the therapeutic window of drugs or plasma levels which will exceed NOAEL levels. Pharmacokinetic differences between children and adults will have the highest impact on the *internal* exposure in neonates. However, in neonates the *external* exposure is very limited via oral intake (breast milk, formula milk, dummy). In some cases child/adult differences towards external exposure, due to a greater inhalation rate and food ingestion rate (particularly for certain foods) per body weight, and greater contact with soil, house dust, and other media which may contain contaminants may be of more importance than differences towards pharmacokinetics. For medicinal products, where external exposure is a much better controlled process, both pharmacokinetic and pharmacodynamic differences will have greater impact on risk assessment. Secondly, the risks of high exposure levels to developing organ systems is unknown. This is especially the case where there is a critical window of sensitivity that overlaps with the period of pharmacokinetic differences from adults. The nervous system, reproductive system, skeletal system, pulmonary system and the immune system all know critical postnatal developmental periods in humans. For example glutamate receptors in the cortex reach at the age of 1-2 the highest levels and then decline to adult levels in the period of 2-16 years. Moreover, IgG levels are at adult levels in the newborn but decrease thereafter and are reach adult levels again at the age of 5 years [Hermanszenski and Webster, 1993]. From a regulatory point of view there is a liberal policy towards implementation of juvenile animal studies. As stated in the FDA Guidance document for preclinical toxicity testing: '…Juvenile animal studies should be considered on an individual basis when previous animal data and human safety data are insufficient….' To our opinion this text is not concrete enough [ICH, July 1997]. At the moment the FDA is performing behavioural studies, in partnership with the Arkansas Children's Hospital. These studies are designed to measure risk associated with certain neuroactive-drugs across species, and in a juvenile population. These studies address the following questions: 1) are animal models appropriate to predict human toxicity, and 2) what is the neurochemical risk of certain drugs in children.

From the above it can be concluded that full adjustment of dosing or ADI's for pharmacokinetic differences can be relatively easily applied, and should to our opinion be seen as a first step in considering risk for the paediatric population. Young animal models will only be relevant in case developing systems are targets for a compound or for its metabolites. Regarding studies in animals it would imply that the choice of an animal model can be substantiated better. This in turn might lead to refinement of the experiments and reduction of the number of animals required.

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Appendix 1 Mailing list

1-5 Directeur Generaal van de Volksgezondheid
6 Voorzitter Gezondheidsraad
7 dr. W.H. van Eck, wnd. directeur Voeding en Gezondheidsbescherming i.o.
8 drs. W. de Leeuw, VWS, KvW/sectie dierproeven
9 drs. I. Arendzen, VWS, VGB i.o.
10 drs. J. Dornseiffen, VWS, VGB i.o.
11 dr. ir. J. de Stoppelaar, VWS/Directie VGB i.o.
12 prof. dr. M. Balls, ECVAM
13 Directie RIVM
14 dr. D. Ruwaard, sectordirecteur sector VGZ, RIVM
15 dr. ir. D. Kromhout, sectordirecteur sector VCV, RIVM
16 drs. J. van Eijkeren, LBV-RIVM
17 dr. J. W. van der Laan, LGM-RIVM
18 dr. H.E.M.G. Haenen, LGM-RIVM
19 dr. C.F.M. Hendriksen, CDL-RIVM/Coördinatiepunt Alternatieven voor Dierproeven
20 dr. A.A.J. van Iersel, Sectorbureau 1-RIVM/Coördinatiepunt Alternatieven voor Dierproeven
21 dr. A.H. Piersma, LEO-RIVM
22 dr. ir. M.J Zeilmaker, LBV-RIVM
23 dr. ir. J.G.M. van Engelen, CRV-RIVM
24 dr. G. Wolterink, CRV-RIVM
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