Guidance for Industry
Nonclinical Safety Evaluation of Pediatric Drug Products

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U.S. Department of Health and Human Services
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I.   INTRODUCTION

This document provides guidance on the role and timing of animal studies in the nonclinical safety evaluation of therapeutics intended for the treatment of pediatric patients. The guidance discusses some conditions under which juvenile animals can be meaningful predictors of toxicity in pediatric patients and makes recommendations on nonclinical testing.

The scope of this guidance is limited to safety effects that cannot be adequately, ethically, and safely assessed in pediatric clinical trials. Serious adverse effects that are irreversible are of particular concern. The guidance also makes recommendations on the timing and utility of juvenile animal studies in relation to phases of clinical development. Sponsors are encouraged to communicate with the appropriate review division to determine whether a juvenile animal study is needed for a particular drug product and to discuss protocol designs before study initiation.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

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[1] This guidance has been prepared by the Pediatric Subcommittee to the Pharmacology and Toxicology Coordinating Committee within the Office of New Drugs (OND) in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration. It does not apply to pediatric products regulated by the Center for Biologics Evaluation and Research (CBER). For information on products regulated by CBER, contact the appropriate CBER office.

II.   BACKGROUND

Many therapeutics marketed in the United States and used in pediatric patients lack adequate information in the labeling for use in that population. A survey conducted by the American Academy of Pediatrics shows that the majority of the drugs listed in the *Physician's Desk Reference* lack information on safety and/or efficacy for pediatric use (Committee on Drugs, American Academy of Pediatrics 1995). However, recent pediatric legislation, including the Best Pharmaceuticals for Children Act (BPCA 2002) and the Pediatric Research Equity Act (PREA 2003), have provided a mechanism to obtain the needed pediatric safety and efficacy information in drug product labels.

Drug development programs have used safety data from clinical studies in adults, supported by nonclinical studies in adult animals, to support the use of a drug in pediatric patients. This assumes that pediatric patients will exhibit similar disease progression, and respond similarly to the intended therapeutic intervention. It is clear, however, that these studies may not always assess possible drug effects on developmental processes specific to pediatric age groups. Developmental processes in pediatric patients may differentially affect drug pharmacokinetics and pharmacodynamics compared to adult therapeutic use. Some adverse effects may be very difficult to detect in clinical trials or during routine postmarketing surveillance. Data obtained from clinical pediatric initiatives have identified ineffective dosing and overdosing of effective drugs as well as unnecessary exposure to ineffective therapies and identification of novel pediatric adverse events. Juvenile animal studies may assist in identifying postnatal developmental toxicities that are not adequately assessed in reproductive toxicity assessments and that may not be adequately and safely tested in pediatric clinical trials.

III.   GENERAL CONSIDERATIONS REGARDING THE NEED FOR STUDIES IN JUVENILE ANIMALS

Considerations such as postnatal development and the utility of studies conducted using juvenile animals are discussed in this section.

A.   Differences in Drug Safety Profiles Between Mature and Immature Systems

Some therapeutics have shown different safety profiles in pediatric and adult patients. Inherent differences between mature and immature systems introduce the possibility of drug toxicity, or resistance to toxicity in immature systems that are not observed in mature systems. Several factors contribute to these potential differences. Postnatal growth and development can affect drug disposition and action. Examples include developmental changes in metabolism (including the maturation rate of Phase I and II enzyme activities), body composition (i.e., water and lipid partitions), receptor expression and function, growth rate, and organ functional capacity. These developmental processes are susceptible to modification or disruption by drugs.

Although some age-dependent effects can be largely predicted by knowledge of the changes in drug metabolic pathways during development, others cannot. There are several examples of

drugs that exhibit differences in toxicity between adult and pediatric patients. These include the following:

- **Acetaminophen** — Acute acetaminophen toxicity is a classic example of how maturation can affect the toxicity profile of a drug. Young children are far less susceptible to acute acetaminophen toxicity than adults because children possess a higher rate of glutathione turnover and more active sulfation. Thus, they have a greater capacity to metabolize and detoxify an overdose of acetaminophen when compared to adults (Insel 1996).

- **Valproic acid** — In contrast to acetaminophen, young children treated with valproic acid appear disproportionately vulnerable to fatal hepatotoxicity (Dreifuss et al. 1987).

- **Chloramphenicol** — Chloramphenicol is associated with mortality in newborns because exposure is increased due to a longer half-life ($t_{1/2}$ = 26 h) compared to adults ($t_{1/2}$ = 4 h) (Kapusink-Uner et al. 1996).

