



事務連絡
平成24年10月15日

各都道府県衛生主管部（局）
薬務主管課 御中

厚生労働省医薬食品局審査管理課

「小児用医薬品開発のための幼若動物を用いた非臨床試験ガイドライン」等の
英文版の送付について

小児用医薬品開発のための幼若動物を用いた非臨床試験ガイドライン及びその質
疑応答集（Q&A）について、添付の英文版を作成しましたので、業務の参考として
ご活用下さい。

（添付資料）

- ① Guideline on the Nonclinical Safety Study in Juvenile Animals for Pediatric Drugs
- ② Questions and Answers Regarding Guideline on the Non-clinical Safety Study in Juvenile Animals for Pediatric Drugs

Guideline on the Nonclinical Safety Study in Juvenile Animals for Pediatric Drugs

Table of Contents

1. Introduction

- Objective
- Background
- Scope of application

2. Examining the Necessity of Study

- Points to consider upon examination
- Cases when juvenile animal toxicity study is recommended

3. Planning of Study

- Purpose of study
- Consideration points at the time of planning
- Study design
- Animals (species, gender, number, and grouping)
- Administration (route, frequency, dose level, timing to start, and period)
- Examination (items, timing, and reversibility)
- Toxicokinetics

4. Timing of Study

5. Use of Study Results

6. References

1. Introduction

• Objective

The objective of this guideline is to present a recommended way of thinking in examining the need for toxicity study using juvenile animals, planning of the study when it is conducted, timing to conduct the study and the use of study results in pediatric drug development. As it is not necessarily required to adhere to the methods described hereafter, those who would plan the study should give due consideration of its scientific validity for safety evaluation.

• Background

So far, while some drugs have been used in the pediatric population, it was often the case that their efficacy and safety were extrapolated just by clinical studies in adults and nonclinical studies in mature animals. Because pharmacokinetics and sensitivity to drugs in the pediatric population are different from those of adults in some cases and organs/functions are in a state of development, it is necessary to consider the effects of the drugs specific to the pediatric population. Depending on the type and the dose of drugs, they may not have efficacy in the pediatric population, or may give serious side effects^{1), 2)}. Recognition on the importance of pediatric drug development is increased in recent years, in order to make more reliable assessments, pediatric clinical studies have been promoted³⁾. Since juvenile animal toxicity study is valuable as a test system that can investigate the toxicity level compared to that in mature animals, the presence of any toxicity specific to juvenile animals which is not normally seen in mature animals, and the effects on development of organs/functions, the study is considered to contribute to the risk minimization in the pediatric clinical use.

• Scope of application

This guideline is applied to the case of paediatric drug development which is newly advanced. ICH M3 guideline is helpful to understand the relationship of toxicity studies with juvenile animals to pediatric clinical studies⁴⁾. For biotechnology-derived pharmaceuticals, principles shown in ICH S6 guideline should be consulted and for anticancer pharmaceuticals, ICH S9 guideline should be followed^{4), 5)}.

2. Examining the Necessity of Study

• Points to consider upon examination

When examining the implementation of juvenile animal toxicity studies, things that should be considered are the results of clinical studies and post-marketing surveillance in adults, information on the usage in the pediatric population, the age of the pediatric population to which the drug is being applied, comparison of the pediatric population to which the drug is applied with juvenile animals (toxicity target, the development of organs/functions, and difference in pharmacokinetics), content of the package of existing non-clinical studies, and the data of drugs which fall into the same pharmacological category.

• Cases when juvenile animal toxicity study is recommended

A juvenile animal toxicity study should be conducted, when there is not sufficient safety information for clinical studies in pediatric populations after the careful examination of the drug safety based on the available nonclinical study results with mature animals and the clinical results including clinical studies in adults and post-marketing surveillance with the age group to which the drug is applied and the design of the pediatric study in mind.

3. Planning of Study

• Purpose of study

The purpose of juvenile animal toxicity study is to ascertain whether the toxicity level compared to that in mature animals, the presence of any toxicity specific to juvenile animals which is not normally seen in mature animals, and the effects on organs/functions development.

