Juvenile Toxicology
- Towards Safe and Effective Paediatric Medicines
Non-Clinical Juvenile Studies

**WHY?**

Given the global consensus on the need for paediatric clinical trials, the use of appropriately designed juvenile animal studies has been recognised as valuable in identifying age-related toxicities that cannot be predicted from existing non-clinical or adult human data. These studies will therefore support dose selection in children and help to bridge the gap between adult and paediatric clinical trials.

**WHEN?**

In order to support paediatric clinical trials, guidance documents on the design and conduct of non-clinical juvenile studies were released by both the FDA and EMA in 2006 and 2008, respectively. Furthermore, in 2009, the ICH M3 (R2) guideline was issued providing further insight into the necessity and timing of such studies; indicating that, where non-clinical juvenile studies are considered necessary, they should be completed prior to long-term paediatric clinical trials and may even be required prior to short-term, multiple dose efﬁcacy studies in the paediatric population. The emphasis being that their timing in relation to the clinical trials should take multiple factors into account such as the therapeutic indication or age of the intended trial population.

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There is unequivocal evidence demonstrating differences between drug safety proﬁles of children and adults with many classical examples such as that of the gray baby syndrome with Chloramphenicol¹ or increased risk of Reyes Syndrome following Aspirin administration to children with Influenza or Varicella infections². Much of this knowledge has been gained from adverse events resulting from the high risk but common practice of off-label paediatric prescribing: administering pharmaceuticals to children outside the terms of the product licence. In 2003, it was estimated that around 75% of marketed drugs were unlabelled for use in children³.

In Europe, the matter was addressed in a similar manner by the passing of the EU regulation No 1901/2006 (and amendment 1902/2006), detailing the requirement to investigate all new pharmaceuticals for children with appropriate clinical trials, as outlined in a Paediatric Investigation Plan (PIP) beforehand. This regulation also provides 6 months extension of the Supplementary Protection Certificate, as an incentive for registering the product for pediatric use.

The bottom line is that, unless there is clear justification to waive a paediatric programme, it is compulsory to conduct one for marketing authorisation of any new pharmaceutical.

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¹ Gray Baby Syndrome
² Chloramphenicol
³ Aspirin
⁴ Pediatric Research Equity Act (PREA)
⁵ Pediatric Rule
⁶ Best Pharmaceuticals Act (BPCA)
⁷ Paediatric Investigation Plan (PIP)
⁸ Supplementary Protection Certificate
⁹ Pediatric Research Equity Act (PREA)
¹⁰ ICH M3 (R2) guideline
¹¹ Pediatric Rule
¹² EU regulation No 1901/2006 (and amendment 1902/2006)
Study Design: points to consider

To ensure that juvenile animal studies provide sufficient and relevant safety data to support the proposed paediatric clinical trials, it is imperative that a considered approach is taken on the basis of sound scientific judgement. Specialist scientific expertise in this area is essential and a ‘one study fits all’ approach will not suffice, given the intricacy of organ and system development in juvenile animals and humans. There are a number of key points which need consideration:

- Therapeutic area
- Target organ toxicity and impact on developing systems
- The proposed paediatric clinical regime
- Pre-existing human and adult/prepubertal animal data
- Route of administration
- Metabolic and secretory route of the compound
- Most appropriate species

Answering these questions is vital in determining the final study design and will affect the age at the start of dosing, dose duration and end points assessed. The timing of dosing should be selected to span any likely affected development phase and match the age range of the target paediatric population, whilst taking any temporal species differences into account. For example, the developmental period for nephrogenesis is pre-natal in the human but in the rodent this is not complete until post-natal Day 11, with the kidney only becoming anatomically complete between 4 to 6 weeks after birth.4

Although studies should always be considered using a case-by-case approach, these are some key end points which should routinely be included:

- Age in Man5
- Equivalent age in the rat (on the basis of CNS and reproductive development)6

| Term / new born Infants (0 to 27 days) | 0 to 10 days |
| Infants / Toddlers (28 days to 23 months) | 10 to 21 days |
| Children (2 to 11 years) | 21 to 45 days |
| Adolescents (12 to 16/18 years) | > 45 days |

5. Age in Man and Equivalents in the Rat (2013). Sequani
6. Equivalent Age in the Rat (2013). Sequani

As with adult toxicity studies, body weights, food consumption, clinical signs, exposure data, organ weights and gross and microscopic pathology examinations are of key importance, as is the assessment of reversibility of any adverse findings. With juveniles it is often also important to assess overall effects on growth, attainment of sexual maturity (usually with the inclusion of a mating phase) and neuro-behavioural observations. There may also be instances when clinical pathology and immunotoxicity prove informative. Sequentia requires comprehensive pathology expertise and extensive experience in the pathology and interpretation of data in juvenile animals.

