S11 Nonclinical Safety Testing in Support of Development of Paediatric Medicines

Core Guideline

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INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

NONCLINICAL SAFETY TESTING IN SUPPORT OF DEVELOPMENT OF PAEDIATRIC MEDICINES

S11

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.

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ICH Consensus Guideline

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LIST OF ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, and Excretion
CNS	Central Nervous System
CT	Computed tomography
DRF	Dose Range-Finding
ePPND	Enhanced Pre- and Postnatal Development
FIH	First in Human
FOB	Functional Observational Battery
GI	Gastrointestinal
ICH	International Council on Harmonisation
JAS	Juvenile Animal Study
NHP	Non-Human Primate
NOAEL	No-Observed Adverse Effect Level
PND	Postnatal Day
PPND	Pre- and Postnatal Development
PK	Pharmacokinetics
PD	Pharmacodynamics
TDAR	T-Cell-Dependent Antibody Response
TK	Toxicokinetic
WoE	Weight of Evidence

1. INTRODUCTION

1.1 Objectives of the Guideline

The purpose of this document is to recommend international standards for, and promote harmonisation of, the nonclinical safety studies recommended to support the development of paediatric medicines. Harmonisation of the guidance for nonclinical safety studies will define the current recommendations and reduce the likelihood that substantial differences will exist among regions. It should facilitate the timely conduct of paediatric clinical trials and reduce the use of animals in accordance with the 3Rs (replace/reduce/refine) principles.

1.2 Background

Several regional guidelines have previously been issued by various regulatory agencies and were not in complete agreement on the need for, timing of, and design of juvenile animal studies (JAS).

There are ICH guidelines that refer to the need for and/or timing or study design of JAS (e.g., ICH E11, M3, S5, S6, and S9); the current guideline is intended to complement the existing ICH guidelines. This guideline reflects current thinking based on collations of examples by regulatory agencies, by industry surveys, and literature.

1.3 Scope

This guideline recommends an approach for the nonclinical safety evaluation of medicines intended for development in paediatric populations. This can include products with prior adult use, as well as products being considered for initial human use in paediatrics (see Section 4).

The ICH S9 guideline should be consulted for recommendations on whether to conduct JAS for those pharmaceuticals included in the scope of the ICH S9 guideline, i.e., anticancer pharmaceuticals. The ICH S11 guideline should be consulted for study design in all cases where a study is considered to be warranted.

Tissue engineered products, gene and cellular therapies, and vaccines are excluded from the scope of this guideline.

1.4 General Principles

Paediatric patients represent a population different from adults when considering the rapid growth and postnatal development of several organ systems. The continued development of these systems can affect drug pharmacokinetics (PK), pharmacodynamics (PD), and/or off-target effects of medicines, potentially leading to differences in toxicity and/or efficacy profiles both between paediatric age groups and when compared to adults.

An early consideration of nonclinical support for paediatric medicine development is recommended. In this respect, changing the design and/or timing of the traditional nonclinical program is one way to address potential safety concerns for the paediatric patient. For example, dosing can be initiated at a younger age in a repeat-dose toxicity study to support the corresponding developmental stages in paediatric patients. Another approach could be to conduct the Pre- and Postnatal Development (PPND) study earlier than usual, with modifications that demonstrate adequate offspring exposure and incorporate additional endpoints (see ICH S5). These changes can obviate the need for, or limit the design of, a dedicated JAS.

An understanding of the overall clinical development plan is needed to design an appropriate, efficient nonclinical plan. Prior to each paediatric trial, a weight of evidence (WoE; see Section 2) based decision should be made to determine whether additional nonclinical investigations are warranted. The outcome of such a WoE assessment can be different for each trial for the same pharmaceutical depending on paediatric age and indication.

The conduct of additional nonclinical investigations should be undertaken only when previous animal and human data are judged to be insufficient to support paediatric studies. JAS are designed to address identified safety concerns that cannot be adequately addressed in other nonclinical studies or paediatric clinical trials, including potential long-term safety effects. This guideline recommends a customized JAS that comprises core design elements and potential additional elements driven by specific concerns.

2. DETERMINING THE NEED FOR ADDITIONAL NONCLINICAL SAFETY INVESTIGATIONS

2.1 Clinical Context

The paediatric clinical development plan for a pharmaceutical is discussed in the ICH E11 guideline, and needs to be understood before an appropriate nonclinical plan can be designed. The paediatric clinical plan includes the indication/condition, the intended paediatric age group(s), and the treatment regimen (particularly, the duration of dosing during the stages of development). The clinical development of a medicine for paediatric patients usually follows initial adult clinical studies. If needed, the design and timing of additional nonclinical investigations are dependent on the identified safety concerns and the intended clinical use.

In case of a severely debilitating or life-threatening disease, or one in which there is serious unmet medical need in a paediatric population, the sponsor and regulatory agencies should consider the timing impact of producing additional data to support patient access to a pharmaceutical. This decision should be based upon a careful and cautious risk-benefit evaluation. If a safety concern is identified for further clinical development, appropriate nonclinical studies (e.g., JAS) should be considered, and could be conducted in parallel with clinical investigation.

2.2 Weight of Evidence Approach

The nonclinical development plan for a paediatric pharmaceutical depends on an integrated assessment based on the totality of the clinical context together with the pharmacology, pharmacokinetic (ADME), and nonclinical *in vitro* and *in vivo* animal and clinical safety data, i.e., a WoE approach. A WoE approach considers multiple factors evaluated together and, therefore, a single factor should not be considered in isolation. The importance of each factor should be weighted such that the final decision concludes whether available data adequately address safety concerns in the proposed paediatric population or whether additional nonclinical studies are warranted.

The WoE evaluation should be conducted when designing the initial paediatric development plan, but revisited if there are changes in age ranges and/or indications. The WoE outcome can be different for each trial depending on the paediatric population and the disease to be treated.

Figure 1 below shows some key factors that should be considered as part of the WoE evaluation to determine the need for further nonclinical investigations. The individual factors are presented below on the left of the figure. The most important factors are the youngest intended patient age and whether there are known (or suspected) adverse effects on developing organ systems of the patients during the conduct of the paediatric trial. The other important factors are not listed in order of weight in the figure. The list is not all inclusive for every situation, as there may be additional specific factors to consider (e.g., clinical management). The WoE factors are further described in the following sections.

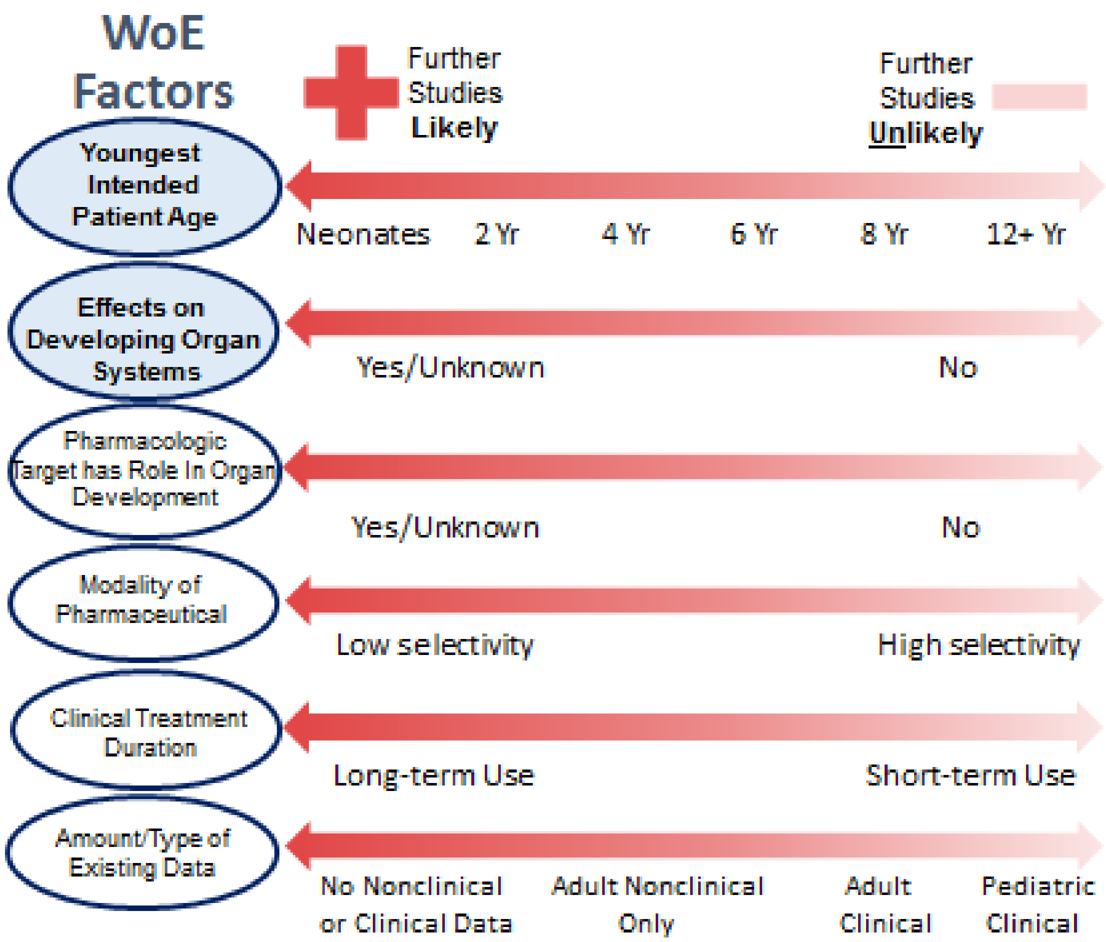

Figure 1: Key Weight of Evidence factors to be considered in determining if nonclinical studies are warranted. The most important factors are the youngest intended patient age and whether there are known (or suspected) adverse effects on developing organ systems of the patients during the conduct of the paediatric trial. The other important factors are not listed in order of weight. The arrows indicate a gradient for the weight of each factor.

2.3 Factors to Inform the Weight of Evidence Evaluation

2.3.1 Clinical Information

The most relevant safety and efficacy data for paediatric patients come from other paediatric subpopulations and adults exposed to the pharmaceutical. This established efficacy and safety profile is usually the first point to consider when determining if additional nonclinical studies are warranted.

The youngest intended patient age is one of the most important factors to be considered. The use of existing clinical data from older subgroups may not necessarily be sufficient (see ICH E11). Further nonclinical studies are more likely to be warranted at the lower end of the age range.