- Consideration points at the time of planning

In designing a juvenile animal toxicity study, an important point that should be considered is the age of the intended pediatric population (developmental stage) of the paediatric drug. Even with the identical drug, the content of the juvenile animal toxicity study that should be performed will differ according to the age to which the drug is applied. Being aware of the organs/functions in development in the target pediatric population (e.g. nervous system^{6), 7)}, reproductive system^{8), 9)}, skeletal system¹⁰⁾, respiratory system¹¹⁾, immune system¹²⁾, urinary system¹³⁾, cardiovascular system¹⁴⁾, metabolic system^{15), 16)}) and the stage of their development, administration and examination in the test animals should be performed at the corresponding timing to that in humans. As for the period of administration to the test animals, do not apply absolute number of days in clinical exposure to it, but to establish it in view of the developmental stage of organs/functions in animals which corresponds to that in humans. Since the effect of exposure on developing organs/functions may be observed even after the completion of their development, evaluation after the completion of organ development would also be useful depending on the type of toxicity. The possibility that stronger toxicity may develop in conditions where organs/functions are immature should be considered. Also, the study with juvenile animals is often accompanied by technical difficulties and the accuracy of study is strongly influenced by the laboratory technique, a plan that can be technically performed without fail should be developed.

- Study Design

Designs of juvenile animal toxicity studies are not routinely. The studies should be planned taking into account to the designs which suite their purposes or the data obtained. If data from nonclinical studies in mature animals and clinical studies in adult or the data from drugs which fall into the same pharmacological category are insufficient, the study designs using juvenile animals should be considered to detect a board range effects of the drugs. If the data obtained suggest concerns about effects on specific organs/functions, the study designs should be considered to focus on detection of effects on the organs/functions. It is principle that nonclinical safety studies using juvenile animals are conducted in accordance with GLP.

- Animals (species, gender, number, and grouping)

It is preferable that animal species and strains in which there are adequate existing nonclinical data be selected. It makes it much easier to compare toxicities between mature and immature animals if the same species and strains as those of mature animals in toxicity study are used. Normally, toxicities can be evaluated by using males and females from a single species. In general, rodent is used. In some case, it is possible to evaluate by single gender when specific organs/functions are investigated, or non-rodent would be used when evaluation in rodent is difficult.

The number of animals should be settled in consideration of the accuracy of evaluation and the 3R (reduce/refine/replace) principles. As for the method of grouping of preweaning animals, it is desirable to give consideration to prevent animals in a control group from being exposed by the test drug, and also to give care to avoid having specific genetic factors of fetal animals be unevenly distributed to a particular group.

- Administration (route, frequency, dose level, timing to start, and period)

The route and frequency of administration are determined by reference to the clinical application of the drug. It can be changed, however, based on kinetics data in mature animals with consideration to technical accuracy.

For dose level selection, the information about studies in mature animals and dose finding studies with juvenile animals is useful. It is desirable that the dosage level in the study is set so as to be able to identify toxic level and dose-response relationship. The dose where slight signs of toxicity in juvenile animals or toxicity in mature animals are observed is recommended as a high dose. Attention should be given, however, that the onset of serious toxicity, in some cases, can make it difficult to find toxicity specific to juvenile animals, degree of its toxicity, and effects on the development of organs/functions, which are original purposes of the juvenile animal study. It is also recommended to establish dose levels with a view to facilitate a comparison including exposure levels to mature animals. It is not always required to determine the non-observed-adverse-effect level (NOAEL) in the juvenile animal toxicity study, though, in the case of dose finding study where toxicity profile in juvenile animals can be predicted to differ substantially from that in mature animals, it is useful to determine the NOAEL.

Suitable timing to start administration in juvenile toxicity study should be selected individually for detection of toxicity in accordance with the age of the pediatric population to which the drug is being applied, examination range of drug effects, the developmental stage of targeted organs/functions.

The period of administration is considered to be possible up to the time of administration start in toxicity study with mature rodent animals except the concern that the drug targets specific organs/functions. It is important to closely evaluate combined results of toxicity studies in both juvenile and mature animals. In a case of the concern that the drug targets specific organs/functions, the drug is administered through the developing stage of organs/functions in the test animals which corresponds to the developing stage of organs/functions in humans by reference to the information on the result of study in mature animals as well as other drugs fall in the same pharmacological category. In monkeys, development of nerve and reproductive systems takes longer time, it is impractical to make evaluation until after the completion of organ development, an appropriate way of evaluation is necessary to be considered.

• Examination (items, timing, reversibility)

Items for examination should be selected with reference to toxicity target organs in mature animals and in view of the objective of juvenile animal study to evaluate effects on the development of organs/functions adequately. The focus should be on the organs/functions grow and develop postnatally such as nervous, reproduction, skeletal, respiratory, immune, urinary, cardiovascular and metabolic systems. The timing of examination should be established in consideration of the period of administration and the duration of organ development so as to obtain information about the effects on the development and its reversibility. In a case of detecting wide range effects of drugs, items and timing of examination in repeated-dose toxicity study and studies on prenatal and postnatal development and maternal function should be referred to. In a case of detecting effects of drugs on specific organs/functions, a wide range of examination items is not necessarily required. Together with at least performance signs and body weight, examination items specific to the detection of targeted toxicity should be selected, and reversibility should also be considered when the targeted toxicity is developed.

