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NON CLINICAL

Juvenile mouse studies

Challenges and capabilities

13 October 2017 - Hollie Blunt & Laura Penn

Overview

Validation of administration routes for juvenile mice

- Cannula placement for oral gavage administration with cadaver animals
- Dosing of live animals
  - Oral gavage from Day 14 and Day 21 of age
  - Subcutaneous from Day 14 of age

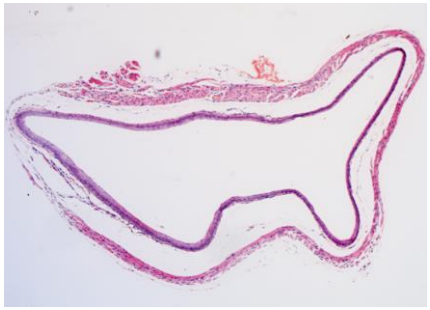
Experience in a juvenile mouse range-finding toxicity study

Available options for inclusion in regulatory studies in juvenile mice

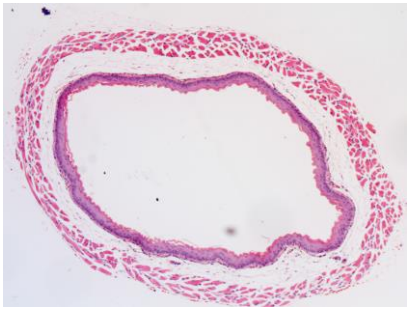
Cannula placement

- 2 litters of Crl:CD-1 (ICR) mice euthanised on either Day 14 or Day 21 of age
- Immediately post-mortem metal cannulae or plastic feeding tube inserted into oesophagus
- Histopathology of oesophagus conducted to establish feasibility of oral gavage procedure

Cross sections of oesophagus after cannula placement from cadaver animals



Day 14 of age

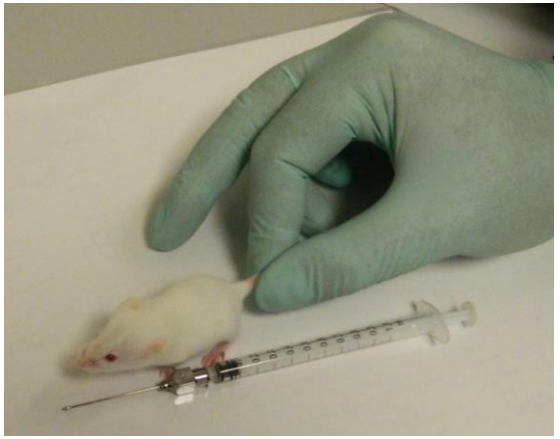


Day 21 of age

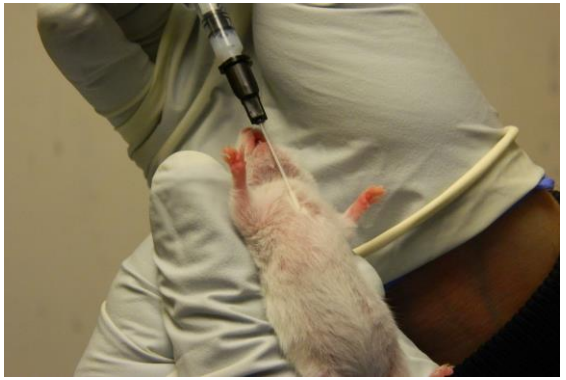
- Microscopic pathology indicated that cannula placement in the oesophagus of cadaver animals was not representative of live animals
- Cannula insertion was feasible on both Day 14 and Day 21 of age

In Vivo study

- Litters of Crl:CD-1 (ICR) mice, standardised to 5M + 5F pups
- Animals were dosed once daily from Days 14 or 21 of age for 5 days and then retained for 2 days before necropsy
  - Oral (gavage) : from Day 14 and Day 21 of age
  - Subcutaneous: from Day 14 only; this was to provide an alternative dosing option to gavage
  - 5 mL/kg dose volume for both dosing routes
- Study end-points included:
  - Clinical observations
  - Body weights
  - Food intake (from Day 21 of age only)
  - Macroscopic and microscopic pathology