The duration of clinical treatment is another factor in determining whether additional nonclinical studies are warranted. Longer durations of treatment are more likely to expose a paediatric subject during a developmentally sensitive window. Whereas short-term use of a pharmaceutical is less likely to affect some aspects of development such as growth, a long duration of use is more likely to warrant further nonclinical studies than short-term treatments.

Additional nonclinical studies are not warranted when existing clinical data are considered sufficient to support paediatric use and/or if identified safety concerns can be clinically managed. A JAS is not warranted to confirm toxicity in target organs in which sensitivity to toxicity is not expected to differ between adults and paediatric patients. Developmental differences in target or off-target tissue maturity do not, in isolation, necessarily mean a JAS is required, but are a concern that needs to be considered.

2.3.2 Pharmacological Properties

Primary or secondary pharmacological properties of a pharmaceutical can be responsible for unwanted side effects. This may raise concerns for paediatric use if effects occur in systems/organs in development and/or if developing organs have a different sensitivity from mature organs. A review of the literature on the developmental expression and ontogeny of drug target(s) (e.g., receptor, enzyme, ion channels, protein), or the known/potential role of the target during development is recommended. Existing data from genetically modified animals (e.g., the knock-out of a receptor) may also identify developmental effects of potential concern for the paediatric population, which could be included in the WoE evaluation.

If the known pharmacology of a medicine has the potential to impact the development of the intended paediatric population, or the role of the pharmacology on development is not understood or reasonably predictable, further nonclinical investigations should be considered. Potential adverse effects of pharmaceuticals with high selectivity for their target (e.g., monoclonal antibodies) are more likely to be related to exaggerated pharmacology and therefore be more predictable than effects of pharmaceuticals with lower selectivity for their pharmacologic target. Pharmaceuticals with lower selectivity may have secondary pharmacodynamic effects and thus are more likely to warrant further nonclinical investigations. Considerations should be given whether conducting *in vitro* or *ex vivo* investigations using juvenile (i.e., animal) or paediatric (i.e., human) tissues would be useful to determine potential age-related differences in sensitivity, density, and distribution of molecular pharmacological/toxicological targets.

Further nonclinical studies might not add value when the underlying pharmacology has already identified a particular hazard.

2.3.3 Pharmacokinetic Data

Important differences can exist in the ADME of pharmaceuticals depending on the age of both patients and animals, leading to potential differences in efficacy and toxicity. These differences are usually most prominent in neonates and infants. Similarly, maturation of the gastrointestinal

(GI), liver, and renal systems can result in rapidly changing systemic exposures, particularly in young animals.

The use of clinical PK modelling and simulation systems for the purpose of predicting PK/ADME characteristics in paediatric populations can be more relevant than conducting JAS. If the results of the PK modelling and simulation indicate that there will be significant differences between adult and paediatric patients, then nonclinical investigations (e.g., *in vitro* studies) can be helpful to determine the potential impact of these differences on toxicity.

2.3.4 Nonclinical Safety Data

Existing nonclinical toxicity study data should be evaluated for signals that could indicate potential effects in organs undergoing development in paediatric subjects. Findings occurring in animals at similar exposures as those likely to be achieved in paediatric subjects are of higher concern, particularly if the findings occur in organs/tissues that undergo significant postnatal development at the intended paediatric age (see Appendix A). Safety signals that occur in adult animals of more than one species are of increased concern. Depending on the age of the animals at study start and the endpoints included, some of these concerns may have been addressed in existing repeat-dose toxicity studies.

Genotoxicity testing and safety pharmacology investigations are normally conducted to support adult clinical trials and, therefore, should be available before paediatric clinical trials commence. If a safety pharmacology study shows an effect in an organ system known to be developing in the intended paediatric patient population, the possible impact of the effect should be carefully considered. Additional genotoxic and safety pharmacology assessments in juvenile animals are generally not needed to support paediatric indications.

Reproductive and developmental toxicity study data may also be available. If PPND study data are available and have shown clinically relevant and sustained systemic exposures in offspring during the relevant postnatal period, these data can contribute to the WoE evaluation. The review of such data should include the maternal tolerance of the drug during pregnancy and lactation, as this could have impacted on the findings in the offspring. Observations of adverse effects in offspring would not, on their own, indicate that a JAS is recommended. However, if there is an identified safety concern that could lead to effects on postnatal development, it should be considered in the WoE evaluation. These data in rodents are primarily relevant to preterm and term neonates if exposure is demonstrated.

In some cases modification of a rodent PPND study can obviate the need for a JAS, provided potential concerns for the paediatric population have been appropriately addressed in the study design (see ICH S5). For enhanced PPNDs (ePPND) studies conducted in the non-human primate (NHP), the data from the offspring can characterize toxicity during early postnatal development, provided relevant exposure and/or PD effects are confirmed in the offspring. When available, ePPND data should be evaluated in combination with data from the general toxicity studies in assessing the value of additional nonclinical investigations.

2.3.5 Feasibility

The decision to conduct an additional animal study should also consider the technical and practical feasibility of the study design and endpoints (see Section 3). If a study in animals cannot be conducted with dose levels that provide acceptable systemic exposures in the range of those expected in paediatric patients, even with an alternative route of administration or frequency of dosing, the conduct of the JAS may not be informative or warranted.

2.4 Application and Outcome of the Weight of Evidence Evaluation

All of the WoE factors described above should be considered when determining whether additional nonclinical investigations are warranted. Additional nonclinical studies are not warranted if identified safety concerns can be clinically monitored and/or managed. When a study is warranted, the specifics of the identified safety concerns will define the objectives of the nonclinical investigation; this could be a JAS or another study (e.g., *in vitro* or *ex vivo* investigations).

Examples of applying the WoE approach are in Appendix B.

3. DESIGN OF NONCLINICAL JUVENILE ANIMAL STUDIES

3.1 General Considerations/Study Objectives

Once it is decided that a JAS is warranted, Section 3 should be consulted to design the appropriate study. This section contains recommendations on study design considerations, core endpoints to be included in all studies, and additional endpoints that can be included to address specific concerns. A JAS design including all potential additional endpoints is not recommended without rationale.

If the reason to conduct a study is primarily driven by a specific, identified safety concern for paediatric patients, the study design should be customized to address particular aspects of function or development of a target organ or system of concern. If the rationale to conduct a study is based on a concern for patient safety due to lack of relevant knowledge of the pharmacology, the study design would generally be broader and include additional endpoints as appropriate.

The maturation of human and animal organ systems can influence susceptibility to toxicity. Understanding the relative level of maturity and function across species during development is needed not only to design the appropriate JAS but also to aid the translation of nonclinical toxicity findings to a specific human age range. This “age” or “stage” mapping can be challenging and is not uniform across different organ systems or species, as the relative maturity at birth, rate of postnatal maturation, and/or regulation of maturation can be quite different between humans and animals. While not comprehensive, Appendix A, Figures A1-A6 provide an overview of age-dependent development of organ systems by species.

3.2 Preliminary/ Dose Range Finding Studies

Preliminary studies such as dose range-finding (DRF) or PK studies with small group sizes of juvenile animals of relevant age are highly recommended to perform tolerability and PK/TK (toxicokinetic) assessments. This is particularly valuable when dosing starts prior to weaning to avoid unexpected mortality, excessive toxicity, and/or irrelevant exposures in a definitive JAS.

Dosing should be initiated at the youngest planned starting age of the animals in the definitive JAS to evaluate the most critical period for tolerability and exposure differences. The DRF dosing period generally lasts a few weeks, e.g., typically until shortly after weaning in rodents. If there are important age-related differences in tolerated dose levels between adults and juveniles, a second DRF study may be needed to select adequate dose levels or a dosing regimen for the definitive JAS. See sections on route of administration (3.6) and dose selection (3.7) for more information on the use of preliminary studies to prepare for anticipated changes in dosing route and/or dose level adaptation during the course of a definitive JAS.

In a preliminary or DRF JAS, lack of tolerability of a pharmaceutical at clinically relevant systemic exposures can indicate a significant concern for the corresponding clinical age range. When the reason for greater sensitivity or significant differences in toxicity profiles between juvenile and adult animals at similar systemic exposure is not understood, additional investigations (e.g., assessment of protein-binding values or blood-brain barrier penetration) can be useful for the interpretation of these differences.

In certain circumstances, DRF studies can explore the usefulness of particular endpoints, tissues, or biomarkers and thus refine the study design of the definitive JAS.

3.3 Animal Test System Selection

When a JAS is warranted, in most cases a single species is considered sufficient. In principle, the rat should initially be considered as the species for a JAS. Other species have been used in JAS (e.g., mouse, rabbit, dog, minipig, NHP). In all cases, the selected species should be justified, as nonclinical studies in a non-relevant species can give rise to misinterpretation and are not recommended.

The following factors should be considered when selecting an appropriate species:

- An understanding of the ontogeny of the pharmacological or toxicological target (e.g., the receptor) in animals in comparison to that in the intended paediatric population
- Preference for a species and strain for which adult repeated-dose toxicity data are available to allow a comparison of the toxicity and systemic exposure profiles between juvenile and adult animals.
- Toxicological target organs
    - the relative stage of organ/system development in the juvenile animal as compared to the intended paediatric population (see also Section 3.4)
    - the ability of the animal model to detect toxicity endpoints of concern
- The technical/practical feasibility to conduct the study in the selected species
- Similarity of ADME characteristics

Advantages and disadvantages of using different rodent or non-rodent species are outlined in Appendix A, Table A1.

While for biopharmaceuticals NHPs are pharmacological responders in many cases, the conduct of JAS in NHPs is challenging for both scientific and practical reasons. There is limited added value of performing JAS in younger NHP as compared to the 2-4 year old NHP used in general toxicity studies and, therefore, alternative approaches to obtaining the necessary data are encouraged. Only in rare cases is the value of JAS conducted in NHP justifiable.

Consistent with ICH S6, a homologous protein, when available, can be considered for the purposes of hazard identification in the rodent or other non-rodent species.

JAS in two species would be warranted only in a paediatric-first situation (see Section 4) or where there are multiple specific concerns for postnatal development and one species alone is not able to address them.

If a paediatric PD model of disease exists (e.g., enzyme replacement therapy), appropriate safety endpoints can be incorporated in these studies. This information could contribute to the WoE evaluation and/or potentially obviate the need for a dedicated JAS.

