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ICH S11 and its impact on Neonatal/Juvenile Toxicity Study Designs

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Agenda

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Introduction

- Since they first became a part of the drug development program, and despite various regional guidelines/guidance documents being issued, the design of pre-clinical neonatal/juvenile toxicity studies has posed our industry problems.
- With so many possibilities for endpoints for inclusion in these studies and in the absence of clear instruction in the guidelines/guidance documents of what the regulatory agencies really wanted, the most common approach taken was to include everything you possibly could … just in case.
- Finally, after over 5 years of discussion, deliberation and review, ICH S11 was adopted by Regulatory Members of ICH Assembly at Step 4 in April 2020, and came into force in Europe at Step 5 on 26 September 2020, so we now have one globally accepted guideline with strong emphasis placed on taking a weight of evidence approach to the design of these studies and the endpoints for inclusion.

Challenges of neonatal/juvenile toxicity studies

- Pre-clinical neonatal/juvenile toxicity studies pose some unique challenges that do not exist in adult toxicity studies
- Children are not just small adults; they need to be considered as a separate entity due to degree of organ system maturity and the rapid changes they undergo, so understanding organ system maturation is critical, and the possibilities for endpoints for inclusion in study designs are almost endless:
  - Routine toxicity parameters?
  - Satellite group needed for bleeds?
  - Behavioural assessments?
  - Reproductive assessment?
  - Immunology assessment?
  - Recovery?
- "Standard designs" do not really exist. The key is to tailor design according to test material class, known effects in adult pre-clinical studies, age of target population, duration of patient treatment etc; this provided an unprecedented opportunity to work closely with our customers.

Challenges of neonatal/juvenile toxicity studies (cont'd)

- Children are not "miniature adults"; predicting responses in children based on adult data can be difficult and unreliable
- Known cases of different sensitivity between children and adults; examples quoted in US FDA guidance (2006):
  - **Chloramphenicol** (broad spectrum antibiotic): Associated with "Grey baby syndrome" in premature babies because of limited clearance » exposure increased due to a longer T½ (26 h) compared to adults (4h)
  - **Valproic acid** (anti-convulsant - epilepsy): Young children treated with VPA appear disproportionally vulnerable to fatal hepatotoxicity
  - **Aspirin:** Increased susceptibility due to abnormal fat accumulation in liver and other organs, and severe increase in intracranial pressure (Reye's syndrome)
- Post-natal growth and development can affect drug disposition and action:
  - Metabolism (maturation rate of Phase I/II enzyme activities)
  - Body composition (water and lipid partitions)
  - Receptor expression and function
  - Growth rate
  - Organ functional capacity
    - These are all susceptible to modification or disruption by drugs

Evolution of neonatal/juvenile toxicity studies

- Until ~17 years ago, targeted pediatric drug development significantly lacking – extensive “off-label” use
- Problem:
  - Assumes that pediatric patients will exhibit similar disease progression and respond similarly to the intended therapeutic intervention as adult patients
  - Estimated 50% - 90% of drugs **never** specifically evaluated for pediatric use
- How can we be sure that adult/pediatric toxicity profiles are the same?
  - Inherent differences between mature and immature systems means that there is a risk of:
    - Unique toxicity profile in children
    - Poor efficacy
    - Exaggerated pharmacology
    - Unexpected adverse effects, even death

Evolution of neonatal/juvenile toxicity studies (cont'd)

- 2002 – EU consultation paper: "Better Medicines for Children" – proposed new legislation
- FDA issued formal guidance in 2006: "Guidance for Industry – Non-clinical Safety Evaluation of Pediatric Drug Products"
- EMA guideline issued in 2008: "Guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications"
- Oct 2012: Japanese MHLW guideline issued: "Guideline on the Non-clinical Safety Study in Juvenile Animals for Pediatric Drugs"
- 2015: Discussions began to create an ICH guideline – ICH S11 Guideline came into force in Europe on 26 September 2020
- Drug development programs for a pediatric population have to take into consideration possible effects on developmental processes specific to the relevant age groups

Development Comparisons

- Where Does a Child Begin and End?