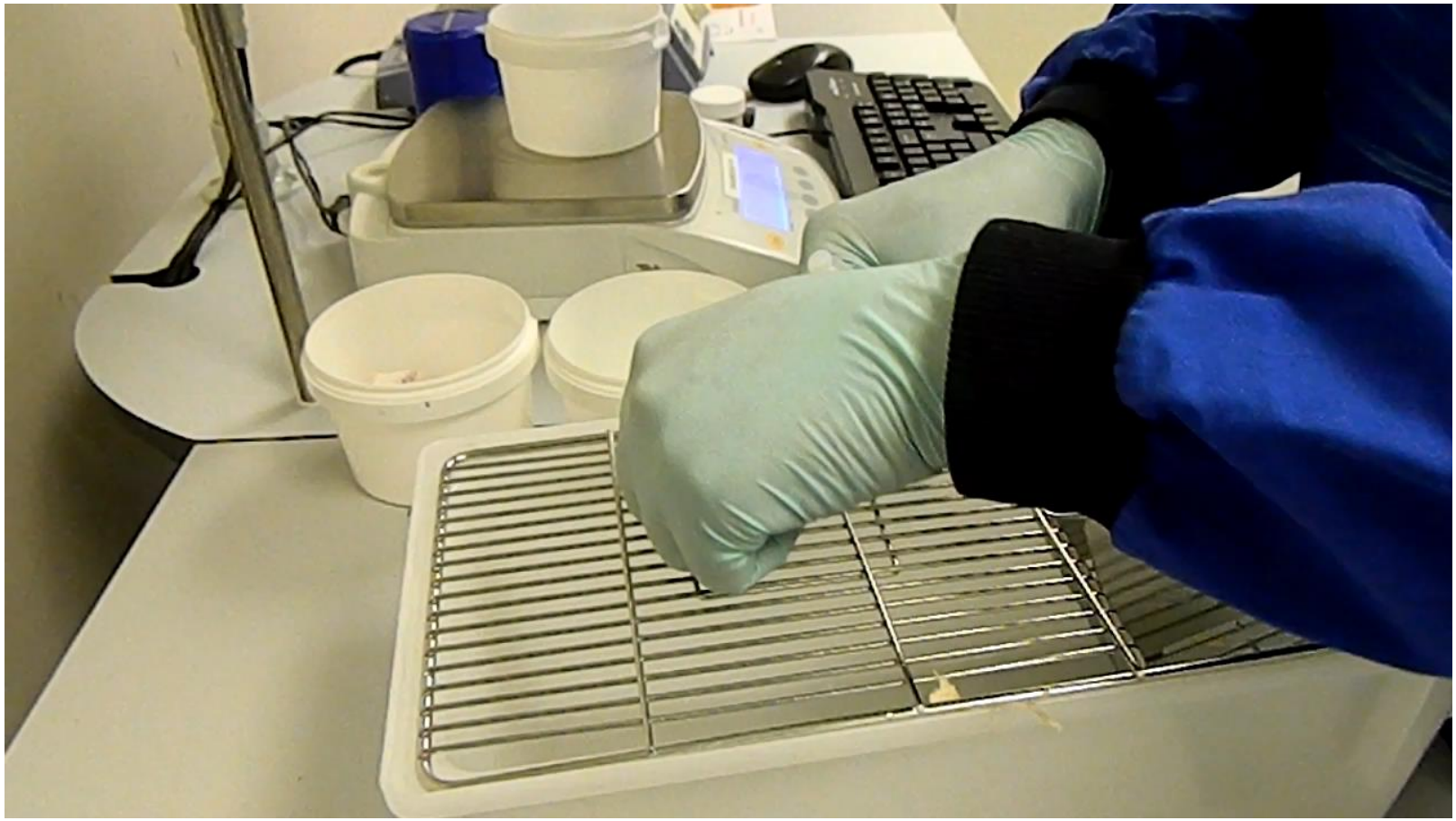


Day 14 of Age Mouse Pup and Metal Cannula (Size 6/30)



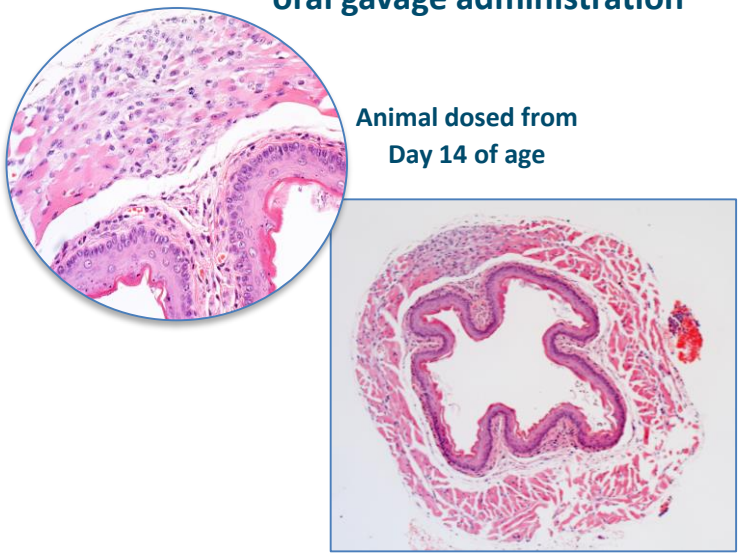
Day 21 of Age Mouse Pup and Plastic Feeding Tube (22 gauge)