3.4 Age of Animals, Dosing Period, and Dosing Regimen

The age of dosing initiation in animals should developmentally correspond to the youngest age of the intended paediatric population, which will depend on a human-to-animal comparison of developmental periods of organ system(s) of toxicological concern. As comparative organ system correlations are not aligned for each organ across species, priority should be given to any target organ/ system of potential concern or to particularly vulnerable developing systems in the intended patient population. The animal age at dosing initiation should be justified using relevant information (see Appendix A).

When determining the duration of administration in JAS, it is important to consider the age range and the shorter developmental period of animals compared to humans, the duration of treatment for the intended paediatric population, the safety concern to be assessed, and the developmental stage of target organs/functions of the intended paediatric population relative to that of the animals used for toxicology studies.

The dosing period in JAS is not only defined by the paediatric age stages (e.g., > 2 years) or the clinical dosing duration but also by the specific stages of organ development for the organs of concern (see Appendix A). To evaluate the impact on a paediatric developmental stage, a longer dosing period in animals can be appropriate to address a concern of a certain organ system that develops late (e.g., central nervous system [CNS]) compared to a system with shorter developmental window (e.g., kidney). In contrast to nonclinical studies for adult populations (see ICH M3), a short treatment duration in paediatric patients can require a longer dosing duration in the JAS to capture the developmental age range of the intended paediatric population. For example, to include the youngest intended patients of 2 years old up to patients 12 years of age with a clinical dosing duration of 14 days, the JAS can have a dosing period longer than 14 days to incorporate exposure at all developmental stages corresponding to human patients from 2 to 12 years old (e.g., in the rat this would be approximately 6 weeks dosing duration, roughly postnatal day (PND) 21 to 65, See Appendix A).

Dosing up to maturation can be feasible in non-rodent species like the dog, minipig, and rabbit, as these species mature over a period of a few to several months, and with relative consistency. In contrast, the interval between birth and maturity for NHPs is several years, making dosing during the entire developmental period not practical. Furthermore, NHPs show considerable inter-individual variation in the age of onset of puberty and maturity.

When a DRF study demonstrates that a dose level or duration of dosing is not expected to be tolerable in a JAS, it may be possible to achieve the clinically relevant exposure at this dose by separating the dosing period into different subgroups (e.g., a required 6-week JAS dosing period is split into two subgroups of 3 weeks dosing, each starting at different ages). This approach may only be needed at the dose that is not tolerated. This approach is especially applicable in cases when the clinical dosing period is comparable to or shorter than the dosing period in the JAS subgroups; it may also have value to identify critical windows of susceptibility. The benefits of this approach should be considered with the drawbacks, such as substantially increasing the required number of animals and difficulties interpreting data at different ages. See Section 3.7 Dose Selection regarding dose adjustment as an alternate strategy to be considered in this situation.

Dosing frequency in JAS may not be exactly the same as in the clinical regimen. For example, even though a clinical regimen is once a week, daily dosing in juvenile animals can be needed to achieve and maintain relevant systemic exposures to evaluate the effects on developing organ systems and/or to maintain systemic exposures at relevant levels during the entire developmental period of concern.

3.5 Off-Treatment Period Assessments

Inclusion of an evaluation period after treatment has stopped in a JAS can help address two issues: 1) whether any effects observed during treatment are reversible, persistent, or progressive and 2) whether any effects emerge later in development as a result of early life exposure (i.e., delayed onset of changes). The need for an off-treatment period is dependent on the outcome of the WoE assessment and the endpoints to be evaluated in the study.

In general, an off-treatment period should be included to understand persistence, progression, or reversibility of a specific effect if this cannot be predicted by scientific assessment (Note 1). The principles of reversibility in ICH M3 apply to JAS endpoints that are similar to those in repeat-dose toxicity studies in adults (e.g., histopathology, clinical pathology). The duration of such an off-treatment period should be sufficient to allow the potential recovery of the effect, and should take into account the elimination of the pharmaceutical. The demonstration of full reversibility is not considered essential. A trend towards reversibility (decrease in incidence and/or severity) and a scientific assessment that this would eventually progress to full reversibility could be sufficient. If reversibility or irreversibility of a specific effect is well characterized in adult animals, it is generally not necessary to confirm this in a JAS. There are endpoints in a JAS that are not amenable to the classic approach of reversibility assessment, such as the timing of onset of puberty and neurobehavioral assessments (e.g., learning). Additionally, the timing of the off-treatment period in relation to the life stage of the animals should be considered

Some alterations can only be identified following an appropriate off-treatment period to allow maturation of an organ system and expression of the alteration. Therefore, some assessments can only be meaningfully performed after a certain level of maturity is expected to be reached (e.g., behavioural assessment, immunological response in T-cell-dependent antibody response [TDAR]). These assessments can be conducted in off-treatment periods after dosing duration has covered all critical developmental windows related to the clinical use. This is especially relevant in cases in which the clinical population is only the very young, such that the JAS dosing duration would cease at an immature age and the animals will continue to mature during the off-treatment period to an age that an appropriate assessment can be conducted.

In non-rodents, the addition of post-treatment groups for JAS can be less useful due to the more protracted development period, high inter-individual variability, and fewer and less well characterized assessments available to identify delayed or altered development.

3.6 Route of Administration

The intended clinical route of administration should be used when feasible, but obtaining adequate systemic exposure is paramount.

Alternative administration routes should be considered in cases of practical difficulties; changing routes during the course of the study can also be considered (e.g., subcutaneous until intravenous is feasible in rodents). The validity of using an alternative dosing route should be justified (e.g., supported by TK data in representative juvenile animals).

If the pharmaceutical is intended for use by two or more clinical routes of administration, a JAS with a single route of administration is sufficient, but should provide adequate exposure in juvenile animals for all intended clinical routes of administration.

3.7 Dose Selection

It is desirable to establish a dose-response relationship for adverse effects and to determine a no-observed adverse effect level (NOAEL) in juvenile animals. Dose levels should be selected to achieve some overlap in the range of exposure in adult animals to enable comparison of effects between adults and young animals. However, the high dose should not result in marked toxicity that can confound the growth and development endpoints and complicate the assessment. Body weight loss or lack of gain during rapid growth periods has the potential to confound results, and is therefore not desirable in a JAS. The low dose should preferably result in exposure levels similar to the anticipated exposure in the intended clinical population. For small molecules, selection of the high dose in accordance with ICH M3 applies. For biotechnology-derived products, the principles for dose selection described in ICH S6 apply.

There can be changes in systemic exposure due to maturation of the ADME systems that can make it challenging to meet the dose selection aims described above. In cases in which preliminary studies demonstrated that juvenile animals are markedly more sensitive than adult animals, or there are substantial changes in systemic exposure as the animals mature, dose adjusting should be considered. Dose adjustment (dose increase or decrease) during a JAS can be appropriate to evaluate endpoints when exposure separation between dose levels can otherwise not be maintained throughout the study. Adjusting doses during the study is intended to keep the exposures somewhat consistent; generally, not more than one or two adjustments during a JAS would be expected.

3.8 Endpoints

Each JAS should include the core endpoints defined in Section 3.8.1 below, unless justified otherwise. Each additional endpoint (see Section 3.8.2) should be considered and justified to address an identified safety concern (Note 2).

For the interpretation of study results in JAS it is important to have appropriate historical control data (HCD) at relevant ages of the species/strain/sex used (Note 3).

3.8.1 Core Endpoints

3.8.1.1 Mortality and Clinical Observations

Mortality should be evaluated throughout the experimental period. Clinical observations, including physical examinations, should be conducted as they can identify overt behavioural effects both on and off treatment

Clinical observations during the lactation period should include maternal nursing behaviour, and should capture clinical observations unique to juvenile animals as much as possible. After weaning, clinical observations should be recorded as for adult animals.

3.8.1.2 Growth

Growth should be assessed by body weights in conjunction with long bone length. As body weight increases dramatically during the early postnatal period, individual weight measurements should be frequently recorded to inform dose calculations. Generally, one long bone (e.g., femur) measured for length at necropsy is sufficient (Note 4).

3.8.1.3 Food Consumption

Food consumption during the postweaning period should be assessed as appropriate for the species.

3.8.1.4 Sexual Development

The physical indicators of onset of puberty (e.g., for rodents, the age of vaginal opening in females and balanopreputial separation in males) are recommended when the treatment period encompasses the relevant developmental window.

3.8.1.5 Clinical Pathology

Standard clinical pathology examinations (serum chemistry and haematology) should be assessed as a terminal endpoint at necropsy if evaluation is planned at an age in which expected clinical pathology ranges are known and can support interpretation of histopathology findings.

3.8.1.6 Anatomic Pathology

At the end of the treatment and/or off-treatment periods, gross pathology, organ weights (Note 5), and comprehensive collection and preservation of tissues should be conducted for animals allocated to necropsy. Histopathology should be performed on major organs (e.g., bone, brain, ovary, testis, heart, kidney, liver) and those with macroscopic lesions. Testicular histopathology should include a qualitative evaluation of spermatogenic progression in mature animals.

3.8.1.7 Toxicokinetics

TK sampling should be conducted near the beginning and end of the dosing period. If dosing is started preweaning, interim TK assessment(s) should be considered. A preliminary or DRF JAS with TK assessment, which is recommended (see Section 3.2), will inform on the sampling day and the timepoints of sample collection.

When designing the TK component of a JAS, microsampling and sparse sampling (if justified) are strongly encouraged (see ICH S3) from the view of 3Rs.

For protein therapeutics, samples for anti-drug antibodies should be collected and evaluated if appropriate (see ICH S6).

3.8.2 Additional Endpoints to Address Identified Concerns

3.8.2.1 Growth

As appropriate for the species, crown rump length, body length (e.g., nose/tail), and/or withers height can be used as an indicator of growth. Serial non-invasive measurement of long bone length using ultrasonic echo or X-ray can be appropriate in non-rodents in addition to a direct measurement at necropsy.

3.8.2.2 Skeletal Examinations

When there is an identified concern about bone metabolism or structure, the measurements of bone-related biomarkers and/or expanded histopathology (e.g., histomorphometry) should be considered. Assessment of bone mineral density (e.g., microdensitometry, dual energy X-ray absorptiometry, peripheral quantitative computed tomography [CT]) or bone structure (e.g., micro CT) can also be conducted as appropriate.