Pre-term or Newborn	Infant < 1 month	Infants/Toddlers (1 m - 1 year)	Children/Adolescents (>2 yrs)
+ Unique pathophysiology	+ Blood-brain barrier (BBB) immature	+ Developing CNS, immune system, growth	+ Mainly growth phase
+ Difficult to extrapolate from adults/children	+ Altered pharmacodynamics	+ Altered pharmacodynamics	+ Developing repro system
	+ Less predictable absorption		+ ↑ compliance issues

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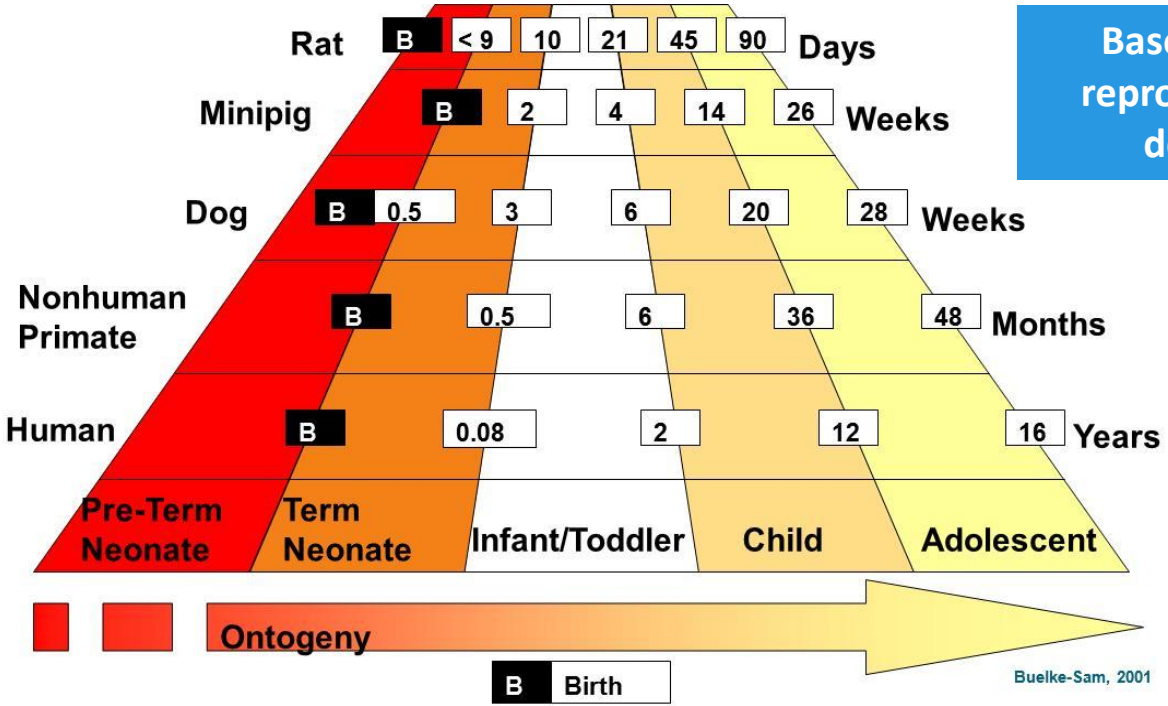
Development Comparisons (cont'd)

- Comparative Age Categories



Based on CNS and reproductive system development

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Study Design Considerations

- Each study is uniquely tailored according to the test material, target population, organ system of interest and duration of use
- Age of animals at start of dosing
  - To match lowest age of target patient group (several regulatory requests to start even younger)
- Duration of dosing period
  - To cover developing organ system(s) of interest
  - To cover target pediatric groups (age range)
  - To reflect planned clinical use (e.g., single dose)
- Number of animals
  - Determined by age at start of treatment and investigations required
  - Pre-weaning: some dose routes = risk of dosing trauma

Study Design Considerations: That Was Then….