- **Inhaled corticosteroids** — Inhaled corticosteroids have been found to decrease growth velocity in children, an irrelevant endpoint in adults (FDA Talk Paper, *Class Labeling for Intranasal and Orally Inhaled Corticosteroid Containing Drug Products Regarding the Potential for Growth Suppression in Children*, 1998).

- **Aspirin** — Aspirin should not be used to treat children with influenza or varicella infections because of their increased risk of developing Reye's syndrome, a complication not seen in adults (Belay et al. 1999).

- **Lamotrigine** — Children are at greater risk for developing hypersensitivity-type reactions, including Stevens-Johnson syndrome, when treated with lamotrigine (Guberman et al. 1999).

B. The Utility of Studies in Juvenile Animals

Adult clinical data can provide useful information regarding study design and dose selection for further study in children in some circumstances. Nonclinical developmental toxicity studies have traditionally focused on prenatal development, with only limited assessment of postnatal developmental effects. Animals used in multiple-dose toxicity studies are usually peripubertal. In some circumstances, data generated from these studies may provide sufficient information to support pediatric clinical trials without additional animal studies, particularly if the intended use includes adolescents but not younger children or infants. Since young animals in general exhibit developmental characteristics similar to pediatric patients, they are considered appropriate models for assessing drug effects in this population. The Agency believes that data from juvenile animal studies can contribute to the assessment of potential drug toxicity in the pediatric population, and can provide information that might not be derived from standard toxicology studies using adult animals, or safety information from adult humans.

It is thought that organ systems at highest risk for drug toxicity are those that undergo significant postnatal development. Thus, evaluation of postnatal developmental toxicity is a primary concern. The structural and functional characteristics of many organ systems differ significantly between children and adults as a result of the growth and development that takes place during postnatal maturation. Examples include the following:

- Brain, where neural development continues through adolescence (Rice and Barone 2000)
- Kidneys, where adult levels of function are first reached at approximately 1 year of age (Radde 1985)

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- Lungs, where most alveolar maturation occurs in the first 2 years of life (Burri 1997)
- Immune system, where adult levels of IgG and IgA antibody responses are not achieved until about 5 and 12 years of age, respectively (Miyawaki et al. 1981)
- Reproductive system, where maturation is not completed until adolescence (Zoetis and Walls 2003)
- Skeletal system, where maturation continues well into adulthood for 25-30 years (Zoetis and Walls 2003)
- Gastrointestinal systems, which may have direct consequences on bioavailability, clearance, and biotransformation of drugs are functionally mature by about 1 year of age (Walthall 2005).

Studies in juvenile animals may be useful in the prediction of age-related toxicity in children, as shown in the following examples:
- The effects of phenobarbital on cognitive performance in children were predicted by experimental studies examining the effects of this drug on the developing rodent nervous system (Farwell et al. 1990; Fonseca et al. 1976; Diaz et al. 1977)
- The vulnerability of human neonates to hexachlorophene neurotoxicity was modeled in developing rats and monkeys (Towfighi 1980)
- The increased susceptibility of infants to verapamil-induced cardiovascular complications would be expected based on animal studies demonstrating a greater sensitivity of the immature heart to calcium channel blockade (Skovranek et al. 1986; Boucek et al. 1984)
- An increased risk of convulsions in young children treated with theophylline was predicted by studies of the preconvulsant effects of this agent in developing rodents (Mares et al. 1994; Yokoyama et al. 1997)

Examples of drug-induced postnatal developmental toxicity demonstrated in animals include the following:
- Neurobehavioral impairment in adult rats following early postnatal exposure to methamphetamine (Vorhees et al. 1994)
- The effects of methylphenidate on growth and endocrine function in young rats (Greeley and Kizer 1980; Pizzi et al. 1987)
- Apoptotic neurodegeneration in neonatal rats treated with NMDA receptor antagonists (Ikonomidou et al. 1999)
- Decreased myelination and axonal damage induced in preweanling rats by vigabatrin (Sidhu et al. 1997)
- Long-term changes in serotonergic innervation in rats exposed to fluoxetine during early juvenile life (Wegerer et al. 1999)
- Chondrotoxicity in immature animals treated with fluoroquinolones (Stahlmann et al. 1997)

Although the significance of these findings for humans is uncertain, there is evidence that some of these effects can be relevant to growing children, notably those of methylphenidate (Mattes and Gittelman 1983; Croche et al. 1979) and fluoroquinolones (Chang et al. 1996; Le Loet et al. 1991).