• Toxicokinetics

In order to confirm whether there is a difference in exposure in juvenile animals and mature animals, toxicokinetics should be performed in juvenile animal toxicity studies.

4. Timing of study

ICH M3 guideline should be consulted with regard to the timing to conduct studies¹⁷⁾. Juvenile animal toxicity study is not required for all the drugs across the board. The need to conduct studies, however, should be determined before starting paediatric clinical studies. In the case where study with juvenile animals is judged to be necessary, the assessment of a juvenile animal toxicity study should be completed before the start of paediatric clinical studies. In addition, it is desirable to be able to use the results of repeated-dose toxicity studies in mature animals at the time when the juvenile animal toxicity study is initiated. The information about the study on prenatal and postnatal development and maternal function should be referred to, if it is available. To investigate the adverse events observed in clinical studies, in some cases, it may be useful to reconsider the toxicity study with juvenile animals.

5. Use of Test Results

The nature and level of toxicity, reversibility of toxic effects, presence of biomarkers, and benefit/risk, etc. are evaluated from the results of juvenile animal toxicity study, comprehensively. Since they may affect whether to conduct pediatric clinical studies as well as their study designs, the results of juvenile animal toxicity study may play pivotal role to the subsequent development of pediatric medicinal products.

At the time of marketing authorisation application, a summary of study is submitted as part of a dossier (CTD), which may be reflected in the description of package leaflets or interview forms and also be associated with the selection of survey items in the post-marketing surveillance.

6. References

- 1) Dreifuss FE et al. 1987. Valproic Acid Hepatic Fatalities: A Retrospective Review. *Neurology* 37: 379-385.
- 2) Kapusnik-Uner JE et al. 1996. Antimicrobial Agents, Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed., Ed. JG Hardman, LE Limbird, PB Molinoff, RW Ruddon, and AG Gilman, McGraw-Hill, New York, p. 1124-1153.
- 3) December 15, 2000, PMSB/ELD Notification No.1334. "GUIDANCE ON CLINICAL INVESTIGATION OF MEDICINAL PRODUCTS IN THE PEDIATRIC POPULATION" (ICH E 11)
- 4) March 23, 2012, PFSB/ELD Notification No.0323-1."CLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS"(ICH S6(R1))
- 5) June 4, 2010, PFSB/ELD Notification No.0604-1. "GUIDELINE ON NONCLINICAL EVALUATION FOR ANTICANCER PHARMACEUTICALS"(ICH S9)
- 6) Wood SL et al. 2003. Species Comparison of Postnatal CNS Development: Functional Measures. *Birth Defects Research (Part B)* 68:391-407.
- 7) Rice D et al. 2000. Critical Periods of Vulnerability for the Developing Nervous System: Evidence from Human and Animal Models. *Environmental Health Perspectives* 108:511-533.
- 8) Marty MS et al. 2003. Development and Maturation of the Male Reproductive System. *Birth Defects Research (part B)* 68:125-136.
- 9) Beckman DA et al. 2003. Landmarks in the Development of the Female Reproductive System. *Birth Defects Research (part B)* 68:137-143.
- 10) Zoetis T et al. 2003. Species Comparison of Postnatal Bone Growth and Development. *Birth Defects Research (part B)* 68:86-110.
- 11) Zoetis T et al. 2003. Species Comparison of Lung Development. *Birth Defects Research (part B)* 68:121-124.
- 12) Holsapple MP. 2003. Species Comparison of Anatomical and Functional Immune System Development. *Birth Defects Research (part B)* 68:321-334.

- 13) Zoetis T et al. 2003. Species Comparison of Anatomical and Functional Renal Development. Birth Defects Research (part B) 68:111-120.
- 14) Hew KW et al. 2003. Postnatal Anatomical and Functional Development of the Heart: A Species Comparison. Birth Defects Research (part B) 68:309-320.
- 15) Kearns GL et al. 1989. Clinical Pharmacokinetics in Infants and Children. A Reappraisal. Clin Pharmacokinet 17:29-67.
- 16) Leeder JS et al. 1997. Pharmacogenetics in Pediatrics: Implications for Practice, New Frontiers in Pediatric Drug Therapy. Pediatric Clinics of North America 44:55-77.
- 17) February 19, 2010, PFSB/ELD Notification No.0219-4. "GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING AUTHORIZATION FOR PHARMACEUTICALS."(ICH M3(R2))

**Questions and Answers Regarding Guideline on the
Non-clinical Safety Study in Juvenile Animals for Pediatric Drugs**

Q1 Are non-clinical safety study guidelines using juvenile animals publicly available overseas?