- EMA and FDA: They did not always agree!
- Although the vast amount of terminology or “you may wish to consider” was the same with EMA and FDA, the interpretation of the guidelines/guidance documents was often quite different at the two agencies. In particular:
  - If dosing required to “adulthood” in rats, FDA requested to 13 weeks of age but EMA only required to 10 weeks of age
  - If CNS active drug, EMA accepted a rat study only to cover all CNS endpoints, but FDA preferred a rat study for neurobehaviour and a dog study for neuropath.
- Yet if a customer submitted their PIP with a proposed design to one agency and it was accepted, the other would accept it too, despite their particular preferences!
- And early on, whilst these studies were new, everyone was learning about them and “data gathering” was ongoing, the approach was very much “throw everything you can at it … because you can” despite what the guidelines/guidance documents said!

Study Design Considerations: This is Now ….

- With ICH S11, finally, we have a single driving guideline, and it's focus is on taking a "weight of evidence" (WoE) approach, both in terms of the need to actually perform pre-clinical juvenile studies, and, if they are needed, what their scope should be (duration, endpoints, phases etc).
- A small Dose Range Finding study is recommended, especially if dosing commences pre-weaning.
- In most cases, a single species is sufficient.
- Clearly defines core endpoints, and additional endpoints to address identified concerns, based on the type and strength of the concern based on the WoE evaluation.
- Encourages taking all available information into consideration to essentially perform the shortest duration and smallest scope study you can, whilst still yielding meaningful results.

Study Design Considerations: ICH S11: Core Endpoints

- Growth (bodyweight and limb length/growth)
- Development (external indices of sexual maturation)
- Survival and Clinical/post-dosing signs
- Food intake (post-weaning only)
- Haematology [not coagulation] and blood chemistry, if evaluation planned at an age where ranges are known and can support histopathology findings
- Organ weights
- Macropathology/micropathology investigations
- Reversibility
- Toxicokinetics (critical endpoint)

Study Design Considerations: ICH S11: Additional Endpoints to Address Identified Concerns

- Neurotoxicity and immunotoxicity assessments:
  - Triggered if the class of compound or previous studies in humans or animals give cause for concern
  - Behavioural tests usually in recovery, but can be evaluated during treatment (generally for CNS active drugs)
- Reproductive function:
  - If positive signal on fertility study (ICH 1.1.1) or among F1 selected animals on pre- and post-natal study (ICH 1.1.3), histopathological changes in male/female reproductive organs in adult studies or delays in attainment of puberty
  - Needs group size of 16+ (ICH 1.1.1 – 1.1.3 Repro guidelines)
  - Usually only assessed after a period of recovery

Study Design Considerations: ICH S11: Additional Endpoints to Address Identified Concerns (cont'd)

- Other growth endpoints
  - Crown rump length, body length (nose to tail), withers height of a dog
- Bone assessments: bone mass and geometry, extra biomarkers of bone formation and resorption, bone histomorphometry
- Additional clinical pathology and/or biomarkers including coagulation and urinalysis
- Anatomic pathology: immunohistochemistry, electron microscopy, imaging
- Ophthalmoscopy examinations: routinely not included because structural development of the eye is largely completed during the prenatal period in humans

Study Design Considerations: “Basic” study

- Example: To provide cover for 1 month old babies and older, dosing of rat pups could start on Day 7 of age

Phase	Typical group size
Toxicity	12M+12F
Recovery/reversibility/off dose	6M+6F
Single dose Toxicokinetic phase ## (6 x TP; 3 samples / TP in treated groups)	18M+18F in treated groups; 6M+6F in Controls

Will be required if bioanalytical method is not compatible with microsampling: up to two 35 µL non-terminal blood samples can be obtained from the tail artery of juvenile rats on Day 7 of age, when samples are taken at least 2 hours apart

