Reduction and Refinement of Rodent Juvenile Toxicity Studies – The Simple Approach to Cross-Fostering.

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The need for juvenile rodent toxicity studies to support paediatric clinical testing of pharmaceutical products has become increasingly commonplace, with appropriately designed non-clinical juvenile studies providing safety data, predictive of toxicity in the paediatric population. Whilst study designs vary hugely due to their tailored approach, almost exclusively these are large and complex studies, with animal usage exceeding any other study in the non-clinical programme. It is vital therefore that all options to reduce and refine these studies are explored. Many publications have focused on the justification of different endpoints and questioned the utility of these studies, however, the simple process of how animals are selected for study is often overlooked, despite the significant impact this can have on total animal usage and the robustness of data. The pup selection approach which is usually adopted, only allocates to study a small number of the dam's natural offspring per litter, due to concern over maternal genetic bias. The cross- fostering approach has often been considered a superior method, whereby the offspring are distributed to multiple litters, removing any genetic bias or culling, and allowing all offspring to be selected for use. This reduces animal usage, provides better quality data and greater study flexibility. However, due to practical feasibility, cross-fostering has been traditionally discounted as idealist. This poster aims to raise industry awareness of a unique cross-fostering approach, developed at Sequani Ltd in a joint collaboration with our animal supplier, Charles River, UK. This approach involves cross-fostering to specific requirements at the suppliers before arrival at the test laboratory and has been shown to reduce total rat usage, typically by > 65 %.

Introduction

Historically, children have been under-served by the drug development process with the risky practice of off-label pharmaceutical use being commonplace. Over the last decade however, this has been addressed internationally, with regulatory legislation and guidance introduced, making it mandatory to perform paediatric clinical trials in order to achieve marketing authorisation for new chemical entities intended for paediatric populations. This has driven an exponential increase in the conduct of non-clinical juvenile studies, intended to support the clinical paediatric programme. Whilst it is recognised that juvenile animal studies should only be initiated if previous animal or human safety data are insufficient (1), it has become increasingly commonplace for juvenile animal studies in at least one species to be insisted by the regulatory agencies in both Europe and the USA. This has led to the current industry position that, in the majority of cases, it is assumed that a juvenile animal study will be required, with rats being the most frequent species of choice (2).

Given the unique nature of these non-clinical studies, which should be tailored to the compound and intended clinical regimen, there has been much attention on how to conduct the most appropriately designed studies, further supported by the issue of industry guidance documents (3, 4). Nevertheless, these studies invariably require large numbers of juvenile animals to cover the various parameters and endpoints that need to be measured. This is further exacerbated in studies starting with the pre-weaning juvenile rat, due to problems such as blood volume limitations, methods of allocation and the requirement for spare litters to counteract the likely natural pup mortality. In an attempt to address this problem and encourage the principals of the three R's (Reduce, Refine and Replace) there has been a considerable effort to raise awareness and educate, with many publications focused on how to reduce juvenile animal usage by removing unnecessary endpoints. However, the simple process of how juvenile animals are provided and allocated to study, and the contribution this can make to the reduction of animal numbers has generally been overlooked.

Methods of juvenile rodent allocation

Ensuring sufficient genetic diversity to fully reflect the population is of fundamental importance in juvenile animal studies, and without this, the quality of the data and conclusions drawn could be flawed. In general, there are three methods by which pre-weaning juvenile rats can be allocated to study. The 'between litters' approach, the 'within litter' approach and by cross-fostering (See Figure 1).

- **The 'between litters' approach:** Selects up to three per sex of the dams natural offspring to allocate to a single study dose level/group. All dams and the remaining, unselected offspring are natural wastage and killed with no study purpose – on average litter numbers this equates to 50 % to 83 % of the litter wasted.
- **The 'within litter' approach:** The dam's natural offspring from the same litter are allocated to different dose levels/groups. Whilst this increases the numbers of animals selected for study and decreases wastefulness, it significantly increases the risk for contamination, allowing for sibling cross-contamination. Given the propensity for mothers to abandon nursing of compromised animals, compound-related effects could also bias pups survival. It should be noted however, that the loss of one litter due to poor maternal behaviour is unlikely to adversely affect the overall study group size.
- **The cross-fostering approach:** On birth, the natural offspring from each dam are fostered to multiple foster dams so that each litter has no siblings, enabling allocation of all offspring to study, and commonly one group/dose level. The impact of a litter loss on the study can be more substantial however.

Whilst the cross-fostering approach would appear superior due to the advantages it offers in reducing animal usage on juvenile animal studies, due to the complex nature of in-house cross-fostering, this approach has been historically disregarded as idealist. Practical challenges were considered to outweigh the benefits for juvenile animal studies, which typically require the supply of multiple batches of animals, each divided into differing groups and further subdivided into subsets/cohorts. This is further exacerbated by the large numbers of dams it would require to ensure that sufficient dams are littered on the same day to provide adequate litters for cross-fostering (one litter of five males and five females requires a minimum of 11 litters born at the same time). As a consequence, despite the benefits of cross-fostering, the 'between litter' approach is routine for laboratories conducting juvenile rodent studies.