Dosing procedure



Study outcomes

Cross sections of oesophagus after oral gavage administration



- Only 1 death during the study (0.2 % of dosing occasions) and limited clinical signs
- No adverse effects on body weight gain
- Microscopic lesions in the oesophagus were minor and considered reversible

Conclusion: Both oral (gavage) and subcutaneous dose routes were considered viable for use for dosing juvenile Crl:CD-1 (ICR) mice and would be sustainable for the entire length of a juvenile study with dosing commencing from at least Day 14 of age

Oral Gavage Range-Finding Study in the Juvenile Mouse

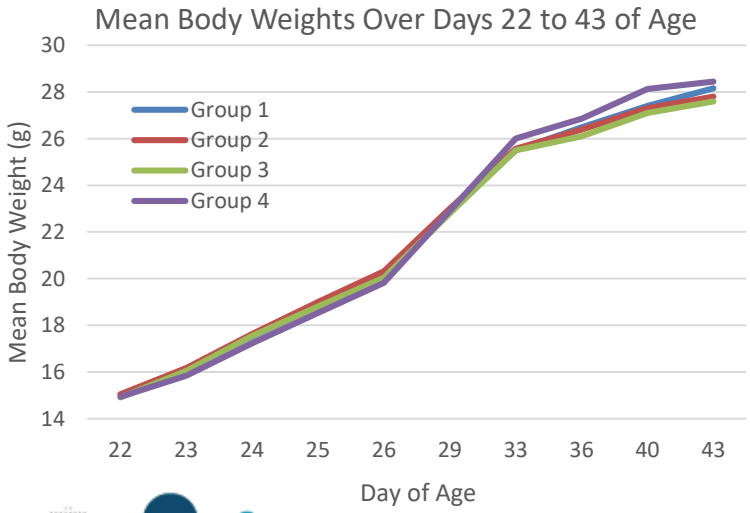
Study Design:

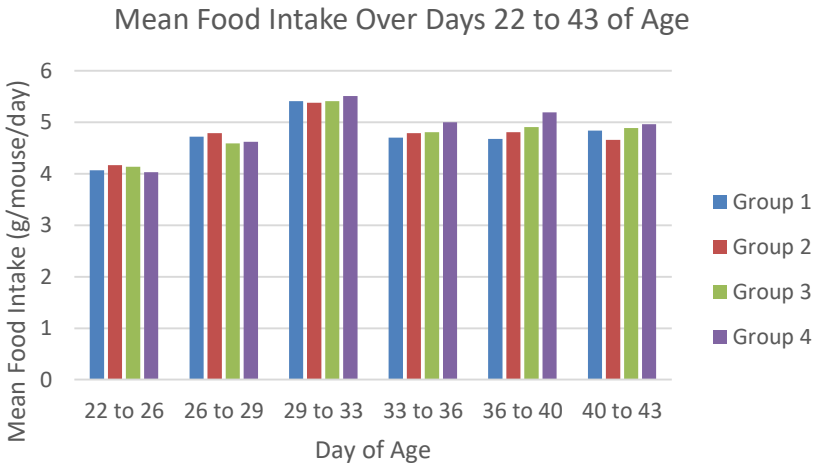
Group	Number of Males	Number of Females
1	9	9
2	12	12
3	12	12
4	12	12
5	12	12
6	12	12
7	12	12

- Standardised litters of 5 male and 5 female Crl:CD-1 (ICR) mice supplied to provide 162 mice
- Groups 1 to 4 dosed Days 22 to 43 of age and Groups 5 to 7 dosed Day 22 of age
- Clinical observations, body weights, food intake, haematology (0.2 mL), TK, macroscopic and microscopic examinations
- TK blood sampling (0.05 mL) at 4 time points; terminal orbital sinus samples on Day 22 of age and tail vein samples on Day 42 of age

Oral Gavage Range-Finding Study in the Juvenile Mouse

- One death attributed to dosing stress (0.05 %)
- No clinical signs
- No effect on body weight
- No effect on food intake
- No macroscopic or microscopic findings
- Limited haematology blood volume allowed interpretation of effects
- Meaningful toxicokinetic evaluation





The study concluded that appropriate dose levels could be selected for the main juvenile mouse toxicity study

Study End-Points

- Pup Development (Pinna detachment, Eyelid Separation, Static Righting Reflex, Startle Response and Long Bone Growth)
- Auditory Function (Preyer Reflex) and Pupillary Light Reflex
- Learning and memory (Water filled E-maze or Morris Maze)
- Motor Activity
- Sexual Development and Reproductive Capacity
- Immunotoxicity (e.g. Anti-KLH Response)
- As well as standard toxicology end-points such as Toxicokinetics, Clinical Pathology and Pathology

Thank you for your attention

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