

A REVIEW OF THE CHOICE OF RAT STRAIN IN TUMORIGENICITY STUDIES.

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ABSTRACT

Data have been analysed from the control groups of up to 54 tumorigenicity studies, using the major strains of rat currently available and used at these laboratories (Sprague-Dawley Charles River International Genetic Standard [SD CR IGS], Sprague-Dawley Harlan UK [SD HA], Wistar [WI Han] and Fischer 344 [F-344]). These dietary or oral gavage studies were completed over 1993 to 2000. The respective Week 104 mortality values (%) and number of studies (n) are given below:

Sex	SD CR IGS	SD HA	WI Han	F-344
Males	56 (n=15)	44 (n=4)	24 (n=18)	51 (n=13)
Females	68 (n=17)	54 (n=4)	31 (n=20)	36 (n=13)

The CR SD rat was our strain of choice for the majority of rat tumorigenicity studies. However, since the mid 1990's the high mortality in this strain, particularly in female rats, has caused concern and the SD IGS rat, which superseded the original strain, has not shown an improved mortality pattern. A review of the data and advantages and disadvantages of alternative rat strains indicates the WI Han rat as a strain of choice, particularly if the main concern is reaching 2 years with an adequate number of surviving rats, without recourse to single housing and diel manipulation methods. The use of the SD HA and F-344 rat strains are viable, but the mortality generally remains higher than the WI rat.

In conclusion, on the basis of regulatory requirements to reach 2 years with an adequate number of survivors, the Wistar (Han) rat would be the strain of choice.

INTRODUCTION

Data have been analysed from the control groups of up to 54 tumorigenicity studies, using the major strains of rat currently available and used at these laboratories. These strains of rat include the Charles River International Genetic Standard (IGS) Sprague-Dawley (SD CR IGS), the Harlan UK Sprague-Dawley (SD HA), Wistar (WI Han) and Fischer 344 (F-344). These dietary or oral gavage studies were completed over 1993 to 2000 and low protein maintenance diet was used in all the studies.

The CR SD rat was our strain of choice for the majority of rat tumorigenicity studies, particularly after changing to low protein maintenance diet in the mid 1980's [1, 2], with the terminal mortality being below 50% for control male and female rats in the majority of studies. However, since the mid 1990's the increasingly higher mortality pattern noted for this strain, particularly in female rats, has caused concern. The SD CR IGS rat, which superseded the original SD CR strain from 1996, has not shown an improved mortality pattern [3-5]. In this review, the mortality, bodyweight and food consumption patterns of the different strains of rat are compared. The advantages and disadvantages of utilising each rat strain are also discussed.

References:

1. Hooks, W.N. *et al.* The Toxicologist, 13: 419 (1993).
2. Hooks, W.N. *et al.* Toxicology Letters 1/74: 37 (1994).
3. Hooks, W.N. and Hooks, W.N. *et al.* Biological Reference Data on CD (SD) IGS Rats - 1999, edited by Dr T Matsuzawa, Yamanouchi Pharmaceutical Co. Ltd., Japan.
4. Hooks, W.N. and Harling, R.J. The Toxicologist, 54: 270 (2000).
5. Harling, R.J. and Hooks, W.N. The Toxicologist, 54: 270 (2000).
6. Hooks, W.N. Biological Reference Data on CD (SD) IGS Rats - 1998, edited by Dr T Matsuzawa, Yamanouchi Pharmaceutical Co. Ltd., Japan.
7. Davies, T.S. *et al.* Food and Chemical Toxicology 38: 219 (2000).

PROCEDURAL DETAILS

Study design

Strain of rat	Source	Studies completed	Number of studies	Number of animals
Sprague-Dawley (SD CR IGS)	Charles River UK	1998-2000	15 (males) 17 (females)	887 (males) 1007 (females)
Sprague-Dawley (SD HA)	Harlan UK	1997	4 (males) 4 (females)	200 (males) 200 (females)
Wistar (WI Han)	Bioresearch Laboratories, Switzerland or Harlan UK	1994-2000	18 (males) 20 (females)	1130 (males) 1250 (females)
Fischer 344 (F-344)	Charles River USA	1993-1998	13 (males) 13 (females)	735 (males) 735 (females)

- The animals were maintained as control rats for dietary or oral gavage tumorigenicity studies, were approximately 6 weeks of age at start of study and mainly gang housed.
- Ground rodent maintenance diet (Special Diets Services Rat and Mouse No. 1: typically 14.5% protein, 3% fat, 4% fibre) was fed *ad libitum*.
- The studies were maintained under standard laboratory conditions, with target ranges of 19-23° for temperature and 40-70% for relative humidity. A 12 hour light and 12 hour dark cycle was maintained.

