Implications for Room Lighting and the Duration of Acclimation Protocols on the Dosimetry of Inhaled Drugs in Rats

Graham R. Paul,[1] Graham I. Somers,[1] and Glyn Taylor[2]

[1] *GlaxoSmithKline, Ware, Hertfordshire, UK*

[2] *School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK*

KEYWORDS: dosimetry, plethysmography, light, tube restraint, rat

INTRODUCTION

Inhaled administration of test materials to animals has technical challenges for quantitative dosimetry. Inhaled "doses" reported in non-clinical studies are often calculated using an equation such as that of Alexander *et al.* [1] to estimate the respired minute volume (eRMV) from body weight data. This approach may overlook physiological effects on lung function associated with a formulation's properties or habituation of animals to the "dosing" technique. Rats are insensitive to red light [2] and anecdotal data (unpublished) suggested red lighting may induce a calmer state in restrained rats. We hypothesized that the lighting color (red or white) and duration of restraint tube acclimation protocols may influence the breath frequency and minute volume of rats during inhalation exposure, representing a potential source of variability in achieved doses. Head-out plethysmography [3] was used to investigate this hypothesis concurrent with inhaled administration of an anti-inflammatory drug.

METHODS

Male rats (Crl:WI(Han); n=8 per group; 11 weeks old) were acclimatized to restraint tubes for two or six days and administered a single inhaled "dose" (600 μg/kg; one hour exposure) of an anti-inflammatory drug under either normal fluorescent lighting or red-filtered lighting ($\lambda \geq 600$ nm). Rats were acclimatized by progressively increasing the period of tube restraint each day, up to a maximum of one hour; rats acclimatized over six days were first subjected to a neck-seal on the fourth day of tube restraint. The micronized drug, 5% (w/w) in lactose, was dispersed into a flow-through chamber using a Wright dust feed [4]. Rats were assessed for breath frequency (BF) and

minute volume (MV) by head-out plethysmography during the snout-only inhalation exposure and euthanized immediately post-dose and sampled for drug analysis of lung homogenate (right and intermediate lobes pooled) by high performance liquid chromatography with mass spectrometry detection (HPLC-MS/MS).

For each rat, a mean BF and MV was calculated for the one hour exposure period and for five minute periods ending 5, 15, 30, 45, and 60 minutes from the start of exposure. Statistical analysis (2-way analysis of variance; ANOVA) was applied to the mean of animal-specific means pooled for acclimation periods or lighting conditions (n=16). A Latin square design was used for statistical analyses (2-way ANOVA) of acclimation period (red and white light data pooled) and lighting (two-day and six-day acclimation protocols pooled).

RESULTS

BF and MV of rats during exposure were not affected by their illumination under red or white light. There was no statistical difference in mean values for the one hour exposure period (Tables 1 and 2), but an initial transient elevation in BF and MV was more pronounced for rats subjected to a two-day acclimatization protocol, relative to a six-day protocol (Figures 1 and 2).

Table 1.

Breath frequency (breaths/minute) of rats during a one hour inhalation exposure period.

	Acclimation [A]		Lighting conditions [B]	
	2 days	6 days	White	Red
Mean (bpm)	168.3	169.0	169.2	170.3
sd	17.9	19.2	15.7	5.93
CV	11%	11%	9%	10%
n	16	16	16	16

No differences of statistical difference (2-way ANOVA)

[A] Acclimation: data for white and red light pooled for statistical analysis

[B] Lighting: data for 2-day and 6-day acclimation protocols pooled for statistical analysis

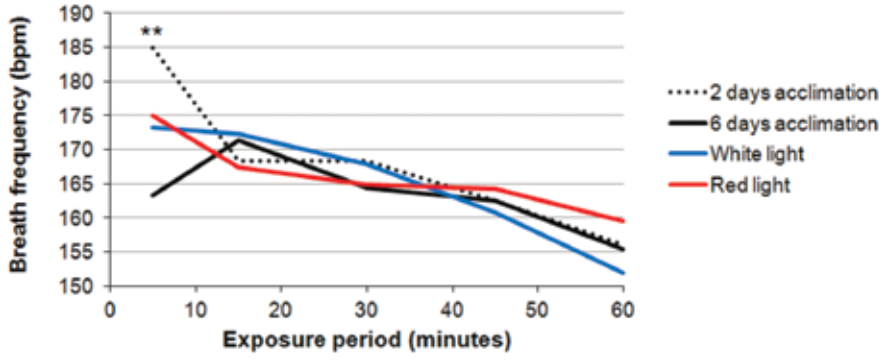

Figure 1. Breath frequency during a one hour inhalation exposure period. **p<0.01 for six-day vs two-day acclimation protocols at five minutes of exposure.

Table 2.

Minute volume (mL) of rats during a one hour inhalation exposure period.