IV. GENERAL CONSIDERATIONS FOR EVALUATION OF PHARMACEUTICALS IN JUVENILE ANIMALS

A. Scope of Nonclinical Safety Evaluation

The nonclinical safety evaluation of pediatric therapeutics should primarily focus on their potential effects on growth and development that have not been studied or identified in previous nonclinical and clinical studies. Juvenile animal testing may be useful in assessing potential developmental age-specific toxicities and differences in sensitivity between adult and immature animals. Although the toxicological assessment should focus primarily on the active moiety, testing the inactive ingredients in the clinical formulation can also be important, particularly when a drug's pharmacodynamics or distribution are altered by the inactive ingredients or when uncharacterized excipients are present. Additional recommendations on testing excipients can be found in the guidance for industry *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*.[2] The toxicological assessment should include local and systemic analyses of effects on postnatal growth and development in the anticipated pediatric population. The known pharmacological and toxicological properties of the drug relative to the proposed patient population should be considered. Any concerns for postnatal developmental toxicity can be addressed either in juvenile animal studies or by modified study designs (e.g., modification of segment III reproductive toxicity studies to include animals of similar developmental status as the pediatric population of concern). Juvenile animal studies are especially relevant when a known target organ toxicity occurs in adults in tissues that undergo significant postnatal development. The extent and timing of nonclinical safety studies will depend on the available safety information for a particular product. For example, the information needed to support a new pediatric indication for an approved product used in adults may be quite different from the information needed to support pediatric use of a new molecular entity because of the postnatal developmental safety concerns in the later population. These concerns will be considered for their particular clinical indications on a case-by-case basis within the drug review divisions.

B. Timing of Juvenile Animal Studies in Relation to Clinical Testing

Specific recommendations regarding the timing of nonclinical toxicology studies are available in the ICH guidance for industry *M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals* (ICH M3 safety studies guidance). The recommendations presented here for juvenile animal studies may assist in identifying postnatal developmental toxicities that are not adequately assessed in general toxicity studies with mature animals and that may not be adequately and safely tested in pediatric clinical trials.

1. Long-Term Exposure in Pediatric Subjects

Most clinical studies in pediatric subjects do not involve long-term exposure to a therapy because they are generally of short-term duration (less than 6 months). This is especially true when the trials are intended to determine pharmacokinetics rather than efficacy. As a result, long-term exposure during postnatal developmental periods is not usually addressed in pediatric clinical

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[2] We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web page at http://www.fda.gov/cder/guidance/index.htm.

trials. If the drug is indicated for chronic use then some assessment of the long-term developmental effects of the drug in animals should be made before marketing. However, in those cases when pediatric clinical studies do involve long-term exposure, we recommend conducting juvenile animal studies *before* initiation of the long-term clinical studies. When designing juvenile animal studies, the age of the pediatric population for which the drug is intended is important. Neonates, infants, and older children are at very different developmental stages, and appropriate nonclinical data should support the drug's use in the intended pediatric population.

2. Short-Term Exposure in Pediatric Subjects

Depending on the indication and use of the drug, safety concerns, and the number of subjects exposed, there may be a need for juvenile animal studies in conjunction with clinical studies even if the trials are designed for short-term exposure. Because juvenile animal studies may identify potential hazards and these hazards may have relevance to human safety, it may be more useful to complete juvenile animal studies before conducting clinical studies so that appropriate monitoring can be incorporated into the clinical trial design to limit human risk.

3. Insufficient Clinical Data to Support Initiation of Pediatric Studies

Typically, pediatric subjects are included in clinical trials after there has been considerable experience in the adult population. When there is insufficient clinical data or experience because of minimal prior adult and pediatric experience, completed juvenile animal studies are needed before initiation of pediatric clinical trials regardless of whether the clinical trials involve long-term exposures. Similarly, when there have been reports of adverse effects with off-label use in pediatric patients and there are inadequate data to evaluate the relationship between the drug and the adverse effects, completed juvenile animal studies are needed before initiation of pediatric clinical studies. The timing of juvenile animal studies relative to clinical testing of therapeutics indicated for serious or life-threatening pediatric conditions will be considered on a case-by-case basis by the review division.