Data presentation:

- **Mortality:** The mean terminal (Week 104) mortality is presented in Figure 1. The mortality pattern for males and females is presented in Figures 2 and 3, over the over the period of Weeks 52 to 104 only, as mortality in the first year is low.
- **Bodyweight:** The growth pattern for males and females over 104 weeks is presented in Figures 4 and 5.
- **Food consumption:** The food consumption pattern for males and females over 104 weeks is presented in Figures 6 and 7.
- **Terminal mortality, bodyweight gain and food consumption:** The mean terminal (Week 104) percentage mortality values, the mean bodyweight gain values over Weeks 0 to 52 (the period of maximal growth) and the mean weekly food consumption (g/rat/week) values over the period of Weeks 1 to 52 are presented with standard deviations in Table 1.

Table 1: Mortality, bodyweight gain and mean food consumption values

Rat strain		Mortality (%) Week 104			Bodyweight gain (g) Weeks 0-52			Food consumption (g/rat/week) Weeks 1-52		
		Mean	SD	n	Mean*	SD*	n*	Mean*	SD*	n*
SD CR IGS	M	56	7.4	15	538	70.9	21	204	9.1	21
	F	68	4.9	17	271	22.9	23	153	6.8	23
SD HA	M	44	15.6	4	419	16.9	4	163	4.6	4
	F	54	8.5	4	203	3.7	4	129	3.9	4
WI Han	M	24	7.3	18	347	47.8	24	159	11.2	24
	F	31	7.2	20	158	18.7	24	123	8.8	24
F-344	M	51	8.6	13	277	30.0	13	124	6.5	13
	F	36	10.8	13	121	13.2	13	89	5.6	13

M Male rats, F Female rats, SD Standard deviation, n Number of studies.
* Values include ongoing studies that have reached 52 weeks.

Figure 1: Mortality at Week 104

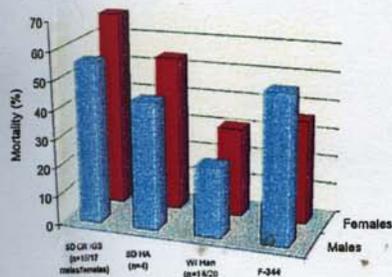


Figure 2: Mortality pattern in male rats

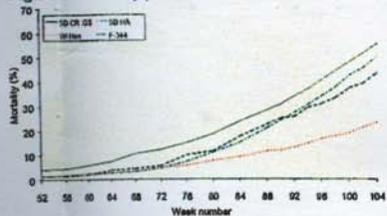


Figure 4: Bodyweight growth pattern in male rats

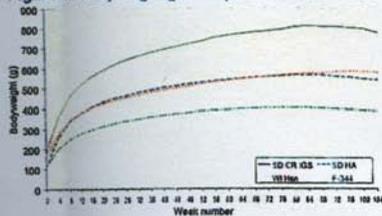


Figure 6: Food consumption pattern in male rats

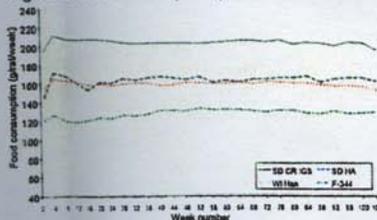


Table 2: Summary of the advantages and disadvantages of the rat strains

Rat strain	Advantages	Disadvantages
SD CR IGS	Vast amount of data available (particularly if the original strain of SD is included). Internationally known strain.	Poor survival at 2 years, particularly in females. Increased group size or a dietary manipulation method required for sufficient survivors at 2 years. Surviving animals are in a geriatric state. High incidence of tumours (e.g. pituitary adenoma and mammary adenoma/adenocarcinoma).
SD HA	Reasonable amount of data available. Survival pattern good in males, reasonable in females. Increase in group size unlikely.	Variable survival at 2 years. Data available relatively small.
WI Han	Large amount of data available. Survival excellent at 2 years. No increase in group size required.	Survival at 2 years is perhaps too good. Strain less well known.
F-344	Large amount of data available. Survival pattern good in females, reasonable in males. Increase in group size unlikely.	Various strain specific pathology (e.g. high incidence of large granular lymphocytic lymphomas and testicular tumours).