	Acclimation[A]		Lighting conditions[B]	
	2 days	6 days	White	Red
Mean (mL)	258.1	253.8	262.0	254.6
sd	18.4	25.2	22.2	25.2
CV	7%	10%	8%	10%
n	16	16	16	16

No differences of statistical difference (2-way ANOVA)

[A] Acclimation: data for white and red light pooled for statistical analysis

[B] Lighting: data for 2-day and 6-day acclimation protocols pooled for statistical analysis

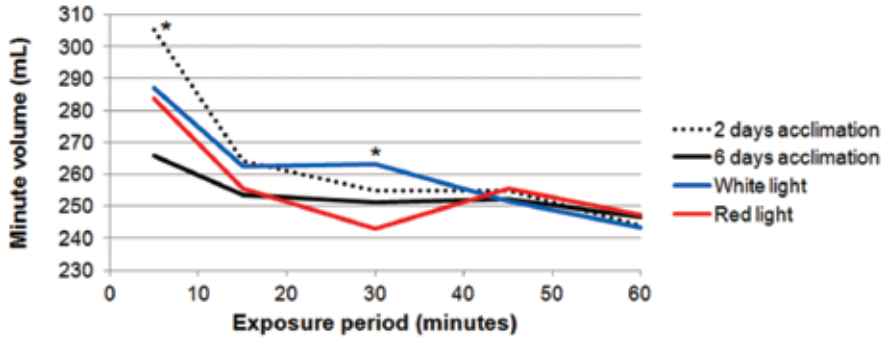

Figure 2. Minute volume during a one hour inhalation exposure period. *p<0.05 for six-day vs two-day acclimation at five minutes; red vs white light at 30 minutes.

The measured MV of rats under the conditions of this study (no pre-exposure settling period) was generally higher and more variable than the body weight-derived estimate of respired minute volume (mean MV ≈ 1.17x eRMV) suggesting a lack of relationship between body weight and MV, or a potential for eRMV to underestimate the "achieved inhaled dose" (Figure 3, Table 3).

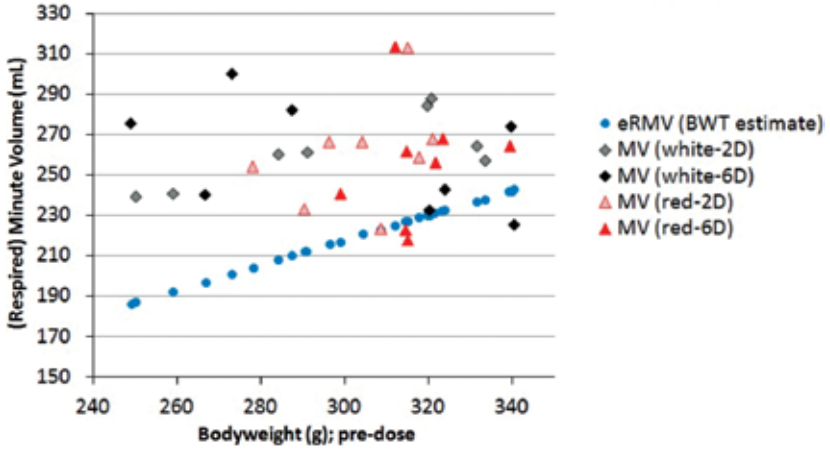

Figure 3. Comparison of the minute volume (MV; measured by head-out plethysmography) and body weight-derived respired minute volume (eRMV).

Table 3.

Comparison of measured and body weight-derived estimates of the minute volume (mL) of rats during a one hour inhalation exposure period.

	Respired minute volume (body weight derived estimate)	Minute volume (measured)
Mean	221	260**
sd	15.9	24.8
CV	7%	10%

**MV vs eRMV; p<0.01 (2-way ANOVA)

Drug-lung homogenate concentrations (Table 4) were more variable than BF and MV. An apparent difference in lung concentrations for red versus white lighting was not corroborated by a similar trend in MV (Table 2) and hence "achieved lung dose."

Table 4.

Lung homogenate concentrations (µg/g) for groups and pooled for lighting conditions or duration of the acclimation protocol.

Pooled data	Acclimation		Lighting conditions	
	2 days	6 days	White	Red
Mean	39.0	35.2	33.5	40.7**
sd	8.19	4.69	4.75	6.81
CV	21%	13%	14%	17%
n	16	16	16	16

** Red vs white light: p<0.01 (2-way ANOVA)
No statistical difference between 2-day and 6-day acclimation protocols

CONCLUSIONS

Results of this study suggest a poor relationship between body weight and MV for relatively small numbers of animals, or a potential for body weight-derived "dose" estimates to underestimate the "achieved inhaled dose." A more pronounced initial transient elevation of breath frequency and MV was evident for rats acclimatized for two days, which could be mitigated if animals are allowed to settle before inhalation exposure. MV and BF were unaffected by red or white lighting. Measurement of MV during non-clinical inhalation studies may refine anomalies in quantitative dosimetry, particularly if respiratory function is affected by treatment. Further work to investigate the relationship between measured MV and body weight increases of rats during repeat "dose" inhalation studies is ongoing.

ACKNOWLEDGEMENTS

The authors thank EMMS (UK) for loan of head-out plethysmography equipment; Charles River Edinburgh (UK; Elaine Rafferty and Steven Oag) for training in plethysmograph restraint; GSK (UK) for resourcing this work, including Mike Denham (statistical support) and Dorian Long. All animal studies were ethically reviewed and conducted in accordance with the Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

REFERENCES

1. Alexander DJ, Collins CJ, Coombs DW, Gilkison IS, Hardy CJ, Healey G, Karantabias G, Johnson N, Karlsson A, Kilgour JD, McDonald P: Association of Inhalation Toxicologists (AIT) working party recommendation for standard delivered dose calculation and expression in non-clinical aerosol inhalation toxicology studies with pharmaceuticals. *Inhal Tox* 2008, 20: 1179-89.

2. Szél Á, Röhlich P: Two cone types of rat retina detected by anti-visual pigment antibodies, *Exp Eye Res* 1992, 55: 47-52.

3. Vijayaraghavan R, Schaper M, Thompson R, Stock MF, Alarie Y: Characteristic modifications of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. *Arch Toxicol* 1993, 67: 478-90.

4. Wright BM: A new dust-feed mechanism. *J Sci Instrum* 1950, 27: 12-15.