Inhalation Dosing in Juvenile Rabbits

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1 INTRODUCTION

The test item, an aqueous phospholipoprotein formulation, has been approved previously for the administration via intratracheal instillation to premature babies. The use of this material by a less intrusive route was investigated. The objective of this study was to assess the toxicity of the test item to juvenile rabbits when exposed via inhalation for 13 days. The study also examined the tolerance of rabbit kits to inhalation exposure/tube restraint for up to 3 hours.

Animals

Four New Zealand White rabbit dams and their kits (7 per litter) were transported from the suppliers at Day 8 of lactation in specially designed crates. They were housed in floor pens (see Fig.1) to increase the floor area compared to standard rabbit caging. Two litters (14 kits) were assigned for test item exposure and 2 litters (14 kits) were assigned as controls and exposed to compressed air.

Dosing Procedure

The kits were conditioned to removal from the dams and to the restraint tubes (see Fig. 2) for an appropriate period prior to initial exposure. Dosing was initiated at Day 15 of lactation when the kits weighed 110-290 g.

Pretrial Litter Observations

The kits were individually inspected and weighed from arrival to assess their conditioning to the environment and fitness for dosing.

Fig 1. Floor pen and environmental enrichment items

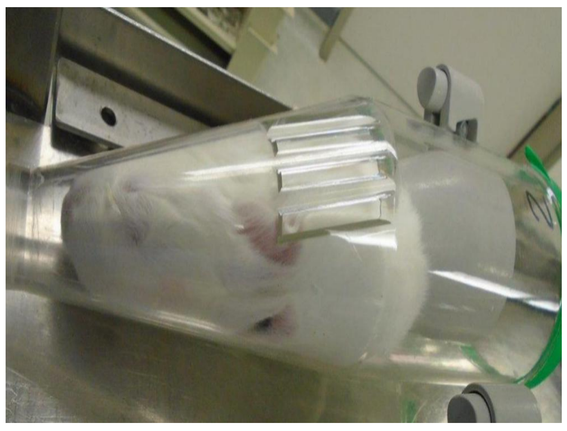

Fig 2. Rabbit kit in restraint tube

2 METHODS

Inhalation Exposure System

The test item was heated in a water–bath at 40˚C for 1 h before generation to reduce test formulation viscosity and aid aerosol generation. The test aerosol was generated using 4 x electronic nebulisers fitted with deep fill reservoirs feeding into a small Perspex antechamber (see Fig. 3). In these devices an internal mesh vibrates loading and ejecting formulation droplets rapidly to produce an aerosol. The nebulisers were primed with warmed test item immediately prior to dosing and topped up regularly to maintain a consistent output rate of the nebulisers.

A nose-only flow-past inhalation chamber (Promech, Sweden) was used to conduct the animal exposures (see Fig. 4). Warmed air (2L/min/neb) was fed through an inlet situated on the side of the nebulisers and conveyed the aerosol to the exposure chamber via the antechamber. The animals were situated on a single tier of the exposure chamber on an integrated support disc

Dosing

- Snout only inhalation exposure
- Once daily
- 180 minutes
- 13 days

Animal Monitoring

- On chamber observations
- Detailed weekly clinical observations
- Daily clinical observations
- Daily body weights
- Daily assessment of condition of the dams

Fig 3. Nebulisers feeding into antechamber

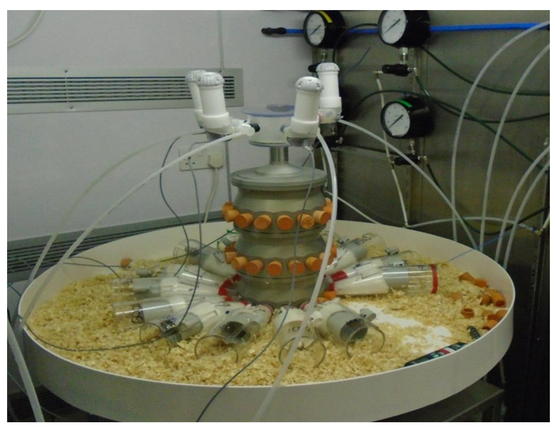

Fig 4. Exposure System (during dosing)

3 RESULTS

In-life Observations

Mortalities

A single control animal, the smallest on study, was euthanised following Day 2 dosing due to poor condition. Considered related to the stress of the procedure.

Clinical Observations:

Wet and un-groomed fur was noted during the dosing period for all test item group animals. These signs were consistent with exposure to a wet aerosol. Control group animals were exposed to dry air and generally exhibited no clinical signs.

Body Weight Profile:

Body weight profile (see Fig. 5) considered normal and comparable between test item and control groups.

Inhalation Exposures:

See Table 1 . Aerosol concentrations showed some variability but were generally stable, reproducible day to day throughout the study and were considered respirable to the animals.

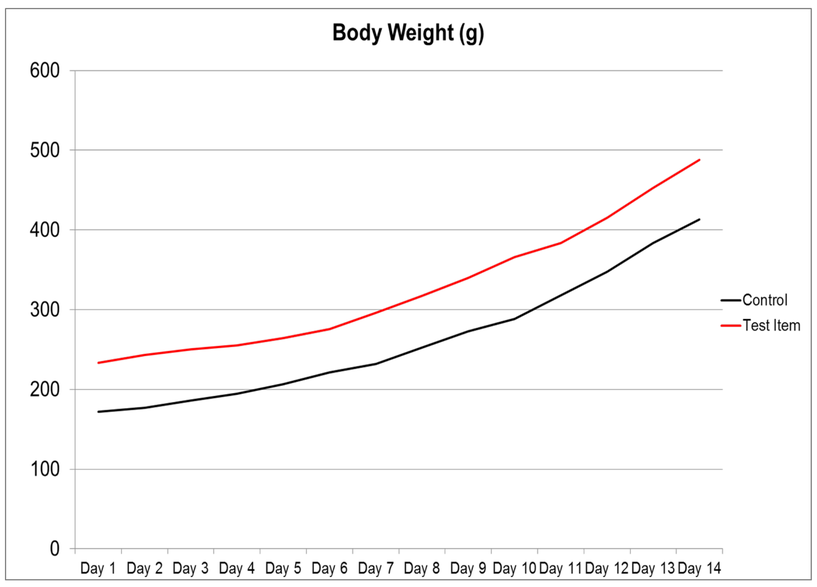

Fig 5. Body Weight Growth Curve

Table 1 – Summary of Aerosol Parameters

Dose Group/ Treatment		Analytical Aerosol Concentration (mg/L)	Estimated (Delivered) Dose (mg/kg/day)
Test Item	Mean	6.08	792
	CV (%)	32.8	27.4
Particle size Distribution		**MMAD (µm) (GSD)**	
Gravimetric Estimation		3.18 (2.112) to 3.86 (1.568)	
Analytical Estimation		3.19 (1.672) to 3.83 (1.565)	

No test item was detected from the air control group.

4 CONCLUSION

In conclusion, the animals tolerated tube restraint for 3 hours and were unaffected by exposure to the test item daily over 13 consecutive days at a dose level of 792 mg/kg/day.

The animal transfer, husbandry and dosing techniques employed in this study were well controlled leading to successful dosing of juvenile rabbits by inhalation.