3.8.2.3 Clinical Pathology

Additional haematology, serum chemistry, and/or biomarkers can be considered to further characterize identified concerns on target organs/tissues. Other parameters such as urinalysis or coagulation assessments can be added when warranted.

Samples collected throughout the study at different ages and/or a series of samples collected within a short time period (e.g., 24 to 48 hours) can also be useful.

Due to the limitation in obtaining adequate sample volumes from juvenile animals (especially rodents), any additional samples that may require additional animals therefore are only recommended when critical to address a concern. When sample volume constraints exist, the parameters to be measured should be selected according to a priority based on the identified concern(s).

3.8.2.4 Anatomic Pathology

Additional tissues/organs can be evaluated to address specific concerns. Immunohistochemical or other special staining methods for tissue sections, electron microscopy, histomorphometry, or other imaging techniques can be warranted for interpretation of some findings.

3.8.2.5 Ophthalmologic Examinations

When there is concern for ocular toxicity, including retina and optic nerve, assessment of ocular endpoints should be considered. Standard ophthalmological examinations (e.g., palpebral reflex, ophthalmoscopy, slit-lamp microscopy) are not a routine endpoint for JAS, because structural development of the eye is largely completed during the prenatal period in humans.

3.8.2.6 CNS Assessments

There are different categories of CNS assessments, such as:

- detailed clinical observations
- behavioural tests

- learning and memory tests, and
- expanded neuropathology evaluations

Selection of any additional CNS assessments should be based only on the particular concerns identified in the WOE evaluation. In addition, the timing of these assessments should take into consideration whether the results will be used to identify adverse effects due to an extension of pharmacology, developmental neurotoxicity (i.e., effects that emerge or are still present after cessation of treatment) or both.

Detailed CNS-related clinical observations document the severity and the onset and duration of the clinical signs relative to dosing (e.g., hyper- or hypoactivity, tremors). These parameters should be assessed when a CNS concern has been identified by the WoE evaluation and should be collected during on- and off-treatment periods as appropriate.

Behavioural testing can include a modified Irwin test, functional observational battery (FOB), assessment of locomotor activity, evaluation of coordination and reflexes, and/or acoustic startle response (e.g., habituation or prepulse inhibition). These tests should be appropriate for the species being tested and the timing of these assessments should be determined relative to the level of maturity in the test species.

In addition, learning and memory can be evaluated by a variety of methods. Different methods assess different aspects of learning and memory. When specific aspects of learning and memory have been identified as areas of concern based on the WoE evaluation, then tests capable of assessing those aspects should be selected. Learning and memory should be evaluated typically during the off-treatment period as this period is most relevant to assess potential persistent or delayed effects. If learning and memory testing is performed during the treatment period, the potential for confounding pharmacological effects (e.g., sedation, decreased motor coordination) should be considered and avoided.

Any CNS areas or components (e.g., hippocampus, myelin) that are identified by the WoE evaluation as potential targets of concern should be assessed with additional neuropathological examinations as appropriate (e.g., additional levels for sections, immunohistochemistry, special stains). These assessments are typically performed at times of scheduled necropsy, unless there is a specific concern related to timing to be investigated. Imaging technologies may also be useful in specific circumstances (e.g., magnetic resonance imaging).

Postnatal CNS assessments are most commonly conducted and characterized in the rat. For those pharmaceuticals where the rodent is an inappropriate species, some behavioural tests are also available in other species (e.g., dogs, minipigs). Learning and memory assessments are infrequently conducted in NHPs. In NHPs, behavioural observations can provide the primary assessment of potential CNS effects in a JAS or ePPND study.

3.8.2.7 Reproductive Assessments

If there is an identified concern for effects on female and/or male reproductive organs or function, histopathology examinations and organ weights can be expanded to include reproductive and/or endocrine tissues in addition to the gonads. Reproductive system effects identified as irreversible in adult animals need not be confirmed in a JAS.

In rodents, for concerns relevant for females, assessment of estrous cyclicity is recommended as an initial assessment of reproductive and endocrine function. For concerns relevant for male rodents, sperm analysis (e.g., counts, motility, morphology) and/or testicular immunohistochemistry can be considered to further characterize effects if they can add critical information not already captured elsewhere.

The timing of the treatment and assessments in relation to that of sexual maturation in the species tested is critical. The timing of folliculogenesis and spermatogenesis should be considered in the study design and timing of reproductive assessments. Assessment of reproductive organs or function (e.g., estrous cyclicity, sperm count, or qualitative histologic assessment of spermatogenesis) can only be conducted in sexually mature animals. If the clinical age range is prepubertal, the concern is whether treatment of a medicine with reproductive toxic potential would cause any delayed effect on sexual maturation or reproductive function in adulthood. In this situation, a study should be designed to treat only during immaturity, and then allow the animal to mature without treatment, and conduct assessments after maturation is reached.

Mating assessments are not generally recommended in JAS. In male rodents, mating assessments have low sensitivity due to a large functional reserve of the testis. In female rodents, assessment of estrous cyclicity and ovarian histology can identify many developmental reproductive liabilities. In non-rodent species mating assessments are not practical due to the protracted duration of development and high degree of individual variability.

The feasibility of other additional reproductive assessments is such that the large majority are conducted in rodents, although they can be considered for those nonrodent species that achieve maturity during the conduct of a JAS. In NHP, additional reproductive assessments are not typically included in JAS.

Hormonal assessments are only recommended in JAS if they can add critical information not already captured elsewhere as there is considerable hormonal variability during puberty. Any hormone assessment should be justified, and the timing and specific hormones assessed should be well characterized for the age the assessment occurs.

3.8.2.8 Immunologic Assessments

If the pharmacological class or data in animals or humans give cause for concern for the development of the immune system, assessments for immunotoxicity should be considered as outlined in ICH S8. Such concerns can include, but are not limited to, a transient, prolonged or permanent decrease or increase in the number or function of a lymphocyte subtype or a sustained increase or decrease in immunoglobulin class. Functional assays such as the TDAR should be performed after appropriate times of development (e.g., after PND 45 for the rat).

3.8.2.9 Other Possible Assessments

If there are additional tissues or endpoints for which concerns are identified and cannot be managed clinically, appropriate evaluations should be planned and performed when nonclinical investigations can add useful information.

3.9 Allocation of Animals to Study Groups

3.9.1 Preweaning Allocation

In most species, initiation of a JAS during the preweaning phase presents a unique situation of dosing offspring within a litter. The maternal animal is a critical component of the study providing nutrition and care, but only the offspring are the test system. The study should be designed to reduce potential confounders of data from offspring related to genetics, maternal care, and littermates (i.e., nature and nurture confounders). Generally, genetic siblings and/or littermates should not be assigned to the same endpoints, especially for the core study endpoints. This can be achieved by the way the litters are constructed in combination with how they are assigned to dose groups and subsets of endpoints.

It is advisable to utilize litter sizes and sex ratios reasonably similar to the natural mean litter sizes for that species and strain. As for the method of assigning dose groups, it is desirable to prevent animals in a control group from being exposed to the test pharmaceutical, thus is it preferred that all animals in a litter be assigned to the same treatment group.

JAS can become large and complex, therefore it is especially important that the study design balances scientific rigor against animal use. Investigators should know all the planned endpoints (core and additional) to design the littering and subset assignment strategy efficiently. Efficiency in study design is critical to reduce animal use as per the 3R principles, and should be measured by the number of maternal animals and litters needed to supply the study. For animal species with low and variable litter sizes or single offspring, the same approach for group allocation design as in general toxicity studies can be appropriate.

After the study has started, each litter size should remain comparable across and within dose groups, as much as possible, while in the preweaning phase because litter size affects pup growth rate. Litter handling, dose group and endpoint subset allocation methods, and specifics of the testing model (e.g., age when litters culled, litter size and sex distribution, fostering, assignment of groups and subsets for evaluation) should be clearly described in the study plan and report. For statistical analysis, data collected from offspring while part of a litter should not be considered an independent variable since an individual offspring is dependent on maternal and littermate factors.

There are different allocation methods for litter management in preweaning, multiparous animals. Appendix C provides one example of an approach for rodents that controls for potential genetic, maternal care, and littermate biases. Other methods are acceptable if they appropriately consider these biases and the study objectives.

3.9.2 Postweaning Allocation

In multiparous animal species, if possible, it is still recommended to allocate the litters to minimize the genetic bias and maternal and littermate variables. In particular when dosing starts in the early postweaning phase, and, when offspring are supplied from a limited number of natural mothers in the test facility, the study should be designed in consideration of the potential confounders similar to those at preweaning allocation.

3.10 Animal Numbers and Sex

A JAS should use an adequate number of animals to evaluate the selected endpoints (e.g., body weights, reversibility, behavioural assessments). To reduce the number of animals, combining assessment of endpoints in the same animals can be effective. It is recommended that JAS be performed in both female and male animals.

4 CONSIDERATIONS FOR PAEDIATRIC-FIRST/ONLY DEVELOPMENT

Section 3 should be consulted to determine study designs needed to address the points below.

A common clinical approach for non-oncology paediatric-only/first pharmaceuticals starts with a First in Human (FIH) study in healthy adult volunteers prior to any paediatric trial. As per ICH M3, this approach generally includes nonclinical repeat-dose toxicity studies of appropriate duration in rodent and non-rodent animals as well as safety pharmacology and genetic toxicology studies before initiation of adult clinical trials. Principles of ICH S6 can also apply. The repeat-dose toxicity studies to support FIH in adults could be performed in several ways; in both species in adult animals or in one or both species by initiating dosing in juvenile animals and continuing treatment into maturity including additional endpoints (see Sections 2 and 3).

Alternatively, there are cases where paediatric patients are treated without any prior adult patient or healthy volunteer data (e.g., for a life-threatening or debilitating disease that only exists in children and when the pharmaceutical cannot be given safely to adult volunteers). In these cases, the FIH trial will be in paediatric patients and the nonclinical program would generally include one JAS in a rodent and one JAS in a non-rodent species, if feasible. Safety pharmacology and genotoxicity testing would be conducted as appropriate for adult use; *in vivo* studies need not be conducted in juvenile animals (see Section 2.3.4).

