The Effect on Body Weight of Rats After Varying Durations of Inhalation Administration Compared with Oral Administration

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Introduction

- To assess the safety of pharmaceutical or biopharmaceutical drugs and to allow industry to manufacture and distribute chemicals, there are regional or country-specific regulatory frameworks that the companies are required to adhere to with the aim of saving lives, improving the health of patients and improving and protecting human health and the environment through evaluation of those chemicals. As part of these assessments, there are certain study types that are required to be conducted.
- For pharmaceutical development, a 13- or 26-week study is required to enable Phase II or III clinical trials, whilst for chemicals, an OECD (Organisation for Economic Co-operation and Development) 13-week study is required, depending on the tonnage band (OECD 408 study design from an oral risk or OECD 413 from an inhalation risk perspective). The route of administration for pharmaceuticals is dependent on the company's development strategy, whilst chemical study conduct is based on the likelihood of exposure.
- To comply with the OECD 413 inhalation chemical study guideline, rodent exposure is 6 hrs/day for 5 days/week. For inhalation pharmaceutical development, a daily exposure of up to 1 hr/day is the standard approach.
- For orally consumed pharmaceuticals and chemicals, rodent administration is daily and only requires a few minutes per animal.
- A comparison of 13-week studies with different dosing regimens using the same rat strain (RccHan WIST) was conducted to assess the impact of inhalation restraint on body weight on control group animals. Data were averaged from the control groups of randomly selected studies for the 3 different dosing scenarios (oral daily 23 studies, inhalation chemical 11 studies, and inhalation pharmaceutical 13 studies).

Methods

Animal Supply and Dosing Methodology

- Studies were selected at random from those conducted at a single Labcorp site using the same strain and supplier to ensure consistency and to minimise potential variability. Rats were RccHan WIST strain provided by Harlan (UK) RMS Ltd.
- The animals ordered ranged from 5 to 6 weeks to 12 to 13 weeks whilst still in accordance with the OECD guidelines or pharmaceutical study design.
- The body weights of these control group animals were captured once weekly using the Pristima software system. Body weights were measured 30 minutes prior to inhalation dosing and immediately prior to oral dosing.
- For inhalation dosing, animals were transferred directly from their home cage and were restrained in snout-only tubes and placed on the inhalation chamber for the period of dosing (6 hrs/day for 5 days/week or up to 1 hr/day) until the animals were returned back to their home cage. All animals were dosed simultaneously. The welfare status of the animals were observed regularly and documented normally between 10 and 15 minute intervals during the restraint period. The sizes of the restraint tubes are dependent on the individual animal's body weight, overall shape of the animal and chamber type. At the back of each tube is a bung and protruding through the bung is a rod and end plate. The bung ensures easy access to the animal whilst being restrained. The rod and end plate ensure that the animal is being dosed effectively by having the snout protruding from the aperture at the front of the tube.
- The orally dosed animals were dosed individually and sequentially (males then females). The animals were dosed with water or a suitable vehicle depending on the test item.
- In all of the inhalation administered chemical 13-week studies, the control group animals were administered air only. For the majority of the pharmaceutical inhalation animals, the control group were administered either a liquid or powder-based vehicle.
- Both food and water were provided ad libitum for all dosing regimens, except during the dosing period.
- Animal care and use was performed according to applicable animal welfare regulations in an AAALAC-accredited facility.

Data Analysis

- Data were analyzed using a repeated measures analysis of covariance (RM-ANCOVA) with Week 0 body weight taken as covariate. This analysis adjusted for differences in initial body weight. Comparisons of these adjusted body weights were made using t-tests for each week separately as well as for the entire period average, and the resulting 95% confidence intervals were plotted.

Results

- The mean body weights for all studies are presented in Table 1 and plotted in Figures 1A and 2A (top) for males and females, respectively. To account for differences in starting weight, a covariate adjustment of the weekly means was performed based on the week 0 data, shown in Figures 1A and 2A (bottom).
- Plots of the differences in adjusted means with associated 95% confidence intervals are shown in Figures 1B and 2B. The differences between oral and inhaled increases over time, with significant differences seen as early as week 2 for males and week 3 for females. There are no notable differences between the two inhaled treatment groups.
- By week 13 of the dosing period, the mean male and female oral animals had a body weight of 405 g and 237 g, respectively. This is compared with male and female inhalation chemical animals having a body weight of 356 g and 220 g, respectively and inhalation pharmaceutical animals of 367 g and 220 g for the male and female, respectively.
- The results demonstrated that the oral dosed males and female animals gained 216 g and 93 g, respectively, over the 13-week dosing period. However, the inhaled chemical male and female animals gained only about half the weight over the same period with values of 110 g and 49 g, respectively. Similarly, the inhaled pharmaceutical male and female animals showed the same trend as the other inhalation cohort and only gained 127 g and 50 g, respectively.
- It is apparent in Figures 1A and 2A that animals in the oral dosed cohorts with lower starting body weights (144 g F; 189 g M) initially grew at steeper rate versus the inhalation cohorts that started at larger weights (171-172 g F; 240-246 g M), which was statistically significant (Type*Week Interaction P-value: <.0001). To demonstrate that the difference in weight gain between the oral and inhaled groups was not entirely due to the faster growth rates of younger animals used in the oral studies, body weight gain at 13 weeks was plotted as a function of initial body weight (Figures 1C and 2C).
- The linear trendlines for all dose cohorts were essentially parallel for their respective sex, indicating that the starting age was not important and that the difference in growth rate for both sexes was primarily a direct result of the route of administration.