A1 In 2006, the United States Food and Drug Administration (FDA) published a guidance, and in 2008 the European Medicines Agency (EMA now EMA) published guideline. Much useful information can be obtained from those sources.

United States Food and Drug Administration. 2006. Guidance for Industry: Nonclinical Safety Evaluation of Pediatric Drug Products.

European Medicines Agency. 2008. Guideline on the Need for Non-clinical Testing in Juvenile Animals of Pharmaceuticals for Paediatric Indications.

Q2 What kind of development is included in pediatric drug development?

A2 We assume the following options for pediatric drug development.

- Development by expanding an indication to pediatric population after approval in adults
- Simultaneous development in adults and pediatric population
- Development by expanding an indication to adults after approval in pediatric population
- Development in pediatric population only

Q3 What age ranges are included in the pediatric population?

A3 Please refer to the guidance regarding clinical studies of drug products for pediatric populations (December 15, 2000, PMSB/ELD Notification No.1334).

- Preterm newborn
- Term newborn infants (0 to 27 days)
- Infants and toddlers (28 days to 23 months)
- Pediatric population (2 to 11 years)
- Adolescents (12 to 16 or 18 years)

Q4 In what cases studies using juvenile animals are recommended?

A4 The conduct of studies using juvenile animals should be considered in the following cases.

- The drug has a novel pharmacologic mechanism (first in class)
- Drugs in the same pharmacological category have shown specific adverse effects or toxicity in pediatric population or in juvenile animals
- Clinical studies in pediatric population will start immediately after completion of the standard toxicity study package, so data from clinical studies in adults is not available (including development of pediatric-only indications)
- Postmarketing data has not yet been obtained for adults, for example, where the development is proceeding simultaneously for indications in pediatric population and adults

- Data from adults, from mature animals, and from pharmacokinetic data indicate a risk of adverse effects (toxicity) affecting a major physiological system such as the nervous system, reproductive organs, bones, lungs, immune system, kidneys, or heart. Please note that the conduction of studies in juvenile animals may not be needed in some cases, depending on the applicable age group for the pediatric indication. For example, even when the drug may affect lung development, if the age to be applied is 6 years of age and older, no studies will be recommended in juvenile animals. This is because the human lung is fully developed by 2 years of age¹⁾.
- When pharmacokinetic discrepancies suggest that exposure may be significantly increased in pediatric population or juvenile animals

1) Zoetis T et al. 2003. Species Comparison of Lung Development. Birth Defects Research (part B) 68:121-124.

Q5 What kind of study designs are applicable?

A5 The following designs provide some general examples. However, not all of these may be uniformly applicable for drugs that have different pharmacologic action or age indications. Technical feasibility should also be considered in designing the study.

Example 1: general evaluation of drug effects

- ✓ Animals: Rats, SD, male and female, 1 day old (for pediatric indications in pediatric population from 0 years of age; 3 weeks old for indications in pediatric population from 4 years of age), 15 male and 15 female animals (30 male and 30 female animals for toxicokinetic studies). Animals are considered 0 days old on the day of parturition. For studies in nursed animals before weaning, it is desirable to limit litter size to 4 males and 4 females, for a total of 8 animals in each litter, prior to the start of administration.
- ✓ Administration: From 1 day (for pediatric indications in pediatric population from 0 years of age; 3 weeks for indications in pediatric population from 4 years of age) to 6 weeks of age, administration once daily by the intended clinical route of administration
- ✓ Configuration of groups: Control, low dose, intermediate dose, high dose
- ✓ Observation: General condition: At least once daily
- ✓ Body weight: At least twice every week
- ✓ Food consumption: At least once every week (after weaning)
- ✓ Water consumption: Measured as necessary (after weaning)
- ✓ Physical development: pinna unfolding, coat growth, incisor eruption, eye opening
- ✓ Reflexes: surface righting reflex, air-righting reflex
- ✓ Sensory functions: vision, hearing
- ✓ General behavior: motor function, learning, and memory
- ✓ Sexual maturity: cleavage of the balanopreputial gland, vaginal opening
- ✓ Fertility: sperm analysis, estrous cycle
- ✓ Function tests: renal function, etc.
- ✓ Urinalysis: at least once during the administration period