Sequentia has over 30 years of experience working with the neonate and juvenile rat and is therefore well qualified to handle studies of this type.

Study Design: looking for the answers

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Over 30 fully managed FIH development programmes in the past 5 years.

Our clients benefit from stronger customer service, greater flexibility and a faster response. In other words, we offer better value for money.
Paediatric investigation plans

In January 2007, the EU brought into effect a new Paediatric Regulation designed to improve the availability of safe, appropriately researched medicines for children.

As part of this regulation, new medicinal products intended for marketing within the EU must be evaluated for use in paediatric populations before marketing authorisation will be granted, unless paediatric use can be waived on the grounds of therapeutic indication or class.

For products with potential for paediatric use, a Paediatric Investigation Plan (PIP) must be submitted, evaluated and agreed with the EMA’s Paediatric Committee (PDCO) in advance of applying for marketing authorisation. The PIP should contain information on all elements of the paediatric development, including:

- the pharmacology/safety/pharmacokinetics of the product,
- proposed age-appropriate formulations (and CMC-related quality aspects),
- designs and rationale for non-clinical and clinical studies,
- timelines for the project.

The PIP must take into consideration all paediatric subsets, and provide appropriate justification for the exclusion of any age groups.

TIMINGS

The timing of PIP submission is open to interpretation by the applicant, and is an area of much discussion within the industry. The Paediatric Regulation states that the PIP (or waiver request) should be submitted no later than the completion of human pharmacokinetic studies, which most applicants are interpreting as the completion of Phase II studies when PK data in the adult patient population is known.

The time taken from PIP submission through to formal agreement is typically around 6-8 months, with time allowed for the applicant to review and address comments from the PDCO.

Once agreed with the PDCO, the PIP is binding and any unapproved changes or non-conformity could result in delays to the overall MAA.

HOW SEQUANI CAN HELP

At Sequani we have helped many customers in the design of non-clinical programs required to support clinical studies in children. This experience, together with our clinical track record means we are also well placed to assist you in PIP production for submission to the paediatric committee.

Hence it is critical that the timing and approach of the paediatric clinical program is justified, given the unique nature of the non-clinical juvenile studies it is also important to elucidate their design within the PIP outlining the scientific basis and how the data should support the clinical program.

INTRODUCTION TO DRUG DEVELOPMENT

CANDIDATE SELECTION

Development

Consultation with regulators: PIP modification

Integration of Paediatrics into Drug Development

Consultation with regulators: PIP modification

Initial PIP in EU

Final PIP

ISA (incl. paediatric data)

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Sequani experience in Juvenile Toxicity

Juvenile toxicity assessments have been performed at Sequani since 2000 and over this time we have developed significant experience in the design, conduct and interpretation of these specialist studies. This, associated with our extensive experience of conducting pre- and post-natal studies makes us a world leader in this area of toxicology.

With more than 60 regulatory juvenile studies to our name, we are uniquely placed to provide authoritative, top quality scientific and practical support. Our specialist teams include a study director team cross-trained in reproduction and general toxicology, and a toxicology team that can advise on study design within the context of European and USA regulatory requirements. After more than a decade’s experience, we are particularly adept at pragmatic management and scheduling. This means that, whatever a study’s scope, size and challenges, we have the skills and ability to ensure it runs smoothly and on time.

Non-rodent alternatives

Whilst the majority of Juvenile Toxicity studies are currently conducted in the rat there are occasions where either the rat is not a suitable model or the regulators request that the study is undertaken in a higher species. Sequani is one of the few CROs to offer juvenile toxicity studies in the minipig.

LANDMARK INITIATIVES

Pioneering a cross-fostering approach to animal supply for juvenile studies, delivering improved data quality whilst reducing total animal use by up to 65%.