After initial clinical trials, JAS can be important to support continued clinical development in paediatric patients on a case-by-case basis, driven by cause for safety concern (see Section 2) and duration of clinical treatment. The principles of ICH M3 should also be considered. If the pharmaceutical is intended to treat a chronic disease, chronic toxicity studies should be conducted in one rodent and one non-rodent species. In at least one of these studies, dosing should start at an age developmentally matched to the lowest age of the intended patient population. In principle, a single set of chronic studies that start dosing from ages that developmentally correlate to the youngest paediatric patient age can provide nonclinical safety data sufficient to cover all ages and durations of paediatric development up to marketing, and can replace adult chronic and separate JAS. Further nonclinical assessments of reproductive toxicity and carcinogenic potential can be warranted.

For biopharmaceuticals, studies in juvenile animals should be limited to relevant species, as per ICH S6. When the NHP is the only relevant species, a JAS in NHPs could support initial clinical use. Non-invasive safety pharmacology endpoints can be included in the juvenile or standard NHP repeated-dose studies. Genotoxic and carcinogenic potential should be addressed as outlined in ICH S6.

JAS in NHP are typically conducted starting at 10-12 months of age, thus limiting the lowest paediatric age ranges. In cases where JAS is not feasible to support the youngest paediatric age, alternative approaches (e.g., in vitro assays, genetically-modified animals, surrogate molecules) should be considered if available and relevant.

A JAS in perinatal and preweaning NHP should only be conducted in the situation of medicines with first and primarily neonatal clinical use, and where alternative approaches to nonclinical safety assessment are not feasible. Studies with direct dosing of offspring can require large numbers of mature dams to populate even a relatively small JAS in NHP. Therefore the design and endpoints should be clearly justified based on the clinical concern. Design expectations should also be flexible; for example, variability in gender distribution and starting weights are expected.

5. OTHER CONSIDERATIONS

5.1 Excipients

Dedicated JAS on excipients are generally not needed to qualify paediatric formulations. To assess the safety of the paediatric clinical formulation, available toxicity information on the excipients should be evaluated. Pharmaceutical formulations used in paediatric indications can occasionally contain novel excipients or excipients not previously used in paediatric populations of a relevant age. If there are insufficient data to support the use of the excipient in the intended paediatric population, a JAS can be warranted. Although JAS that are primarily intended to assess the safety of active ingredients need not always be conducted with the clinical formulation, an excipient could be assessed in a JAS along with the active ingredient, if such studies were being conducted.

5.2 Combination Pharmaceuticals

The development of combination pharmaceuticals for paediatric use should have a nonclinical evaluation consistent with the principles outlined in ICH M3 (R2) for combination products in general together with the WoE principles outlined in this guideline. For example, a combination JAS would generally not be recommended for a combination of two late stage entities for which there is adequate paediatric clinical experience with co-administration. Whereas, a combination JAS might be warranted for a combination of two early stage entities if a WoE evaluation suggests that a JAS would address identified concerns. If additional nonclinical information is needed, the study design should consider what assessment endpoints are appropriate to address any concerns of administering the particular combination. If a JAS is considered appropriate, assessment of the combination as it is to be used clinically is generally sufficient and testing of the individual active ingredients may not be critical. Alternatively, an extra group with the combination could be added to a JAS that is already being conducted with one of the single entities. This could eliminate the need to do a separate study with the combination product.

GLOSSARY

Enhanced Pre- and Postnatal Development Study (ePPND):
This study design is based on biopharmaceutical (NHP) experience and is a PPND study which includes elements of the embryofetal development (EFD) study in newborns and infants instead of the fetus.

Juvenile:
Any postnatal stage not fully matured in terms of morphology and function

Paediatric First:
Paediatric-first development is when the pharmaceutical is developed for paediatric patients before any clinical data are available in adults for any indication.

Paediatric Only:
Paediatric-only development describes development for an indication requiring treatment exclusively in paediatric ages (e.g., neonatal respiratory distress syndrome).

Weight of Evidence:
An approach that evaluates a combination of information from several independent sources to determine if there is sufficient evidence to support paediatric clinical trials or whether additional nonclinical assessments are recommended to address safety concerns that cannot be managed clinically.

The weight given to the available evidence depends on factors such as the quality of the data, consistency of results, nature and severity of effects, and relevance of the information. The weight of evidence approach requires use of scientific judgment and, therefore, should consider the robustness and reliability of the different data sources.

NOTES

Note 1 If the off treatment period begins prior to maturity, the capacity and character of the recovery can be influenced by the continued growth and development of some organ systems, and should be carefully interpreted.

Note 2 The propensity for mortality to occur is generally higher in juvenile animals compared to adult animals. Study-related procedures should be limited as much as possible before and at the time of weaning as they can contribute to mortality.

Note 3 Assessments on immature animals should be done with reference to age-matched control data (e.g. body weights, clinical pathology, organ weights, histology) either from concurrent control animals or from other reference background data. This is especially important to consider in cases of unscheduled assessment of endpoints. JAS animals are generally not screened prior to initiation of treatment. Therefore, background rates of abnormalities in juveniles can differ from animals of the same age used in adult toxicity studies.

Note 4 Since growth happens in spurts, frequent assessments of bone length for ‘transient’ effects on growth is challenging to appropriately power and offers limited value. An assessment using data from the end of treatment is more useful. An effect solely on decreased body weight gain is not necessarily an effect on growth.

Note 5 Assessment of organ weight data should be done in the context of growth. For instance, if growth was restricted then absolute weights of most organs decrease in proportion to body weight; however, some organs have different sensitivity to growth effects.

REFERENCES

1. ICH E11 Guideline: Clinical Investigation of Medicinal Products in the Paediatric Population; 2017
2. ICH M3 Guideline: Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals; June 2009.
3. ICH S5 Guideline: Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility; 2000
4. ICH S6 Guideline: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals; 2011
5. ICH S9 Guideline: Nonclinical Evaluation for Anticancer Pharmaceuticals; 2009

APPENDIX A: OVERVIEW OF AGE-DEPENDENT DEVELOPMENT OF ORGAN SYSTEMS BY SPECIES

These tables reflect a high level overview of organ system development by species to illustrate similarities and differences between the commonly used toxicology species, as compared to humans, for the timing and relative duration of development. Specific milestones include birth, introduction of solid foods, weaning, puberty, and adulthood. The tables are intended to aid in the assessment of the relevance of existing nonclinical data, as well as the selection of species, starting age, and dosing duration for a JAS. These summary tables are based on a review of current knowledge, but are not comprehensive. Species-specific and/or organ system reviews in the literature can provide additional detail and should be consulted for each specific situation.

APPENDIX A: OVERVIEW OF AGE-DEPENDENT DEVELOPMENT OF ORGAN SYSTEMS BY SPECIES

Figure A.1: Age-dependent Development of Human Organ Systems

System	General Considerations	Neonate (< 1 mths)	1st Solid Food (~ 6 mths)	Weaning Toddler (~ 2 years)	Puberty (~ 11-15 years)	Adulthood (> 18 years)
Integument	• Critical neonatal function (barrier, water and thermoregulation, conductance, sensation) • then progressive surface acidification, local microbiome and immune function					
CV	• Critical neonatal physiologic transitions (pulmonary and systemic vascular resistance) • Adaptive myocardial and vascular changes • Progressive increase in ion channels.					
GI	• Functional at birth, with adaptations especially over first year to accommodate shift in diet/complexity and populate microbiome					
Renal	• Nephrogenesis complete at term birth • Progressive increase in GFR and renal function over first year					
Hepato-biliary	• Structurally well developed at birth • Progressive increase in metabolic functionality, especially over first 6 months to 1 year of age					
Pulmonary	• Increased alveolization and surface area over first year					
Immune	• Progressive population of secondary immune tissues and development of memory as a function of time and environment					
Endocrine	• Most glands are well developed at birth and critical for growth • Zona reticularis of adrenal cortex and gonads undergo expansion in late childhood/early puberty					
Repro-ductive	• Testes descended at birth, populated by germ cells, Sertoli cells and Leydig cells • 'Mini puberty' at 2 to 4 months of age, adrenarche in late childhood • Subsequent reproductive changes occur at onset of puberty and continue until adulthood					
Nervous	• Defined sequential and progressive development into adulthood • Maximum neuron count and brain:body weight at birth, with postnatal apoptosis, pruning and migration • Myelin and glia present at birth • Neurotransmitter and conduction systems mature at variable rates (ie: opiate receptors/metabolism, GABA, serotonin & noradrenalin differ)					
Skeletal	• Growth plates present at birth • most rapid postnatal growth occurs prior to age of 4 years, with slower growth through childhood primarily mediated by GH and TH • pubertal growth spurt driven by sex hormones • growth plates close during adolescence/early adulthood					

	Major period of functional and structural growth and development
	Completion of structural development; active period of growth and/or functional maturation
	Slow continued growth or refinement of function; also can reflect a period of relative inactivity, as in prepubertal reproductive tissues
	Structurally and functionally fully mature

Figure A.2: Age-dependent Development of Rat Organ Systems

System	General Considerations	Neonate (~ PND 1-10)	1st Solid Food (~ PND 15)	Weaning (~ PND 21-25)	Puberty (M ~ PND 42, F ~ PND 35)	Adulthood (~ PND 70)
Integument	• Critical neonatal function (barrier, water and thermoregulation, conductance, sensation) • Thicker epidermis first 2 weeks of age • Adnexa and hair develop postnatally • Structurally resembles adult by PND 21 • sexual dimorphism by PND 35 to 42					
CV	• Critical neonatal physiologic transitions (pulmonary and systemic vascular resistance) • Adaptive myocardial and vascular changes • Progressive increase in cardiomyocytes and ion channels to PND 21					
GI	• Immature at birth; lack gastric acid and poor pancreatic enzyme production until after PND 14 • Highly permeable proximal small intestine allows absorption of intact proteins • Adaptations in 3rd week postnatal to accommodate shift in diet					
Renal	• Nephrogenesis incomplete at birth • Progressive increase in GFR and renal function over first 3 to 5 weeks of age					
Hepato-biliary	• Structurally immature at birth • Progressive development of organized hepatic cords and plates, with increase in metabolic functionality, over first 4 weeks of age					
Pulmonary	• Saccular at birth • alveolization occurs over first 2 to 3 weeks of age					
Immune	• Progressive population of secondary immune tissues and development of memory as a function of time and environment • TDAR typically assessed after PND 45					
Endocrine	• Most glands are well developed at birth and critical for growth					
Repro-ductive	• Period of decreased androgen production by Leydig cells during 3rd week postnatal necessary for expansion of germ cells and Sertoli cells • Remaining reproductive changes and appearance of sexual dimorphism occur at onset of puberty (postnatal week 5 to 7)					
Nervous	• Structural maturation of olfactory bulbs, retina/eye, cerebellum, hippocampus, and cerebral crotex occurs postnatally • Maximum neuron count and brain:body weight at PND7, with extensive postnatal apoptosis, pruning and migration • Myelin not present at birth • Conduction systems, opiate receptors/metabolism, GABA, serotonin & noradrenalin pathways mature at different rates					
Skeletal	• Rapid postnatal growth through adulthood • Long bone growth plate structure not evident until PND 14 to 21, and remain open into adulthood					