Study Design Considerations: Inclusion of Neurotoxicity Evaluations

- US FDA CDER Guidance 2006:
  - "For Developmental neurotoxicity assessments, well-established methods should be used to monitor key central nervous system functions, including, assessments of reflex ontogeny, sensorimotor function, locomotor activity, reactivity, learning and memory."
- A neurobehavioral testing battery could be:
  - Ontogeny of Air righting reflex from Day 14 of age
  - Auditory startle response and pupil closure reflex on Day 20 of age
  - Post weaning automated motor activity monitoring
  - FOB – In The Hand and Arena observations
  - Learning and Memory – Morris water maze place task
  - Automated auditory startle response pre-pulse inhibition

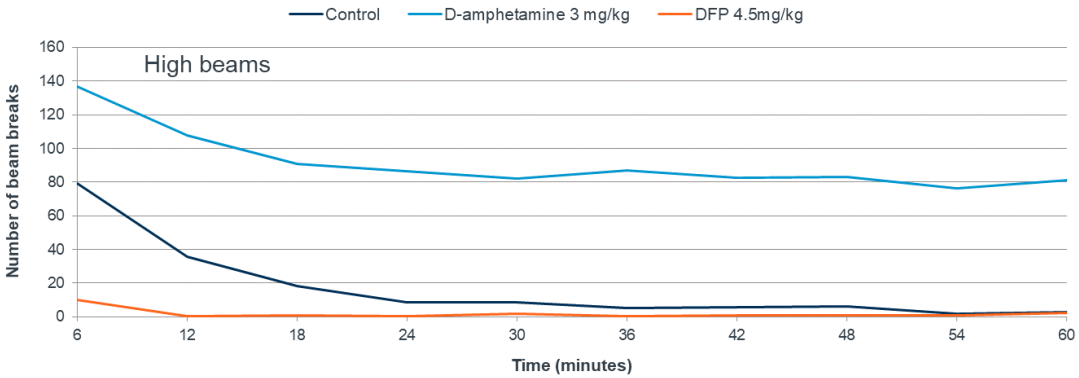
Study Design Considerations: Inclusion of Neurotoxicity Evaluations (cont'd)

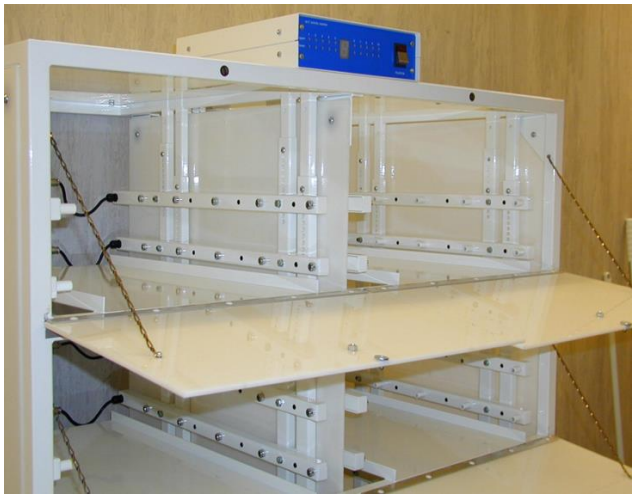
- Group size:
  - Suggest should be 20m & 20f to match group size on the pre- and post-natal study (ICH S5(R3) Annex 1: 1.1.3) for medicinal products and the group size for most of the neurobehavioral tests on the OECD 426 Developmental Neurotoxicity study for chemicals – gives strong statistical power

Phase	Typical group size
Toxicity	12M+12F
Recovery/reversibility/off dose	20M+20F
Single dose TK phase (6 time points; 3 samples per time point in treated groups) – not required if microsampling can be used	18M+18F treated groups; 6M+6F Controls

Study Design Considerations: Inclusion of Neurotoxicity Evaluations (cont'd)

- Locomotor Activity
- High beam breaks (rearing activity) – positive control data





Data: Covance

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Study Design Considerations: Inclusion of Neurotoxicity Evaluations (cont'd)

