The Han Wistar rat as an experimental model
Content

- Comparison of carcinogenicity data
- Bodyweight impact on cage density
- Comparison of toxicity data
- Comparison of reproductive toxicity data
- Wistar rats: mGlu2 receptor loss case study
Carcinogenicity studies
Numbers of animals per group and termination procedures

- Each test guideline for lifespan studies require a minimum of 50 males and 50 females per group.
- However, there are also considerations that need to be accounted for in relation to the number of animals that should survive to the end of the treatment period.
- Mortality rates for the selected strain of rat or mouse must therefore be taken into consideration in selecting the group size to ensure acceptability of the study.
### Mortality patterns (from studies at Envigo 1995-2015)

+ **Summary of survival (%) for most commonly used animal models**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Envigo Han Wistar rat</td>
<td>73</td>
<td>60-85</td>
</tr>
<tr>
<td>CR Sprague-Dawley rat</td>
<td>46</td>
<td>28-71</td>
</tr>
</tbody>
</table>

+ **Above information in terms of No. animals/group based on 50/sex/group**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Envigo Han Wistar rat</td>
<td>36</td>
<td>30-43</td>
</tr>
<tr>
<td>CR Sprague-Dawley rat</td>
<td>23</td>
<td>14-35</td>
</tr>
</tbody>
</table>
Termination criteria

+ Pharmaceuticals
  + Ideally there should be 50% survival beyond 18 months
  + Termination criteria involves dialogue with US FDA
  + “Usual” guidance is:
    + High dose group: discontinue treatment at 20 survivors and terminate group on reaching 15 survivors
    + Low/intermediate dose groups: discontinue treatment at 20 survivors and terminate gender when one of these groups reaches 15 survivors
    + Control group: on reaching 20 survivors terminate all groups of that gender

(But do not depend on this as there have been variations on this theme.)
Termination criteria

+ Chemicals / agrochemicals
  + Survival should be above 50% at 15 months in mice and at 18 months in rats and above 25% at 18 months in mice and at 24 months in rats (USEPA Guideline 870.4200)
  + The OECD guidance (Guidance Document 116; April 2012) is:
    + High dose group: terminate gender when survival falls below 25% (12 animals in a 50/sex/group study)
    + Control/low/intermediate dose groups: terminate gender when survival falls below 25% (12 animals in a 50/sex/group study) in any one of these groups
Termination criteria

Intentional food additives (USFDA Redbook 2000)

+ “The Agency recommends that petitioners / notifiers carefully consider their choice of rat strains for carcinogenicity bioassays, since some strains have more serious problems with survival than other strains.
+ It is recommended that carcinogenicity studies begin with at least 50 animals per sex/group.
+ Petitioners / notifiers are encouraged to begin bioassays with more than 50 animals per sex per group if survivorship is expected to be a problem with the rat strain used in the study.
+ If fewer than 25 animals per sex per group are expected to survive to the end of the study (24 months), petitioners / notifiers should take particular care to ensure and document early detection of dead animals through attentive and frequent cage-side observations, thus minimizing the loss of tissues from autolysis.
+ In addition, they should consult with the Agency as soon as a problem with survivorship in a carcinogenicity study becomes apparent.”
## Recommended group sizes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Route</th>
<th>Group size</th>
<th>Minimal survival†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Han Wistar rat</td>
<td>Diet</td>
<td>50/sex/group</td>
<td>30 25</td>
</tr>
<tr>
<td></td>
<td>Other routes (inc. inhalation)</td>
<td>55/sex/group</td>
<td>32 28</td>
</tr>
<tr>
<td>Sprague-Dawley rat</td>
<td>All routes</td>
<td>65-70/sex/group</td>
<td>18-20 11-40</td>
</tr>
</tbody>
</table>

† Based on lowest observed survival in previous studies
New EU Legislation - Bodyweight impact on cage density
On 22 September 2010 the EU adopted Directive 2010/63/EU which updates and replaces the 1986 Directive 86/609/EEC on the protection of animals used for scientific purposes. The aim of the new Directive is to strengthen legislation, and improve the welfare of those animals still needed to be used, as well as to firmly anchor the principle of the 3 Rs, to Replace, Reduce and Refine the use of animals, in EU legislation. Directive 2010/63/EU took full effect on 1 January 2013.
On 1 January 2017 new minimum standards for caging come into force in the EU.