- ✓ Hematology: at necropsy (in the main group on the day after completion of administration; in the satellite group after the completion of organ development)
- ✓ Necropsy: on the day after completion of administration (7 weeks of age) in 10 animals/group and at the completion of organ development (13 weeks of age) in 5 animals/group. Macroscopic observation of organs and tissues, measurement of organ weights and tibia length, histopathologic examination
- ✓ Toxicokinetics: 1, 4, and 24 hours after single-dose administration at 1 week of age, 1, 4, and 24 hours after single-dose administration at 3 weeks of age; 5 animals at each time point

Example 2: Evaluation of the effects of the drug on specific organs and functions

The study design should be optimized to detect toxicity during the development of the targeted organ or function. For example, if renal development is targeted, the study is conducted within the first 3 weeks after birth in rats²⁾. Ototoxicity of aminoglycoside antibiotics is studied in 1-week-old rats³⁾, and joint toxicity of quinolone antibiotics is studied in 3-month-old dogs⁴⁾.

- 2) Zoetis T et al. 2003. Species Comparison of Anatomical and Functional Renal Development. Birth Defects Research (part B) 68:111-120.
- 3) Lenoir M et al. 1983. Comparative ototoxicity of four aminoglycoside antibiotics during the critical period of cochlear development in the rat. A functional and structural study. Acta Otolaryngol Suppl 405:1-16.
- 4) Takayama S et al. 1995. Toxicity of quinolone antimicrobial agents. J Toxicol Environ Health 45:1-45.

Q6 How many animals are used for assessment?

A6 Ordinarily, rodent studies will be performed in 10 or more males and 10 or more females per group, and studies in non-rodents will use 3 or more animals of each sex in each group. If a group is to be monitored after the completion of organ development, enough additional animals will be needed to accurately evaluate that group. For toxicokinetics, 3 to 5 animals of each sex per group will be sufficient for each sampling point.

Q7 What are general considerations for group allocation before weaning?

A7 Group allocation method before weaning should be decided considering the possibility of contamination between animals and the litter effect. The recommended method is to allocate pups of each litter into test groups at birth, and then to raise each test group with a foster dam.

Q8 How do we select the starting age for administration in nonclinical studies based on a pediatric indication in a specific human age group?

A8 Technically feasible day (0 to 5 days after birth) in the facility should be selected as an initiation day of administration, when the effects of drug that for indication in infants are

investigated comprehensively in rats. For indications in pediatric population 4 years of age and older, since postnatal development of organs and functions is nearly complete by that age except for the reproductive organs and the skeletal system, in some cases it is permissible to start administration at weaning (3 weeks of age) in rats. For indications limited to pediatric population 12 years of age and older, safety evaluation can be possible from standard toxicity study data (repeated dose study initiated in rats at 6 to 7 weeks of age).

Q9 In what cases is it permissible to select a different administration route from the one intended for clinical use?

A9 In some cases, depending on the age in weeks of the juvenile rats, an intended clinical route of administration such as intravenous administration may be technically difficult. Until the clinical administration route becomes feasible, it is permissible to use a substitute administration route (such as subcutaneous administration), as determined through reference to pharmacokinetics data, etc.

Q10 Is it possible to evaluate the nervous system and reproductive system in monkeys?

A10 For studies in cynomolgus monkeys, it is impractical to postpone tests until the animal reaches maturity (3 to 4 years of age). It can also be difficult to evaluate fertility by mating 1 male and 1 female. For example, methods for the evaluation of the nervous system and reproductive system at a relatively young age (6 months after birth) include tests of learning and memory (the monkey moved a food reward through a maze to the retrieval point)⁵⁾ and evaluation of histological changes in the ovaries and testes from the administration of HMG and HCG are under development.

5) Tsuchida J et al. 2003. New Type of Puzzle-Task Finger Maze Learning in *Macaca fascicularis*. *Int J Primato* 24:261-70.

Q11 What are general considerations for toxicokinetic analysis in juvenile animals?

A11 If pharmacokinetic profiling data is available for mature animals, it may be sufficient to monitor the exposure levels for juvenile animals. To facilitate the comparison between juvenile and mature rats, the sampling points in juvenile animals should be selected with reference to those in mature animals. In some cases, if the values of 24 hours after the administration of a single dose are available, no data is required from multiple-dose administration. Conversely, pharmacokinetics may change considerably during the maturation of the juvenile animal (transporter expression, etc.), so that useful data can be obtained from toxicokinetic assessment not only on the first and last days of administration, but also over the period of development into later age. In rat studies, animals in satellite groups are ordinarily used for this purpose. In unweaned rats, such as neonates, since only a small amount of blood can be collected, the pooling of blood samples from several animals is acceptable if needed. In some cases, the drug concentration within a target organ may also provide useful information.