C. Issues to Consider Regarding Juvenile Animal Studies

These considerations are important in determining the appropriateness and design of juvenile animal studies: (1) the intended or likely use of the drug in children; (2) the timing of dosing in relation to phases of growth and development in pediatric populations and juvenile animals; (3) the potential differences in pharmacological and toxicological profiles between mature and immature systems; and (4) any established temporal developmental differences in animals relative to pediatric populations. We also recommend that endpoints relevant to identifying target organ toxicity across species be included in the juvenile animal study design. Juveniles generally undergo more dynamic development than is seen in the relatively stable adult. Although the greatest concern is with chronic, long-term therapy, the duration of anticipated treatment of the pediatric population should be considered in relation to the duration of developmentally sensitive phases. For instance, a relatively short exposure time for neonates may cover a period of more substantial development than would a longer exposure in prepubescent children where development occurs over a much longer time frame. It is important

for juvenile animal toxicology studies to be designed efficiently, using the least number of animals to identify potential pediatric safety concerns. Whenever feasible, we recommend designing an initial study to address endpoints of concern for multiple potential pediatric populations. In all cases, studies using juvenile animals are appropriate when adequate information cannot be generated using standard nonclinical studies or from clinical trials. The following issues are specific to studies in juvenile animals for assessing toxicity.

       1.   *Developmental Stage of Intended Population*

Consideration should be given to the age of the intended population and thus the stage of postnatal development. The condition to be treated may also influence the type, extent, and timing of testing considered appropriate. Selection of appropriate endpoints in the nonclinical studies to address concerns for the specific pediatric populations is important. Recommendations regarding specific age ranges of pediatric subpopulations are discussed in the ICH guidance for industry *E11 Clinical Investigation of Medicinal Products in the Pediatric Population*.

       2.   *Evaluating Data to Determine When Juvenile Animal Studies Should Be Used*

Evaluation of the available data is important when considering the need for studies in juvenile animals. Toxicity studies in juvenile animals may be appropriate when available nonclinical or clinical data are insufficient to support reasonable safety of a therapeutic for pediatric patients. Gaps in the age ranges of rodent and nonrodent species used in standard toxicity testing are widely acknowledged. These age gaps can affect assessment of nervous system toxicity endpoints in particular because of the extended process of maturation. Standard toxicity studies with adult animals cannot assess all of the relevant endpoints, especially growth present in the immature animal. In other circumstances, however, juvenile animal studies would be neither informative nor necessary. For example, juvenile animal studies might not be necessary when: (1) data from similar therapeutics in a class have identified a particular hazard and additional data are unlikely to change this perspective; (2) there are adequate clinical data and adverse events of concern have not been observed during clinical use; (3) target organ toxicity would not be expected to differ in sensitivity between adult and pediatric patients because the target organ of toxicity is functionally mature in the intended pediatric population and younger children with the functionally immature tissue are not expected to receive the drug.

Most drugs that are intended for use in pediatric patients have established efficacy and safety profiles in adult humans. Some data may also be available from pediatric patients aged 12 years or older. For some drugs a preponderance of clinical data will be obtained from children, as in the case of inhaled corticosteroids (FDA Talk Paper, *Class Labeling for Intranasal and Orally Inhaled Corticosteroid Containing Drug Products Regarding the Potential for Growth Suppression in Children*, 1998). For approved drugs that have already undergone extensive clinical testing, substantial nonclinical pharmacology and toxicology data will have already been performed. The toxicology assessment generally includes studies of general toxicity, reproductive toxicity, genetic toxicity, carcinogenicity, and special toxicities, as well as studies in juvenile animals, if available. Target organs of toxicity of the drug both in humans and animals should have been identified in these studies. A thorough evaluation of these data should

enable scientists to: (1) judge the adequacy of the nonclinical information; (2) identify some of the potential safety concerns for the intended population; and (3) identify any gaps in the data that might be addressed by testing in juvenile animals.

>   3.   *Considering Developmental Windows When Determining Duration of Clinical Use*

Based on the observation that embryo-fetal development is especially sensitive to perturbation during organogenesis, tissues that undergo significant postnatal development in pediatric patients and juvenile animals may also have greater sensitivity to certain drug-induced toxicities than mature tissues. Organ systems identified as undergoing considerable postnatal growth and development include the nervous, reproductive, pulmonary, renal, skeletal, gastrointestinal, hepatobiliary, and immune systems. Given the variable rate of postnatal development during different periods of childhood, the definition of long-term treatment can vary by pediatric population. Intended treatment of several weeks may not be considered long term in early adolescence, but might involve considerable development for the neonate given the duration of some developmental windows.