Figure 3: Mortality pattern in female rats

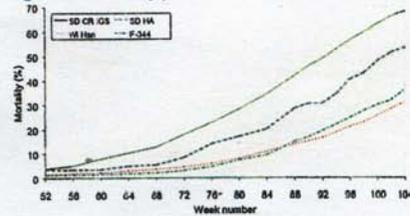


Figure 5: Bodyweight growth pattern in female rats

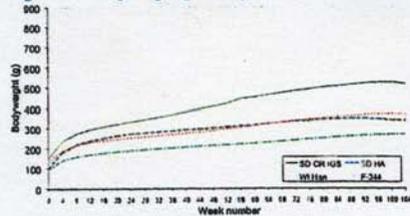
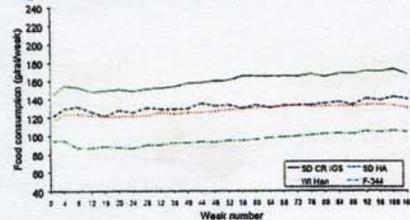


Figure 7: Food consumption pattern in female rats



RESULTS AND DISCUSSION

The CR SD IGS male and female rat studies show the highest mortality, bodyweight and food consumption patterns when compared with the other strains of rat. Conversely, the lowest mortality patterns are seen in WI Han male and female rats, and in F-344 female rats. The lowest bodyweight and food consumption patterns are seen in male and female F-344 rats. The bodyweight and food consumption patterns for male and female SD HA and WI Han rat strains are similar.

The SD CR IGS strain is the most commonly used strain for rat tumorigenicity studies, despite the high mortality pattern. The data accumulated for this strain is vast, particularly if the original strain of CR Sprague-Dawley rat is included. At these laboratories, no remarkable differences have been observed in the in-life parameters or tumour profile between the two Charles River SD rat strain designations [3-6]. However, the high mortality pattern obtained for this strain necessitates the development of strategies to ensure sufficient surviving animals at 2 years. Increasing the group size from the standard minimum of 50 males and 50 females per group to 65 males and 65 females ensures sufficient surviving animals at 2 years to satisfy the regulatory authorities, but increases the study costs and does not agree with the principle of animal use reduction. The Sprague-Dawley strain would be a suitable animal model (without increasing the group size) if the studies were terminated when the 50% mortality point was reached (approximately Week 100 in males and Week 92 in females), treating the sexes separately. This strategy would ensure that studies have reached the current natural lifespan for this strain of rat, ensure that there were sufficient survivors available for terminal tumorigenic evaluation, and decrease the undue influence of geriatric lesions in the pathological examinations. It should be noted, however, that this strategy is not currently acceptable to the regulatory authorities. Although the use of one of the many diet restriction methods to improve survival is now acceptable to the regulatory authorities, this may result in changes the tumour profile of the strain and consequently compromise the available background data. Additionally, the animals in diet restriction studies have to be singly housed and this practice is generally not accepted in the UK and Europe on animal welfare grounds, unless scientifically justified.

The WI Han rat has been used in an increasing number of tumorigenicity studies at these laboratories and the background data available is increasing. The mortality of this strain is always well below 50% at 2 years and therefore no increase in group size is required. The tumour profile is not potentially masked by undue geriatric lesions. In fact, it could be argued that the WI Han strain has not reached its lifespan at 2 years. However, the regulators currently accept termination of studies using this strain at 2 years rather than at the 50% mortality point. Additionally, it has been suggested that tumorigenicity studies need not be conducted beyond 18 months, as any tumorigenic potential would have been realised between 12 and 18 months [7]. This suggestion would also be appropriate for terminating the SD CR IGS rat studies at the 50% mortality point.

The use of the SD HA (as an alternative source of the Sprague-Dawley rat if this strain must be used for developmental reasons) and F-344 rat strains are viable, but the mortality generally remains higher than the WI rat. Additionally, the F-344 rat shows a high incidence of large granular lymphocytic lymphomas and testicular tumours that could potentially mask tumorigenicity.

In conclusion, on the basis of regulatory requirements to reach 2 years with an adequate number of survivors, the Wistar (Han) rat would be the strain of choice.