- Learning and Memory

Image: Covance

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Study Design Considerations: Inclusion of Reproductive Evaluations

- Usually evaluated at Day 14 of gestation (as per the fertility study ICH S5(R3) Annex 1: 1.1.1)
- Can be more extensive: parturition and post-natal survival & development assessed, if these stages have not been evaluated on earlier studies:
  - Needs group size of 16+ (ICH S5(R3) Annex 1: 1.1.1 and 1.1.3 Repro guidelines)
  - Usually only assessed after a period of recovery
  - Quite common to pair treated males with untreated females and treated females with untreated males

Phase	Typical group size
Toxicity	12M+12F
Recovery/reversibility/off dose	6M+6F
Reproductive function	20M+20F
Untreated females for pairing with treated males	20F
Single dose Toxicokinetic phase (6 time points; 3 samples per time point in treated groups) – not required if microsampling can be used	18M+18F in treated groups; 6M+6F in Controls

Study Design Considerations: Inclusion of Immunotoxicity Evaluations

- Evaluations during treatment and recovery could include:
  - Immunophenotyping of peripheral blood leukocytes
  - T-cell dependent antibody response (TDAR) to Keyhole limpet hemocyanin (KLH) = IgM response
  - Enhanced microscopic examination as per recommendations laid out in: Hayley P. et al (2005) STP Position Paper: Best practice guideline for the routine pathology evaluation of the immune system

Phase	Typical group size
Toxicity	12M+12F
Recovery/reversibility/off dose	20M+20F
Immune system function	20M+20F
Single dose Toxicokinetic phase (6 time points; 3 samples per time point in treated groups) – not required if microsampling can be used	18M+18F in treated groups; 6M+6F in Controls

Study Design Considerations: “Complex” design

- Incorporates all aforementioned evaluations: neurotoxicity, reproductive function and immune system (and similar in design to studies conducted prior to ICH S11, when we did pretty much everything feasible). The design would be as follows:

Phase	Typical group size
Toxicity	12M+12F
Recovery/reversibility/off dose	6M+6F
Reproductive/behavioural function	20M+20F
Untreated females for pairing with treated males	20F
Immune system function	20M+20F
Single dose Toxicokinetic phase (6 time points; 3 samples per time point in treated groups) – not required if microsampling can be used	18M+18F in treated groups; 6M+6F in Controls

Study Design Considerations: Age and Species

- Age at start of treatment will affect how animals are sourced
  - Age stagger on arrival dependent on investigations required during study
- Species Selection:
  - Must be appropriate for evaluating tox endpoints relevant for intended pediatric population
  - Rats are traditionally the species of first choice; dogs are usually the second species
  - Testing in one appropriate species using both sexes will normally be sufficient
- Why the Rat?
  - Major species in general toxicity data
  - Studies run in a short time-frame
  - Can synchronize breeding/animal supply
  - Pup dosing possible from an early age
  - Good statistical power by using a large number of animals [the number of animals determined by consideration of accuracy, evaluation and the principles of the 3Rs (reduce, refine, replace)]
  - Established functional/behavioural tests
  - Reproductive function easy to assess
  - Can easily randomize litters pre-weaning which spreads genetic pool, eliminating genetic bias and all litters born can be used (no wastage due to abnormal sex ratio/litter size)

Study Design Considerations: Age and Species (cont'd)

- When NOT the Rat?
  - When it does not respond to compounds in similar way to humans
  - Its small size:
    - May compromise some routes of dose administration
    - Makes toxicokinetic and/or clinical pathology sampling difficult:
      - Uses many animals because pre-weaning bleeds are generally terminal
      - Achievable blood volumes low, so may need to pool samples
- Use of Dogs
  - Usually regulators request to answer a specific question, e.g., specific FDA request to assess CNS pathology, to further investigate findings in the rat juvenile studies
  - Not always necessary to perform all "standard" toxicology endpoints
  - Long lead-in time
    - Suppliers do not wish to release their breeding females
  - Cannot synchronize breeding of parent females
    - Supply will be over several weeks
  - Long duration studies due to longer maturation
  - Low statistical power
  - Behavioural/reproductive assessments difficult