>   4.   *Timing of Exposure*

The timing of the intended use of the drug as it relates to periods of rapid postnatal growth and development is important. If the drug is intended for use in children undergoing phases of rapid overall growth and development, it is important to evaluate an animal model undergoing a corresponding growth phase. Organ systems mature at specific times in specific species. Human-to-animal comparisons of developmental periods for the nervous, reproductive, skeletal, pulmonary, immune, renal, cardiac, and metabolic systems are presented in Section VII at the end of this guidance. These can be used as general guides to appropriate periods of treatment to assess the development of specific systems in various animal models. Immature animals have accelerated chronological development compared to humans, which can facilitate evaluation of long-term effects following acute or chronic exposure using well-defined endpoints (e.g., assessment of reproductive or nervous function).

>   5.   *Selection of Study Models*

In addition to consideration of models and endpoint assessments based on the intended pediatric human use, target organs for toxicological and pharmacological activity identified in adults need special consideration. It is important that organ systems identified as specific targets of drug toxicity in adults and that undergo significant postnatal development be studied in juvenile animals for those specific effects, even when the primary postnatal developmental period in humans does not coincide with the intended treatment phase. This is based on the observation that development is generally a continuous event. Additionally, a therapeutic target tissue may be developmentally regulated by other tissues or organ systems. In such cases, it may be advisable to examine the effects of the drug during the stages of development relevant to all of those tissues/organs in a test species.

V.   GENERAL CONSIDERATIONS IN DESIGNING TOXICITY STUDIES IN JUVENILE ANIMALS

A.   Types of Studies

Testing approaches can use generalized screening tests to provide hazard identification or can be designed to specifically address identified concerns. We recommend the selection of an appropriate, scientifically justified study design. The effects of dosing and handling on immature animals can be systematically assessed. Studies conducted in juvenile animals to support the safety of pediatric therapeutics may either be protocols designed to address a specific safety concern, or modified peri- and postnatal developmental study protocols. Dedicated juvenile animal protocols can be designed to address specific concerns based on known properties of the drug, product class, or other information. Modified repeat-dose toxicity studies can provide a more general screen for potential hazards in some instances. However, we recommend that such studies modify the animal age at study initiation, duration of treatment, and endpoints assessed to address the specific concerns. Modification of standard ICH studies designed to address developmental stages C-F[3] would include ensuring adequate exposure in juvenile animals during the postnatal period and assessment of developmental endpoints appropriate for the intended pediatric population. Assessment of developmental endpoints not usually included in standard repeat-dose toxicity studies also may be appropriate. In addition to ensuring adequate exposure to the drug, histopathologic examinations and effects on specific growth parameters and functionally immature tissues in the juvenile animal would be important. In these modified designs dosing can be initiated with animals younger than usual and extended until the developmental period for the intended pediatric population has been completed in the animal species in accordance with the age of the pediatric patients who would use the drug. Information from such studies can be compared with the findings from treated adults of the same species to evaluate whether the effects are specific to juvenile animals.

B.   Animals

1.   Species

The species of the juvenile animal tested should be appropriate for evaluating toxicity endpoints important for the intended pediatric population. Traditionally, rats and dogs have been the rodent and nonrodent species of choice. In some circumstances, however, other species may be more appropriate. For example, when drug metabolism in a particular species differs significantly from humans, an alternative species (e.g., minipigs, pigs, monkeys) may be more appropriate for testing. When determining an appropriate species, sponsors are encouraged to consider certain factors, such as the following:
* Pharmacology, pharmacokinetics, and toxicology of the therapeutic agent
* Comparative developmental status of the major organs of concern between juvenile animals and pediatric patients
* Sensitivity of the selected species to a particular toxicity

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[3] ICH guidance for industry *S5A Detection of Toxicity to Reproduction for Medicinal Products*

A study in juveniles from one animal species may be sufficient to evaluate toxicity endpoints for therapeutics that are well characterized in both adult humans and animals. It is anticipated that this evaluation often can be accomplished in the rodent using modified perinatal and postnatal developmental studies, although other approaches can be used.

> ## 2. *Age*

The age of the animals at initiation of dosing should be determined by the postnatal development parameters of interest. It is important that the stage of development in the animals being studied be comparable to that in the intended pediatric population.

> ## 3. *Sex and Sample Size*

We recommend including both male and female animals in these studies. It is important that adequate numbers of animals are used to demonstrate the presence or absence of effects of the test substance. When determining the sample size, consideration of the magnitude of the biologic effect that is of concern is also important. The particular study design used (e.g., a screening study or one designed to address an identified concern, modification of a standard design, composite or split litter design) will influence the number of animals it takes for an adequate evaluation.