	Major period of functional and structural growth and development
	Completion of structural development; active period of growth and/or functional maturation
	Slow continued growth or refinement of function; also can reflect a period of relative inactivity, as in prepubertal reproductive tissues
	Structurally and functionally fully mature

Figure A.3: Age-dependent Development of Beagle Dog Organ Systems

System	General Considerations	Neonate (< 3 wks)	1st Solid Food (~ 3 wks)	Weaning (~ 8 wks)	Puberty (M ~ 5-8 mths) (F ~ 6-12 mths)	Adulthood (> ~ 12 mths)
CV	• Critical neonatal physiologic transitions (pulmonary and systemic vascular resistance) • Adaptive myocardial and vascular changes • Cardiac innervation continues development during approx. 2 to 4 months of age • Significant increase in blood pressure and decrease in heart rate from week 1 to 6 months of age					
GI	• Similarities in the stomach to that seen in human • At birth gastrointestinal tract is fully formed (functional development primarily between birth and weaning)					
Renal	• Kidney is structurally and functionally immature at birth • Completion of nephrogenesis at approx. 2 weeks of age • Acid-base homeostasis develops postnatally • Concentrating ability develops prenatally					
Hepato-biliary	• Hepatic structural maturation reached at approx. one week of age • Bile secretory function not fully mature at birth (at 4 to 6 weeks of age: 30 to 70 % of adult values)					
Pulmonary	• Considered acceptable model for the study of pulmonary toxicity in juvenile population					
Immune	• Immunologic tissues are largely structurally mature at or shortly after birth • Development of the immune system very similar to that seen in human, but placental transfer of IgG is poor • IgG transfer from dam primarily occurs during first 24 h postnatally via the colostrum • thymus undergoes rapid postnatal growth and reaches maximum size at 1 to 2 months of age					
Repro-ductive	• Testicular descent incomplete at birth: occurring at 5 to 6 weeks of age • Males reach sexual maturity at approx. 7 to 8 months of age • Females reach sexual maturity at approx. 8 to 12 months of age					
Nervous	• Rapid cognitive development through 12 to 16 weeks of age with critical developmental period for learning at approx. PND 18 to 28 • Neonatal (primitive) reflexes also disappear at approx. PND 28 • Functional locomotor development occurs postnatally (standing approx. 3 weeks of age with rapid progression through first month)					
Skeletal	• Long bone ossification primarily occurs postnatally with appearance of ossification centers between 1 to 10 weeks of age • Most rapid long bone growth is complete by 5 months of age, with slower continued growth through puberty					

	Major period of functional and structural growth and development
	Completion of structural development; active period of growth and/or functional maturation
	Slow continued growth or refinement of function; also can reflect a period of relative inactivity, as in prepubertal reproductive tissues
	Structurally and functionally fully mature

Figure A.4: Age-dependent Development of Göttingen Minipig Organ Systems

System	General Considerations	Neonate (< 2 wks)	1st Solid Food (~ 2 wks)	Weaning (~ 4 wks)	Puberty (M ~ 3/4 mths, F ~ 4/5 mths)	Adulthood (> ~ 6 mths)
Integument	• Critical neonatal function (barrier, water and thermoregulation, conductance, sensation) • similarities in development to human					
CV	• Critical neonatal physiologic transitions (pulmonary and systemic vascular resistance); • Adaptive myocardial and vascular changes • Similarities in development to human					
GI	• Maturity reached by approx. 4 weeks of age • Model for human stomach development					
Renal	• Nephron formation up to approx. 3 weeks after birth • Functional mature at approx. 3 months of age					
Hepato-biliary	• Structurally and functionally immature at birth • Adult appearance at approx. 4 weeks of age and full function at 3 to 4 months of age					
Pulmonary	• Lungs are well developed at birth • Alveolization occurs over first 1 to 2 weeks of age and completed within 2 weeks of age					
Immune	• Very little function at birth • Anatomically full developed at approx. 4 weeks of age • Model for human immune development					
Repro-ductive	• Sexual maturity in males with approx. 3 to 4 months of age and in females with approx. 4 to 5 months of age					
Nervous	• Growth mainly in the late prenatal to early postnatal period • Nervous system complete by 6 months of age • Brain development of the neonatal pig is similar to the human term neonate • Neuromuscular system is more functionally mature at birth than in human					
Skeletal	• Rapid postnatal growth through adulthood; closure of the epiphysial growth plates at 18 months of age • Full grown adults at approx. 24 months					

	Major period of functional and structural growth and development
	Completion of structural development; active period of growth and/or functional maturation
	Slow continued growth or refinement of function; also can reflect a period of relative inactivity, as in prepubertal reproductive tissues
	Structurally and functionally fully mature

Figure A.5: Age-dependent Development of Cynomolgus Monkey Organ Systems

System	General Considerations	Neonate (< 1 mth)	1st Solid Food (~ 3 mths)	Weaning (~ 6 mths)	Puberty (~ 3-4 years)	Adulthood (~ 4 years)
Integument	• Functional (barrier, water and thermoregulation, conductance, sensation) with hair and adnexa present at birth					
CV	• Critical neonatal physiologic transitions (pulmonary and systemic vascular resistance) • Adaptive myocardial and vascular changes • Myocardiocyte expansion through 3 months of age, then progressive growth					
GI	• Functional at birth, with adaptations especially over first year to accommodate shift in diet/complexity and populate microbiome					
Renal	• Nephrogenesis complete at term birth • Progressive increase in GFR and renal function over first 6 months of age					
Hepato-biliary	• Structurally well developed at birth; progressive increase in metabolic functionality, especially over first 3 to 6 months					
Pulmonary	• Structurally mature at birth with progressive growth					
Immune	• Progressive population of secondary immune tissues and development of memory as a function of time and environment					
Endocrine	• Most glands are well developed at birth and critical for growth • Zona reticularis of adrenal cortex expands at 3 to 6 months of age (adrenarche) • endocrine function of gonads expands at puberty					
Repro-ductive	• Testes descended at birth, populated by germ cells, Sertoli cells and Leydig cells • Follicular development and atresia begins at 3 to 6 months • Subsequent reproductive changes (menarche and spermarche) occur in at onset of puberty and continue until adulthood					
Nervous	• Defined sequential and progressive development into adulthood • Postnatal apoptosis, pruning and migration most prominent before weaning • Myelin and glia present at birth • Neurotransmitter and conduction systems mature at variable rates (i.e.: serotonin and noradrenalin differ)					
Skeletal	• Growth plates present at birth • Most rapid postnatal growth occurs prior to weaning, followed by slower growth until growth plates close during adulthood					

	Major period of functional and structural growth and development
	Completion of structural development; active period of growth and/or functional maturation
	Slow continued growth or refinement of function; also can reflect a period of relative inactivity, as in prepubertal reproductive tissues
	Structurally and functionally fully mature

Figure A.6. Comparison of Rat and Human Ontogeny

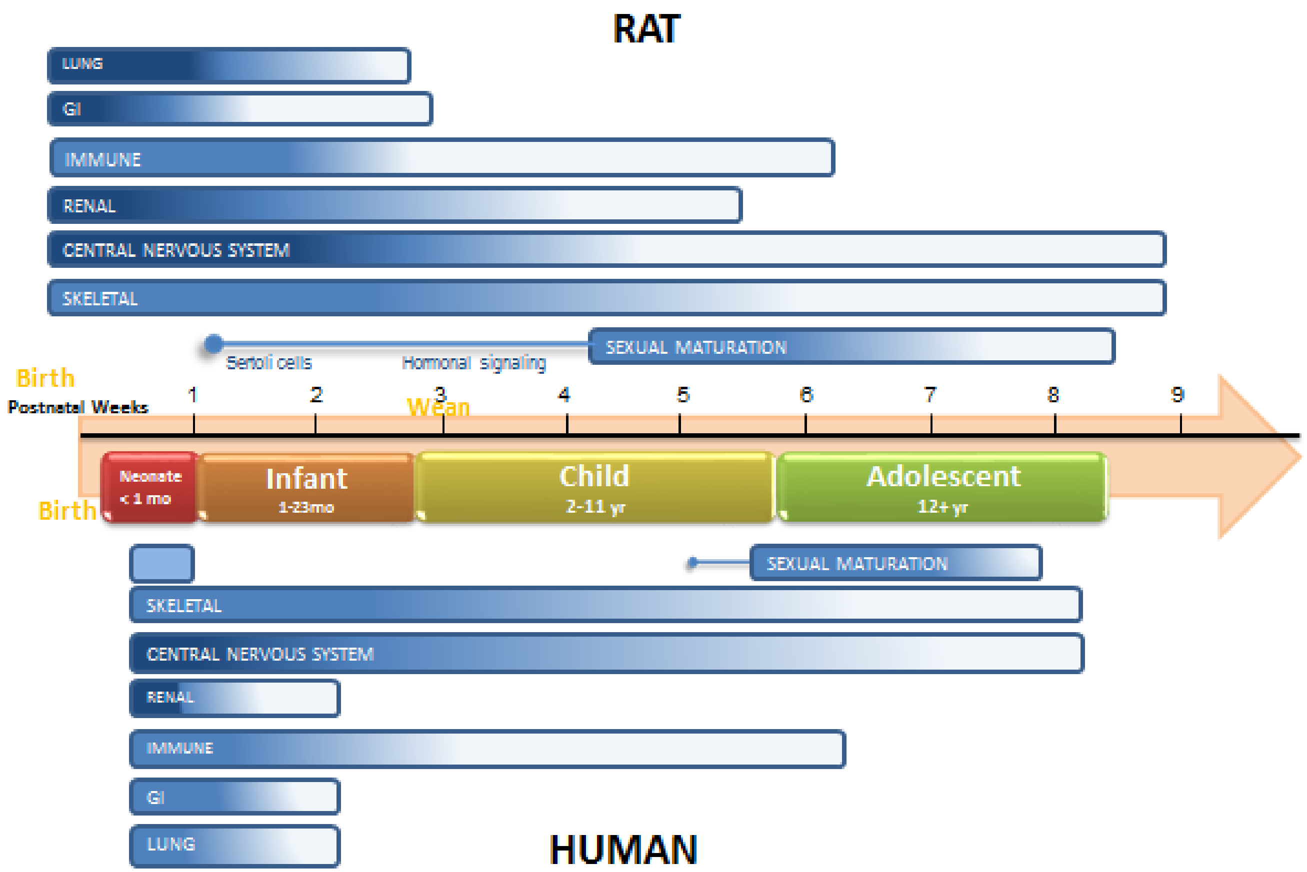
RAT
LUNG
GI
IMMUNE
RENAL
CENTRAL NERVOUS SYSTEM
SKELETAL
Sertoli cells
Hormonal signaling
SEXUAL MATURATION
Birth
Postnatal Weeks
1
2
3
4
5
6
7
8
9
Wean
Neonate
< 1 mo
Infant
1-23mo
Child
2-11 yr
Adolescent
12+ yr
Birth
SEXUAL MATURATION
SKELETAL
CENTRAL NERVOUS SYSTEM
RENAL
IMMUNE
GI
LUNG
HUMAN