- Research project to develop microsampling techniques and bioanalytical methodology to reduce animal use in juvenile rat studies. Results published in the International Journal of Toxicology.
- Development of an extensive collection of comparative pathology data in juvenile rats and minipigs at a range of ages, including scanned digital images for training and reference use.
- Validation of toxicity studies in juvenile minipigs for regulatory submission, with continuing research projects to further develop the model’s utility.
- Collaborative research leading to enhanced understanding of toxic effects in the developing juvenile rat brain.

The minipig offers significant advantages over other non-rodent models:

- Good litter size,
- Ability to dose from a very young age (Day 1 by oral gavage),
- Fewer emotive issues compared with the use of juvenile dogs or NHPs,
- Cross fostering possible from Day 1 of age and,
- Early sexual maturity possible (4 to 5 months).

<table>
<thead>
<tr>
<th>Age in Man</th>
<th>Equivalent age in the minipig on the basis of CNS and reproductive development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term / new born Infants (0 to 27 days)</td>
<td>0 to 4 weeks</td>
</tr>
<tr>
<td>Infants / Toddlers (28 days to 2.5 months)</td>
<td>2 to 6 weeks</td>
</tr>
<tr>
<td>Children (2 to 11 years)</td>
<td>4 to 14 weeks</td>
</tr>
<tr>
<td>Adolescents (12 to 15/18 years)</td>
<td>&gt;14 weeks</td>
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As the stage of target organ development is an important consideration when designing a Juvenile Toxicity study and yet only limited data were available in this area, Sequani undertook a research project to document the development of juvenile rat organs from Day 4 of age to Day 35.

The study looked at a total of 28 tissues, examples of which are given in the table opposite.

### Juvenile organ development at Sequani

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The study looked at a total of 28 tissues, examples of which are given in the table opposite.

### Upcoming publications/presentations on juvenile toxicology

For details of relevant Sequani publications and events please visit our website:

[www.sequani.com](http://www.sequani.com)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Status at birth and early development</th>
<th>Age to maturity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Anatomically complete, haemopoiesis marked at birth, disappears rapidly. The perinatal period is the time at which a marked increase in P-450 occurs in hepatocytes throughout the liver lobule.</td>
<td>Adult levels in rodents by day 45.</td>
<td>P450 levels 50-80% of adult levels at 1 year. The period before weaning is the time at which the sublobular heterogeneous distribution of P-450 appears. The period after weaning is the time at which a slight increase in P-450 content in perportal hepatocytes and a marked increase in the enzyme in centrilobular hepatocytes takes place.</td>
</tr>
<tr>
<td>Brain</td>
<td>Not fully developed. Neuroendocrine development takes place in utero in man (complete by 7 months in utero) but is post-natal in rats. Glial genesis initiated at birth and continues at a high rate till day 45.</td>
<td>Half of the cell population of the cerebrum forms in the first 3 weeks of life. Brain weight a good general indication of development.</td>
<td></td>
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<tr>
<td>Lungs</td>
<td>No true alveoli, smooth walled air channels and sacculae only.</td>
<td>3 weeks. Lung volume increases fourfold in the first 3 weeks and continues to grow for around 5 months. Development is in three phases. Expansion (birth-day 4), tissue proliferation (day 4-13) and equilibrated growth (3 weeks - 5 months).</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>Little or no white pulp. Immunologically functional by day 14, although peak function is achieved at puberty.</td>
<td></td>
<td>Lymphocytes appear in perivascular tissue around day 2. T cells and monocytes precursors of interdigitating cells occupy PALS by day 5.</td>
</tr>
<tr>
<td>Adrenal</td>
<td>Cortex fully functional. Rapid growth occurs in the early post-natal period.</td>
<td>1 week. Final stages of medullary maturation dependent on splanchnic innervation.</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>Well differentiated at birth. Weight doubles from 3-6 gm in week 1.</td>
<td>Weight stabilises at around 20 weeks. Development of many organs heavily influenced by circulating levels of thyroid hormones.</td>
<td></td>
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<tr>
<td>Kidney</td>
<td>Still developing. Proximal tubule appears the slowest at around 40 days. Number of glomeruli more than double in the first 3 weeks of life.</td>
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Everyone will tell you they try to build trust with their clients, but at Sequani we really feel we’re getting it right. It’s what drives everything we do.

5) ICH Guideline E11. Clinical Investigation of Medicinal Products in the Paediatric Population; finalised (Step 4, July 2000)