> ## C. **Exposure**

> ## 1. *Route of Administration and Dosage Formulation*

When performing nonclinical studies, the intended clinical route of administration and dosage formulation[4] should be used unless an alternate route of administration and dosage formulation provides greater exposure or is less invasive with adequate exposure. Assessment of toxic effects by more than one route of administration can be appropriate if the drug is intended for clinical use by more than one route of administration. When different routes are expected to result in differences in systemic and local exposure of such magnitude that occurrence of postnatal toxicity would be expected, sponsors should consider testing by multiple routes. When the intended clinical administration is intravenous, this route should be sufficient. Since the primary purpose of these studies is to identify potential hazards, small changes in exposure/distribution by route generally would not be considered important.

Since adverse effects can sometimes be related to metabolic differences between adult and juvenile animals, toxicokinetic studies can provide useful information for assisting in study interpretation. Assessment of developmental differences in parent drug disposition and profiles of significant metabolites in juvenile animals should be made according to established guidelines (see the ICH guideline for industry *S3A Toxicokinetics: Assessment of Systemic Exposure in Toxicity Studies*).

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[4] We recommend safety evaluations of inactive ingredients be conducted to determine potential adverse effects in pediatric subjects. The type of testing is dependent on the extent to which this information is already well understood.

2.   *Frequency and Duration of Exposure*

The frequency of administration should be relevant to the intended clinical use of the drug. In some cases, however, the use of dosing frequencies similar to those anticipated for clinical administration are not feasible because of technical considerations for the animal models used. Changes in frequency can be made when variables such as metabolic and kinetic differences are considered.

The duration of treatment in animals should include at least the significant periods of relevant postnatal development for the selected species. When the aim of the study is to evaluate potential long-term effects, dosing duration should be increased relative to the intended therapeutic use. One approach to consider is establishing exposure and initial tolerability in a dose-range finding study followed by a definitive study powered to assess specific concerns. Treatment-free periods designed to assess reversibility of possible adverse effects should also be considered. Inclusion of recovery periods in studies can be valuable in distinguishing acute to intermediate pharmacodynamic effects from frank developmental toxicity, and this information could influence the evaluation of potential human risk. Depending on the concern being addressed, it may be sufficient to assess delayed toxicity through organ maturity or it may be necessary to continue until the juvenile animal reaches adulthood.

3.   *Dose Selection*

It is important to establish a clear dose-response relationship for adverse effects in juvenile animals, when possible. The high dose should produce identifiable toxicity (either developmental or general). The intermediate dose should produce some toxicity so that a dose-response relationship can be demonstrated if one exists. The low dose should produce little or no toxicity, and a NOAEL should be identified, if possible. We recommend evaluating and potentially modifying intermediate and low doses in relation to those that produce the desired pharmacodynamic effect in the test species.

D.   Toxicological Endpoints and Timing of Monitoring

The selection of toxicological endpoints to be monitored in a juvenile animal study is critical for assessing the effects of a drug on development and growth. Designing studies to determine drug effects on overall postnatal growth as well as postnatal development of specific organ systems (e.g., skeletal, renal, lung, nervous, immunologic, cardiovascular, and reproductive) is appropriate. It is important that studies include measurement of overall growth (e.g., body weight, growth velocity per unit time, tibial length), clinical observations, measurement of organ weights, gross and microscopic examinations, assessment of sexual maturation (mating, fertility), and neurobehavioral testing. More specific measurements can be reserved for case-specific evaluations based on the knowledge of the pharmacologic or toxicologic target. Clinical pathology determinations can also be useful but can be limited by the technical feasibility of obtaining adequate samples for analysis, particularly in the case of rodents. For developmental neurotoxicity assessments, well-established methods should be used to monitor key central nervous system (CNS) functions, including assessments of reflex ontogeny, sensorimotor

function, locomotor activity, reactivity, learning, and memory. Modifications of existing toxicity designs or *de novo* juvenile studies should be used depending on the concerns to be addressed.

It can be helpful to determine the relationship between toxicologic endpoints and drug exposure (e.g., predosing, immediately postdosing, time of peak plasma concentration). To differentiate long-term effects on development from acute effects, it might be appropriate to measure certain endpoints immediately before daily administration of the drug. Also, adding recovery group animals is helpful in determining whether the drug-induced effects are reversible. The more specific the concern, the more directed the study design approach can be. A more generalized screening approach may be useful if little information is available.