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Table A1. Principal Advantages and Disadvantages of Various Mammalian Species for Use in Juvenile Animal Studies

Species	Advantages	Disadvantages
Rat	● Well-studied species in juvenile animal studies with extensive historical control data ● Several consistent developmental milestones (general growth, preputial separation/vaginal opening, puberty) ● Often used for (adult) general and reproductive toxicology ● Body size allows most manipulations/administrations starting early preweaning ● Litter size allows allocation of pups to different endpoints and dedicated cohorts of pups ● Compressed development (~10 weeks) allows for inclusion of wide range of endpoints during the short period ● Compressed development allows for inclusion of endpoints which are difficult to perform using large animals (such as FOB, developmental neurotoxicity, immunotoxicity, fertility/breeding) due to longer developmental period ● Compressed development allows for inclusion of nonstandard endpoints if warranted (FOB, developmental neurotoxicity, immunotoxicity, fertility/breeding) ● Small body weight requiring low amount of test material ● Relatively easy transportation, housing and management ● Pups and dams are amenable to fostering ● Easy to obtain many pups with the same postnatal stage	● Small body size, high metabolic rate and rapid growth can lead to fast decline in general condition and death. ● Several organ systems are less developed at birth relative to man (particularly CNS, lung, kidney, GI tract and immune system; eyes do not open until PND 12-14) ● ADME characteristics of oral pharmaceuticals given in the preweaning phase often translate poorly to humans due to immaturity of the GI tract ● Compressed development can make it difficult to identify distinct windows of vulnerability ● Conventional blood samples are often terminal collections, particularly preweaning ● Can easily become very large studies as most endpoints or collections require dedicated cohorts of pups
Mouse	● Generally similar to rat, some differences may make mouse a better model for specific organ systems ● Many genetic modification models available	Similar to rat, additionally: ● Allows fewer manipulations /administrations than rat from early on ● Requires dedicated cohorts of pups for each endpoint or collection and can require sample pooling ● Less historical information than the rat.

Species	Advantages	Disadvantages
Dog	• Often used in general (adult) toxicology • Relatively large at birth • Relatively easy to handle • Litter size allows allocation of pups to different endpoints • Puppies can be separated from dams for a few hours • Breeding can be planned in advance	• Protracted development (~7-14 months to sexual maturity, ~18-24 months to skeletal maturity) with variable developmental milestones • Altricial at birth (i.e. eyes do not open until ~ 2 weeks postnatally) • Variable litter sizes and sex distribution can make it difficult to populate study with minimal bias (genetic/litter, sex distribution) across groups • Limited historical background data, especially for nonstandard endpoints • Substantial inter-individual variability in growth and development • Seasonal breeder (supply & study start over weeks or months) • Not amenable to fostering • Large body size requires comparably large amounts of test compound compared to rodents
Minipig/ Pig	• Many similar developmental milestones as humans • Relatively large at birth • Relatively easy to handle • Breeding can be planned in advance • Litter size allows allocation of piglets to different endpoints • Amenable to cross fostering • Relatively large litters usually allow balanced sex distribution • Neonatal GI tract similar to human for orally administered drugs • All routes of administration feasible (except inhalation); best model for dermal studies • Short development (~6-9 months), relatively easy transport and housing compared to other large non-rodents	• Less well established historical control data than dog or NHP toxicology species • Require colostrum for passive transfer of maternal Ig in perinatal period • Large body size requires comparably large amounts of test compound compared to rodents • IV and gavage administration can be challenging in very young piglets
Species	**Advantages**	**Disadvantages**

NHP (cynomolgus; rhesus and marmoset also feasible)	● Many similar developmental milestones as humans ● Neonates/infants similar to human for GI tract, immune system, cardiovascular, renal and special sense (eye, ear) development ● Macaque infants are relatively large at birth ● Extensive reference data from birth available ● Often used for (adult) general and reproductive toxicology (e,g., ePPND), especially for biopharmaceuticals ● Often the most pharmacologically relevant animal model for highly targeted therapies	● Protracted development (~3-6 years for sexual maturity, ~5-8 years for skeletal maturity in macaques) makes an extensive juvenile study to cover all developmental phases not practical ● Single offspring for macaques with high inter-individual variability in growth and development ● Marmosets typically have twins and require both maternal and paternal care in preweaning phase; offspring are relatively small ● Offspring highly dependent on maternal care over first month (minimal procedural intervention recommended; pre-weaning manipulation & dosing feasible with risk of maternal rejection), and are cohoused with dam for first 3-6 months; with shipping and quarantine requirements it is rarely feasible to initiate studies in juvenile monkeys < 9 months of age ● Neonatal NHP are precocious relative to human neonates in terms of musculoskeletal, CNS, endocrine and respiratory system ● Cannot synchronize breeding (supply & study start over weeks or months for seasonal breeders such as rhesus) ● Ethical reservations (need strong rationale to justify use of juvenile NHP for toxicity testing)
Rabbit	● Compressed development (~5-6 months) and small body size requiring comparably low amount of test material ● Relatively easy to handle ● Often used for reproductive toxicology; also can be used for ocular administration, evaluation of bone growth ● Litter size allows allocation of kits to different endpoints ● Relatively easy transport and housing	● Developmental milestones less well established than other nonrodent species ● Not routinely used / well accepted in (adult) general toxicology ● Handling young offspring can provoke cannibalism or rejection by the mother

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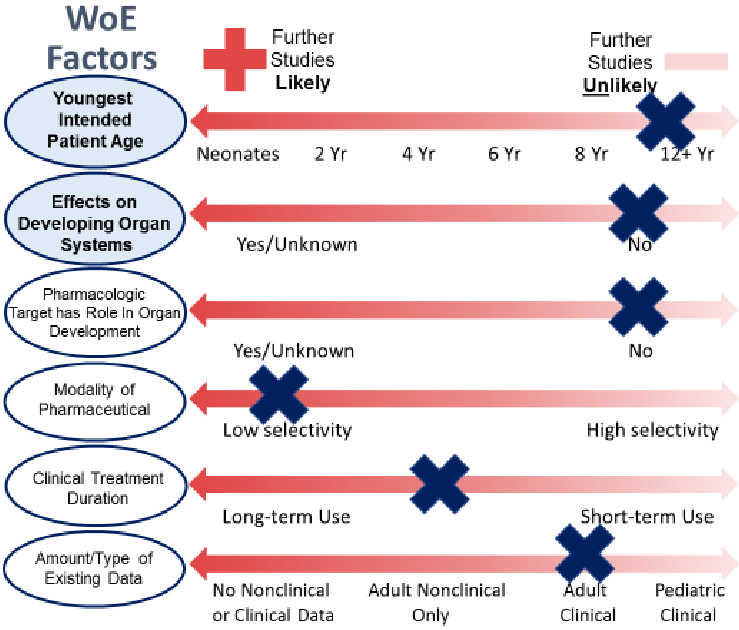
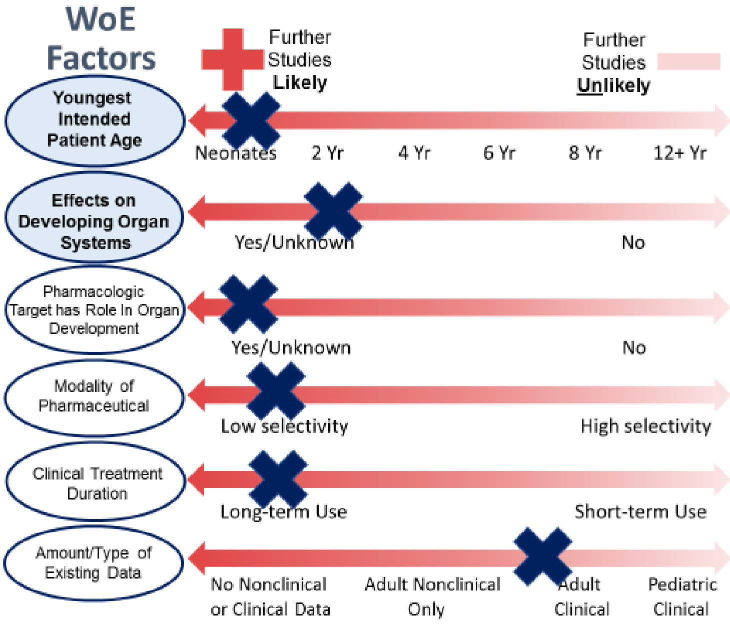
Other Species	Other species could be considered for cause when pharmacologically and toxicologically relevant. Examples of alternative mammalian test systems include the hamster, guinea pig, tree shrew, ferret, cat, sheep and goat. Advantages tend to be species and program specific, but often reflect use of that species in genetic or disease models, or when there is data supporting interpretation and translatability of specific endpoints. ● Developmental milestones less well established than in rat, mouse, dog, minipig/pig and NHP ● Not routinely used / well accepted in (adult) general toxicology ● Limited historical control toxicology data ● Limited use (model in special indications such as heart failure) ● Many require colostrum for passive transfer of maternal Ig in perinatal period ● Limited availability of purpose-bred animals and suitable laboratory housing