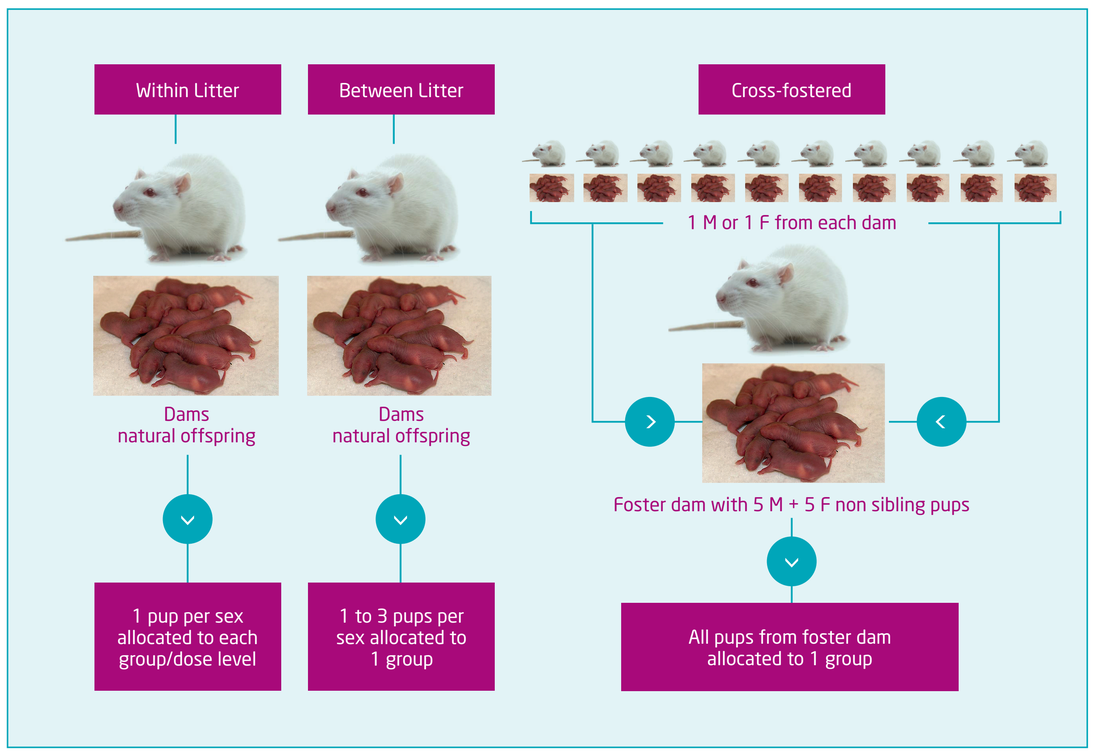

Figure 1. Types of pre-weaning allocation for juvenile rodent studies

Table 1: Pre-weaning rodent juvenile study design example

Subset/Cohort	Group/Dose Level			
	1	2	3	4
I - Main Study	12 M + 12 F	12 M + 12 F	12 M + 12 F	12 M + 12 F
II - Recovery	6 M + 6 F	-	-	6 M + 6 F
III - Repro/behavioural development	20 M + 20 F	20 M + 20 F	20 M + 20 F	20 M + 20 F
IV - Toxicokinetics*	18 M + 18 F	18 M + 18 F	18 M + 18 F	18 M + 18 F

**Assumes 1 terminal sampling occasion, 6 time points, 3 animals/sex/time point*

Table 2: Percentage difference of animal usage of 'between litter' allocation when compared with the cross-fostered approach

Allocation Method*	Number of dams**	Number of pups***	Total animal usage	% reduction possible using cross-fostered approach
Between litters (1/sex /litter selected)	236	3068	3304	81.4
Between litters (3/sex /litter selected)	96	1248	1344	54.2
Cross-fostered (5/sex/litter selected)	56	560	616	-
Cross-fostering uses on average, 68% fewer animals than between litter allocation				

**Assumes average litter size of 13 for 'between litter' dams; cross-fostered litters standardised to 5/sex/litter. **Includes spare dams required.*
**** Numbers assumed prior to culling for 'between litters' allocation; culling not required for cross-fostered litters.*

Simplified cross-fostering

At Sequani, a unique and simplified approach to cross-fostering has been developed and adopted on all pre-weaning rodent juvenile studies conducted at our laboratories since 2011. This has wide ethical implications by dramatically reducing animal usage and refining the quality of data due to the increase in genetic diversity. In some circumstances, the use of cross-fostered litters has also shortened lead times to commence studies and reduced cost.

Given the huge numbers of dams required to provide sufficient litters for cross-fostering, the cross-fostering procedure was modified to enable this element to be conducted at our suppliers, Charles River laboratories, UK. Our method uses the pre-existing Sprague Dawley breeding stock at the supplier's facility to provide the foster dam and offspring of known number (usually 5 per sex). It ensures that sufficient dams are available for selection on any given day, and can also eliminate the need for time-mating, reducing lead time to animal delivery. Due to the exclusion of poor breeding dams at the suppliers, this also ensures that only dams of proven maternal quality are supplied with cross-fostered litters from Day 2 of age and practically eliminates litter losses at the testing facility due to inadequate maternal behaviour. Given the outstanding maternal nature of the Sprague Dawley rat, losses of pups in transit have been negligible. Furthermore, by cross-fostering to a known litter size at the supplier's facility, any offspring or dams not used for the cross-fostering procedure are returned to breeding stock at the supplier, eradicating natural wastage or culling.

Impact of the simplified cross-fostering approach

In Table 2, a comparison has been made between the number of dams and pups used for the simplified cross-fostering allocation versus the most common alternative method of allocation, the 'between litter' approach. This assumes a standard study design as depicted in Table 1.

Conclusion

Whilst other approaches to reducing juvenile animal usage are acknowledged and encouraged, in making a simple change in allocation procedures, and adopting the cross-fostering approach developed at Sequani, overall animal usage (including dams and offspring) at our laboratories has been reduced by greater than 65 % for each main pre-weaning juvenile rodent study. Furthermore, by increasing the genetic diversity and reducing variations in maternal behaviour, it has refined the quality of data and therefore the robustness of the conclusions drawn. As such the approach we have described makes a substantial contribution to the principles of the 3Rs.

References

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