VI.   GENERAL CONSIDERATIONS FOR APPLICATION OF JUVENILE ANIMAL DATA IN RISK MANAGEMENT

A.   Use in Clinical Trials

It is important that nonclinical toxicology studies designed to support the safety of clinical trials in pediatric subjects identify hazards specific to the treated population. These studies can provide information useful in limiting the risk of experiencing adverse events and identify appropriate clinical monitoring. When adverse effects are observed in nonclinical toxicology studies, there are a number of possible uses of these findings. Biomarkers of adverse effects could be identified in nonclinical studies that would be useful in monitoring subjects in clinical trials. In cases where biomarkers cannot be identified or safely used in clinical studies, nonclinical pharmacokinetic data could be useful because a given adverse effect would be associated with a particular level of systemic exposure which might be extrapolated to clinical use. Blood level monitoring could then be used in clinical trials to minimize the probability of such an adverse effect occurring. If toxicities identified in juvenile animal studies are likely to occur in pediatric patients, cannot be monitored clinically, and would not be considered an acceptable potential consequence of exposure, it may not be possible to safely conduct pediatric clinical trials. Consideration of the risk-benefit analysis of a given drug therapy is important.

B.   Use in Product Approval

Nonclinical toxicology studies in juvenile animal models could demonstrate adverse effects that should be considered in seeking postmarketing commitments by the sponsor, in labeling a product for pediatric use, or in determining the approvability of a drug for pediatric use. Delayed or irreversible adverse effects might be identified in animal studies but not in clinical trials because the pediatric clinical trial might have been of insufficient duration to demonstrate the adverse effect. It is possible that biomarkers of adverse effects could be identified in nonclinical studies that were not seen in clinical trials, but might nevertheless be important to include in the product label. Depending on the nature and severity of these adverse effects and the risk-benefit relationship of the intended use, the sponsor might conduct long-term follow-up human safety studies as a postmarketing commitment. The sponsor might conduct long-term follow-up studies even following acute drug exposure if these effects were found to be delayed or irreversible. Use of the drug could be restricted to serious indications based on nonclinical findings even if the

adverse effects were not demonstrated in clinical trials. In this case, the product label would include information on the relevant adverse effects observed in nonclinical studies. Adverse effects associated with chronic drug exposure in nonclinical studies might not have been observed in clinical trials of comparable length. In such a case, the label might be written to reflect these findings. Juvenile animal studies might also be useful in identifying specific age groups in which the drug should not be used or in determining unsafe parameters of exposure. Finally, it is possible that nonclinical findings could result in a product label that specifically warns against use in pediatric patients based on a risk-benefit analysis.

VII.   HUMAN-TO-ANIMAL COMPARISONS OF DEVELOPMENTAL PERIODS

The information on comparative developmental timing, shown in Tables 1 – 8, was considered current at the time this guidance was developed. These comparisons should be considered with new information as it becomes available in deciding how best to design appropriate juvenile animal studies to address risks to the pediatric population. Neither the human nor the animal data represent a precise determination of the timelines of development due to the inherent variability and different endpoints examined. Because of the nature of science, these tables should only serve as a general starting point.

Table 1: Nervous System

Developmental Event	Postnatal Developmental Period			
	Human (Years)	Primate (Weeks)	Dog (Weeks)	Rat (Days)
Glutamate receptors[1] (Maximal binding)	1-2 Cortex Decline to adult 2-16			28 Decline to adult >28
Monoamine system[2]	2-4 Maximum receptor density			21-30 Adult levels
Ocular dominance[3]	0-3			21-35
Cerebellum persistent external germinal layer[3]	0.6-2			0-21
Rapid phase of myelination ends[4]	2			25-30
Cognitive development Delayed response learning[5]	1-2	9-36	12-16	10-35

[1] Ikonomidou et al. 1999
[2] Rice and Barone 2000
[3] Sidhu et al. 1997; Kimmel and Buelke-Sam 1994
[4] Radde 1985
[5] Wood et al. 2004

Table 2: Reproductive System

Developmental Event	Postnatal Developmental Period				
	Human (Years)	Rhesus Monkey (Years)	Dog (Days)	Mouse (Days)	Rat (Days)
Puberty[1]	11-12	2.5-3	180-240	35-45	40-60

[1] DeSesso and Harris 1995; Marty et al. 2003; Beckman and Feuston 2003; Lewis et al. 2002

Table 3: Skeletal System

Developmental Event	Postnatal Developmental Period					
Fusion of 2° Ossification Centers[1]	Human (Years)	Monkey (Years)	Dog (Years)	Rabbit (Weeks)	Rat (Weeks)	Mouse (Weeks)
Femur Distal Epiphysis	14-19	3-6	0.7-0.9	32	15-162	12-13