Study Design Considerations: Dose Administration

- Dose Route/Volume in Rats:
  - Stage of physical development determines feasibility of dose route:
    - SC/IP/Oral (buccal cavity): Day 1 of age
    - Oral gavage: From ~Day 14 of age
      - Dose volume ideally 5 mL/kg for oral route
      - 10 mL/kg possible with care (if maximum practical formulation concentration is low)
    - Intravenous: single dose from Day 14 of age; repeat dose from Day 21 of age
    - Intramuscular: Day 21 of age
    - Inhalation: 1-hr snout-only exposure feasible from Day 2 of age
  - Use intended clinical route, where practical; sometimes use substitute route until clinical route is feasible, e.g., subcutaneous administration instead of repeat IV dosing
    pre-weaning

Study Design Considerations: Inhalation Administration

- Poses some unique challenges when compared to other routes:
- Size of animals
  - Young rat pups much smaller than mice (handling issues)
  - No fur to ~Day 11 of age (maintenance of body temp during exposure)
- Age of start of treatment?
  - Is there time for and/or is it practical to acclimatise to the restraint tubes?
- Duration of removal from dam?
  - Dose determined by length of exposure due to maximum practical concentration
  - Needs to be minimised to help maintain body temperature and to prevent maternal rejection
- Method of exposure
  - Snout only a challenge due to size (pivot ability → turn around in tubes?; tubes small enough?)
  - Whole body → risk of maternal exposure through grooming → possible “double-hit” exposure through milk?

Study Design Considerations: Inhalation Administration (cont'd)

- Particle size
  - Differences in ventilation rates and in structure/size of the upper respiratory tract in different age groups → significantly different patterns of particle deposition, and gas transport due to the variations in air flow patterns.
  - Physicochemical characteristics of inhaled particles/gases also influence particle disposition (deposition/ADME).
  - Ideally need <1µm particle size due to stage of lung development – technically difficult to make smaller, particularly with nebuliser as most are clinical and designed to deliver particle size for the adult population.
- Stage of respiratory system maturation:
  - A multi-event, continuous process, not restricted to pre-natal life.
  - Dramatic embryonic changes occur, however majority of lung changes occur post-natally → prime target for toxic insult → increased potential for developmental delay/disturbances.
  - Only a limited number of maturational events need to be finished by birth for successful survival.
  - Exposure to pollutants/toxins/repeated pulmonary infections cause lung alterations that are difficult to distinguish from changes related to aging.

Study Design Considerations: Inhalation Administration (cont'd)

- 5 main stages of human lung development, all discrete windows of vulnerability in respiratory system development:
  - Embryonic stage: Week 1-5 of gestation
  - Pseudo-glandular stage: Weeks 5-17 of gestation
  - Canalicular stage: Weeks 17-26 of gestation
  - Saccular stage: ~Week 24 until birth
  - Alveolar stage: Birth until 2-3 years of age
    - 15-20% of adult alveoli have formed at birth.
    - Stage of microvascular maturation: 3 months-3yrs
    - Normal growth: Until adulthood

Study Design Considerations: Inhalation Administration (cont'd)

- Rats as models of human lung development:
- Early Alveolarisation
  - Human: Birth to 1-2 years
    Rat: Postnatal Day 4 to Day 13
- Microvascular maturation
  - Human: Ca. 3 months to 2-3 years
    Rat: Postnatal Week 2 and 3
- Late Alveolarisation
  - Human: Ends at ca. 8 years. From 2 years to adulthood, growth proportional to body weight
    Rat: Slow progression throughout life

Study Design Considerations: Inhalation Administration (cont'd)