APPENDIX B: CASE STUDIES APPLYING THE WEIGHT OF EVIDENCE APPROACH

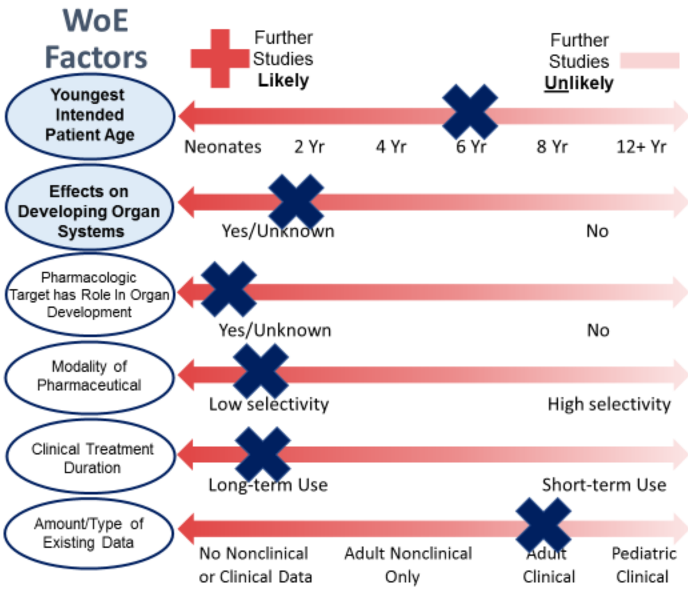
A. A small molecule with known pharmacology has available adult clinical and nonclinical data including repeated dose toxicity data. None of these data suggest a safety concern in a developing organ for the intended paediatric population of adolescents (12 years and above), for a one-month duration of clinical treatment. The WoE analysis indicates that no additional nonclinical investigations are needed.



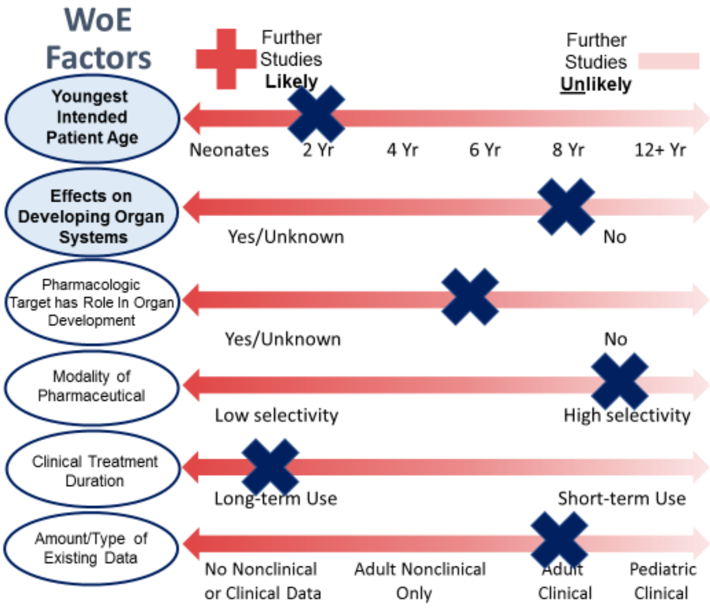
B. A small molecule with a novel mode of action intended for chronic use starting in neonates or infants has limited Phase 1 clinical and nonclinical safety data with no significant safety concerns identified. There are potential pharmacologic effects on developing organ systems. The WoE analysis indicates further nonclinical investigation, such as a core JAS with additional endpoints based on the targeted developing organ systems, would be useful.



C. A small molecule with known pharmacology with a well characterized critical role in CNS development intended for chronic use in children (6 years and above) has nonclinical and adult clinical data. The concern for a potential effect on the developing CNS cannot be addressed clinically by monitoring and management. Existing data adequately addresses other developing systems. The WoE analysis warrants a post-weaning JAS study design that includes core endpoints and additional endpoints limited to CNS, including detailed clinical observations, behavioral assessments, a learning and memory evaluation, and expanded neuropathological examinations.



D. A monoclonal antibody targets a soluble cytokine and is intended for chronic paediatric use in rheumatologic and allergic diseases (>2 years old). The only findings are reversible decreased serum Ig and occasional injection site reactions (in both animals and adult patients). In a monkey ePPND study, offspring exposure was comparable to dams through PND 28 and decreased pharmaceutical Ig levels was detected on PND 28 and 56 postnatally. T-cell-dependent antibody response (TDAR) results were similar to controls (between 3-6 months postnatally). The WoE analysis does not warrant a JAS.

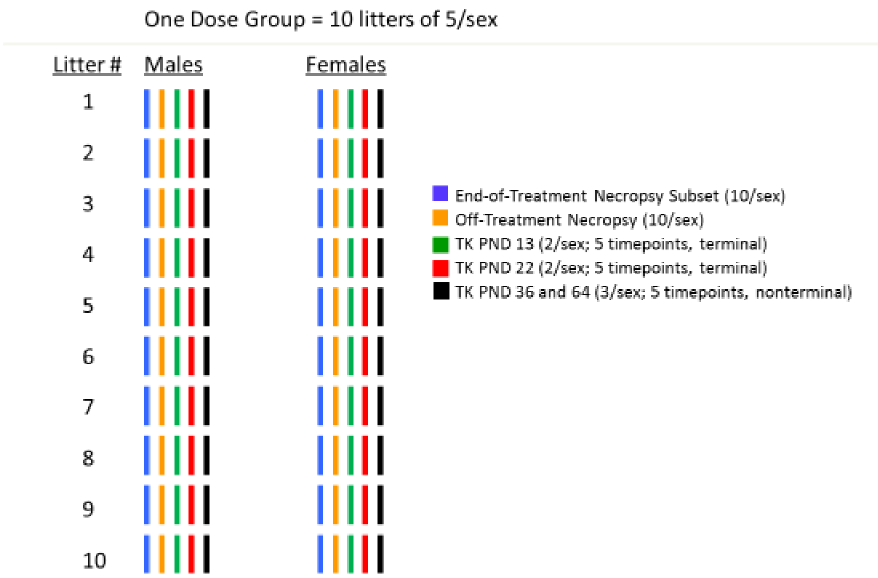


APPENDIX C: EXAMPLE OF AN APPROACH TO RODENT PREWEANING LITTER ALLOCATION:

Natural Litters + Whole Litter Group Assignment + Inter-Litter Endpoint Subset Assignment

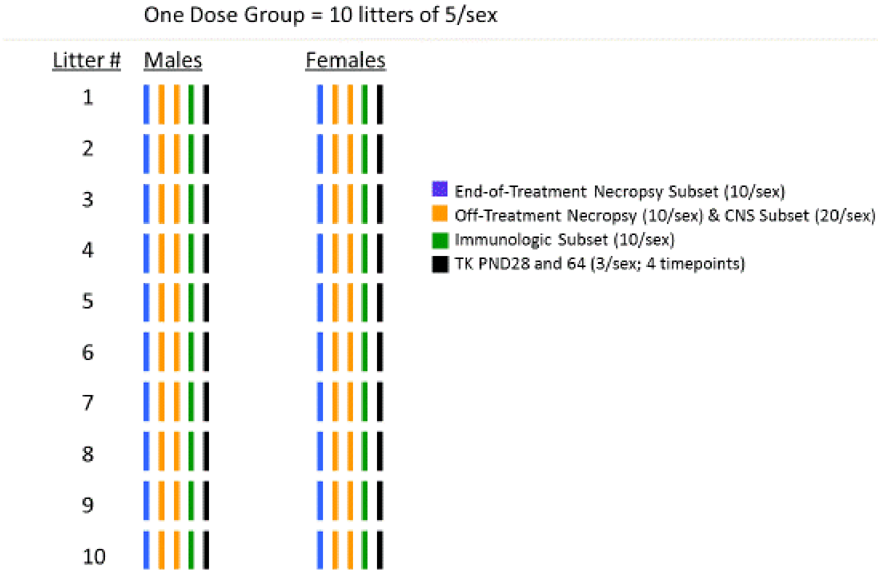
Initiation of a JAS during the preweaning phase presents a unique situation and should be designed to reduce potential confounders related to genetics, maternal care, and littermates. This is achieved by how the litters are constructed in combination with how they are assigned to dose groups, and then to subsets of endpoints. In this approach, the offspring stay with their natural mother and are culled to the desired litter size with a balanced sex ratio. When necessary to minimize the required number of litters to supply the study, a very small percentage of pups are fostered to other litters. Here, Wistar Han rat litters are culled to 10 offspring per litter composed of 5 males and 5 females (the mean natural litter size is ~11). The whole litter is then assigned to the same dose group with 10 litters each assigned to each dose group. Offspring are arbitrarily assigned to subsets for specific endpoints in an inter-litter fashion, i.e., as one male or female from each litter in a dose group to the specific endpoints. The advantage of the whole litter group assignment is the littermates receive the same dose level so there is a low risk of cross contamination and confounding variables of high dose and control offspring competing for suckling position and time. Also, keeping the pups with genetic dams and assigning the endpoints in an inter-litter fashion ensures genetic, maternal care and littermate influences are distributed evenly.

Example A



For Example A, the definitive JAS design includes the core assessments with the only additional assessment of off-treatment/recovery necropsy. The pups are allocated 1/sex/litter for n=10/sex for the end-of-treatment necropsy subset which would also have sexual development, clinical pathology, and long bone length. TK is collected frequently based on dose range data with two sets of 1/sex for TK on PND13 and 22 (composite terminal sampling) and 1/sex for postweaning TK collections which are nonterminal. Microsampling minimizes animal use. In this case, dosing starts on PND 7 and the first TK sampling after the first dose would be collected from separate dams and litters available after randomization, because litter and maternal cofounders would not be relevant for a single dose TK assessment.

Example B



For Example B, the definitive JAS design includes the core assessments and additional assessments of off-treatment/recovery necropsy, full CNS assessments and immunologic assessment and dosing from PND 9 to 63.   The pups are allocated 1/sex/litter from each litter for n=10/sex for the necropsy (with expanded neuropathology) and immunologic (TDAR) subsets each; and 2/sex for the subset for CNS testing (clinical observations, behavior and learning and memory) using half of these also for the off-dose necropsy obviating the need for extra animals, and 1/sex for postweaning toxicokinetic (serial sampling).   TK sampling after the first dose would be collected from separate dams and litters available after randomization, because confounders would not be as relevant for single dose TK assessment.