Table 1. Mean Body Weight for All Data Sets

	Mean Body Weight (g)													
Data Set	**Wk 0**	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 5**	**Wk 6**	**Wk 7**	**Wk 8**	**Wk 9**	**Wk 10**	**Wk 11**	**Wk 12**	**Wk 13**
Oral Males	189	224	255	282	304	321	336	350	363	373	384	393	400	405
Oral Females	144	160	175	187	197	205	211	218	223	226	231	234	237	237
Inhaled Chemical Males	246	256	270	285	297	307	316	325	332	339	344	350	353	356
Inhaled Chemical Females	172	178	185	192	196	202	206	209	212	214	216	218	219	220
Inhaled Pharmaceutical Males	240	255	269	283	295	308	318	328	337	345	352	360	364	367
Inhaled Pharmaceutical Females	171	177	183	190	194	200	204	206	210	213	216	219	221	220

Mean control group data are shown for 23 oral studies, 11 chemical inhalation studies and 13 pharmaceutical inhalation studies.

Discussion

- In summary, there was a significant reduction in body weight gain for both male and female inhalation-dosed chemical and pharmaceutical control animals when compared with the oral dose administration scenario over the 13-week dosing period.
- The extent of reduced of body weight gain appears to be irrespective of the duration of inhalation restraint (either 6 hrs/day for 5 days/week or up to 1 hr/day) and time deprived of water and food, even though the rats are principally active nocturnally and dosing is undertaken during daylight hours. It has been observed previously that depravation of water will result in reduced food intake in mice (Bekkevold 2013) and in rats (Dietze 2016), which would affect any body weight gain.
- It is unlikely that the design of the restraint tube caused constriction or hindered growth for the inhalation dosing as otherwise the growth rate curve for the female inhaled animals would be more reflective to that of the orally dosed animals.
- To assess the reduced body weight gain effect, further investigation is required. This could include assessing:
  - Body weight for studies that are longer than 13 weeks in exposure duration
  - Food consumption data
  - Blood parameters such as white blood cells count, platelet count or corticosterone
- This body weight trend for the inhalation-dosed animals does not affect the accuracy of dose calculation or local toxicity, clinical overage or dose level-based risk assessment, since weekly body weights with concurrent dose groups are used.

Conclusions

There was a reduced body weight gain for both male and female inhaled chemical and pharmaceutically dosed animals when compared to the male and female orally dosed animals of the same strain over the same dosing period (13 weeks).

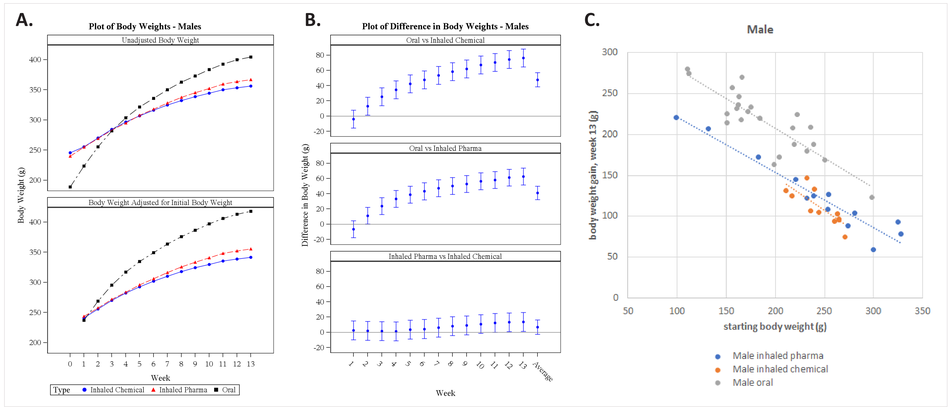

Figure 1. Male body weights. A) Weekly body weights are plotted as both unadjusted (top) and covariate adjusted (bottom) means. A significant difference in the treatment regimens was detected in the RM-ANCOVA analysis (Type Effect P-value: <.0001). **B)** The difference in weekly body weights between treatments are plotted for oral vs. inhaled chemical (top), oral vs. inhaled pharma (middle) and inhaled pharma vs chemical. Data are show as the mean ± 95% confidence interval (CI). Exclusion of zero from the 95% CI indicates significance at p<0.05. **C)** Plot of body weight gain after 13 weeks versus initial body weight. Each data point represents an individual study.

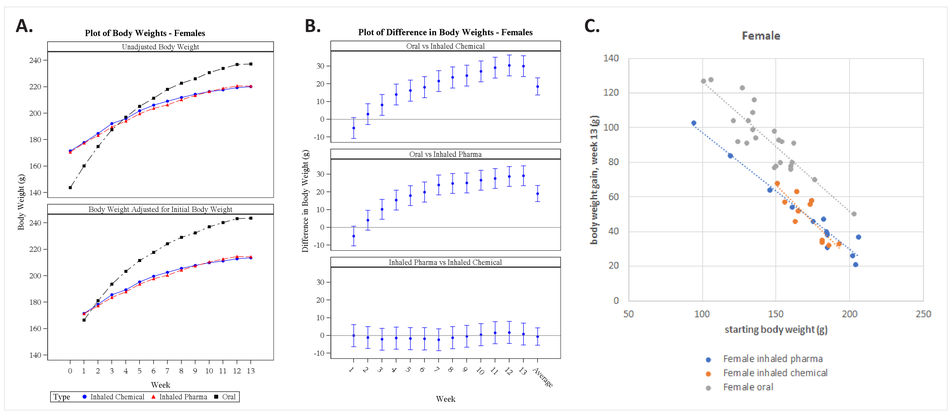

Figure 2. Female body weights. Panels are as described in Figure 1.

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References

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