[1] Zoetis 2003

Table 4: Pulmonary System[1]

Developmental Event	Postnatal Developmental Period (Days)		
Alveoli Formation[2,3,4]	Human	Rat	Mouse
Onset	Prenatal	1-4	1-2
Completion	730	28	28

[1] The stages of lung development (glandular, canalicular, saccular, alveolar) at birth varies with the species. Human lungs have few alveoli and are considered in the alveolar stage at birth. Rodent lungs are less developed and considered in the saccular stage without alveoli at birth (Zoetis and Hurtt 2003).
[2] Burri 1997
[3] Merkus et al. 1996
[4] Tschanz and Burri 1997

Table 5: Immune System

Developmental Event	Postnatal Developmental Period (Days)	
	Human	Mice
B-cell Development[1]	Prenatal	Prenatal
T-cell Development[1]	Prenatal	Prenatal
NK-cell Development[1]	Prenatal	21
T-dependent Antibody response[1]	0	14 41-56 Adult level
T-independent Antibody response[1]	45-90	0 14-21 Adult level
Adult level IgG[1]	1825	42-56

[1] Holladay and Smialowicz 2000

Table 6a: Renal — Functional

Developmental Event	Postnatal Developmental Period (Days)	
	Human	Rat
Glomerulo-/Nephrogenesis[1,2]	Prenatal	8-14
Adult GFR and tubular secretion[1,2]	45-180	15-21

[1] Snodgrass 1992
[2] Travis 1991

Table 6b: Renal — Anatomical

Developmental Event	Postnatal Developmental Period (Weeks)					
	Human	Dog	Rabbit	Rat	Mouse	Pig
Completion of Nephrogenesis[1]	Prenatal Week 35	2	2-3	4-6	Prenatal	3

[1] Zoetis 2003

Table 7: Metabolism

Developmental Modulation of Phase I/II Metabolism			
	Maturation of Enzyme Activity		
Enzyme	Human (Years)	Rat (Days)	Rabbit (Days)
CYP2D6[1,2]	0-3	NA*	NA*
CYP2E1[2,3,4]	0-1	4-17 ↓ Post weaning male>female	14-35 2X adult @35
CYP1A2[1,5,6,7]	0.5 1(> adult)	7-100 Low levels	21-60
CYP2C8[1,2]	<1	NA*	NA*
CYP2C9[1,2]	<0.5 0.5 (> adult)	NA*	NA*
CYP3A4[2]	0-2	NA*	NA*
Acetylation[1,2]	1 (35% adult)	NA*	NA*
Methylation[1,2]	<1 (50% adult)	NA*	NA*
Glucuronidation[1,2]	0 (>adult) 12		NA*
Sulfation[1,2]	0	NA*	NA*

* NA = not available
[1] Kearns and Reed 1989
[2] Leeder and Kerns 1997
[3] Waxman, Morrissey, Le Balnc 1989
[4] Peng, Porter, Ding, Coon 1991
[5] Ding, Peng, Coon 1992
[6] Imaoka, Fujita, Funai 1991
[7] Pineau, Daujat, Pichard, Girard, Angevain 1991

Table 8: Cardiac[1]

Cardiac Parameter	Postnatal Developmental Period (Maturation Level Similar to Adult)		
	Human (Years)	**Dog**	**Rat (Weeks)**
Electrophysiology (ECG)	5-7 years	NA*	3-8
Cardiac Output (CO) and Hemodynamics	Birth 138 bpm; Adults 85 bpm. <2 yrs: Smaller ventricular vol., stroke index, ejection fraction vs. adult Birth BP 62/40; 2 months 85/47; 0.5-8 yrs. Diastolic 58-62	Increase in BP and decrease in HR from 1 week to 0.5 years	Early increase in HR then constant into adulthood High CO and low PVR Neonate-puberty systolic BP doubles reaches maturity by 10 weeks
Myocytes	Diploid at birth compared to 60% in adults (40% polyploidy)	NA*	Primarily diploid in infant and adult
Coronary Vasculature	Diameter of arteries doubled at 1 yr. max at 30 yrs. Capillary angiogenesis occurs postnatally and density decreases with age	Capillary angiogenesis occurs postnatally and density decreases with age	Capillary angiogenesis occurs postnatally arterial maturation by 1 month
Cardiac innervation	Neuron number increase and reach adult pattern/density in childhood	Continued development during 2-4 months	Adrenergic pattern mature by 3 weeks and nerve density mature by 5 weeks. Cholinergic matures postnatally

* NA = not available
[1] Hew and Keller 2003

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