- Upper respiratory tract development:
  - In the rat, the upper respiratory tract is generally considered mature by ~ 23 days of age.
- Nasal Cavity
  - 4 days : Nasal turbinates plump rudimentary structures supported by cartilage. All 3 types of lining epithelium present. NALT present and very small. Bowman's glands consist of small acini embedded in abundant loose connective tissue.
  - 8 days : Nasal turbinates incompletely formed and mainly supported by cartilage, with few small ossified areas. Vomeronasal organ appears mature. NALT is present, and Bowman's glands appear mature.
  - 23 days: Nasal cavity, nasolacrimal duct, and vomeronasal organ with their epithelia (olfactory, respiratory and squamous) and turbinates appear mature. NALT is present at the adult amount.
- Nasopharynx
  - 4 days: Epithelia are thinner and salivary glands smaller than in the adult.
  - 8-23 days : Nasopharynx appears mature.
- Trachea
  - 4-8 days: Tracheal glands in bud stage. Majority of tracheal epithelial cells not ciliated.
  - 15 days: Majority of tracheal epithelial cells not ciliated.
  - 23 days: The trachea appears mature.

Study Design Considerations: Practicalities of Procedures

- Ophthalmoscopy: *not a core endpoint in ICH S11*
  - Eyes closed at birth, so not possible prior to Day 15 = pre-treatment assessment often not feasible; can confound data interpretation
- Urine collection: *not a core endpoint in ICH S11*
  - Not practicable prior to weaning
  - Early post-weaning: individual volumes low – pooled sample/limited list of parameters
- Behaviour assessments: *not a core endpoint in ICH S11*
  - Essential to tailor to age at testing
  - During treatment – commonly conducted prior to dosing
- Ensuring correct identification of pups pre-weaning:
  - Accurate toe marking (or alternative) required

Study Design Considerations: Practicalities of Procedures (cont'd)

- Blood Sampling in the Rat
  - In neonatal rats, blood samples may need to be pooled, unless microsampling can be used
  - Pre-weaning samples are generally terminal
  - Post-weaning may be only 1 sample/occasion due to limited circulating blood volume
  - Blood sample site: varies according to age and sample volume required
  - Microsampling
    - Blood sample of ≤75 µL
    - Capillary action
    - 10-25 µL of plasma
    - Can replace conventional TK where 200-300 µL of plasma was required

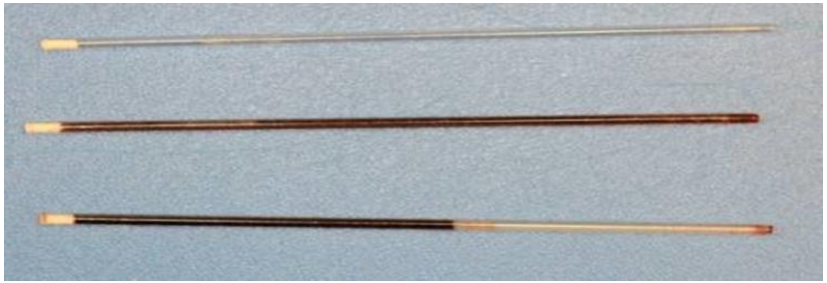

Image: Labcorp

The Road Ahead

- Will S11 be the panacea for neonatal/juvenile tox study design?
  - … maybe, maybe not.
- In many sections of the guideline, ICH S11 is very clear in its expectations, with greater use of terminology such as “should be assessed”, “should be performed”, “strongly encouraged” or “recommended”, particularly (but not exclusively) in the core endpoints.
- There remains a number of “can be expanded”, “can be useful”, “may be considered” entries, but mostly in the additional endpoints which would be covered by the scope of the WoE evaluation, during which the decision to include/not include endpoints would be a made.
- But these retained “vague statements” still allow the different agencies to apply some “flexibility” in their interpretation.
- Overall though, the implementation of S11 will hopefully make the job of our customers (and us) a great deal easier – one driving document accepted (in theory) by all agencies
- … so we’ll wait and see!

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Thank you for listening

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