<table>
<thead>
<tr>
<th>Weight of animal (g)</th>
<th>Minimum floor area per gang housed animal (cm²)</th>
<th>Permitted animals per cage (P2000, Cage floor area 2090 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>200</td>
<td>10.45</td>
</tr>
<tr>
<td>200-300</td>
<td>250</td>
<td>8.36</td>
</tr>
<tr>
<td>300-400</td>
<td>350</td>
<td>5.97</td>
</tr>
<tr>
<td>400-600</td>
<td>450</td>
<td>4.64</td>
</tr>
<tr>
<td>&gt;600</td>
<td>600</td>
<td>3.48</td>
</tr>
</tbody>
</table>

In long-term studies, if space allowances per individual animal fall below those indicated above towards the end of such studies, priority shall be given to maintaining stable social structure.
Impact on cage density for Envigo, Huntingdon and Eye

<table>
<thead>
<tr>
<th>Study Duration (age of rats)</th>
<th>Envigo Han Wistar</th>
<th>Sprague-Dawley (CD) rat</th>
<th>Charles River - Wistar Han Crl:WI(Han)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 6 weeks (~12 weeks of age)</td>
<td>5/cage</td>
<td>5/cage</td>
<td>5/cage</td>
</tr>
<tr>
<td>13 weeks (~20 weeks of age)</td>
<td>5/cage</td>
<td>4/cage</td>
<td>5/cage</td>
</tr>
<tr>
<td>26 weeks (~32 weeks of age)</td>
<td>5/cage</td>
<td>4/cage</td>
<td>4/cage</td>
</tr>
<tr>
<td>52 weeks</td>
<td>4/cage</td>
<td>4/cage</td>
<td>4/cage</td>
</tr>
<tr>
<td>104 weeks</td>
<td>4/cage</td>
<td>4/cage</td>
<td>3/cage</td>
</tr>
</tbody>
</table>
New European legislation

+ Our (Envigo, UK) current standard is to house rats 5/cage in Tekniplast P2000 cages
+ From 1 Jan 17, rats over 400g will need to be housed 4/cage
+ Animals over 600g should be housed 3/cage. However, because of the poor survival in the SD rat, 4/cage rather than 3/cage should be used to avoid having individually housed animals in the latter stages of a study
+ May have an impact on the room(s) a study may take which could impact price
<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sprague Dawley</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>790</td>
<td>500</td>
</tr>
<tr>
<td><strong>Han Wistar</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>600</td>
<td>360</td>
</tr>
</tbody>
</table>
Comparative bodyweight

- Male SD Rats
- Female SD Rats
- Male HW Rats
- Female HW Rats

Bodyweight (g) vs. Week Number
Comparative mortality

- Male SD Rats
- Female SD Rats
- Male HW Rats
- Female HW Rats

Mortality (%) vs. Week Number
Incidence of most common tumours (>4%) males

- Pancreas: Islet cell (B)
- Mesenteric LN: hemangiolma (B)
- Thymus: thymoma (B)
- Thyroid: follicular cell (B)
- Thyroid "c" cell (B)
- Adrenal medulla (B,M)
- Pituitary: distalis (B)
- Skin: fibroma (B)
- Skin: keratoacanthoma (B)

Legend:
- Male SD Rats
- Male HW Rats
Incidence of most common tumours (>4%) females

- Thymus: thymoma (B)
- Thyroid: c cell (B)
- Adrenal: medulla (B)
- Pituitary/diabetes (B.M)
- Mammary fibroadenoma (B)
- Mammary adenoma (B)
- Mammary adenocarcinoma...
- Uterus: polyp (B)

Incidence (%)
Tumours with most differences - males

Incidence (%) - Males SD Rats vs. Males HW Rats

- Liver: hepatocellular (B,M)
- Pancreas: acinar cell (B)
- Mesenteric LN: hemangio (B)
- Thymus: thymoma (B)
- Thyroid: follicular cell (B)
- Adrenal medulla (B,M)
- Skin: fibroma (B)
- Skin: lipoma
- Skin basal cell (B)
- Testis: Leydig cell (B)
- Lymphoid: histiocytic-sarcoma
- Lymphoid: Ail (M)
Tumours with most differences - females

![Bar chart](chart.png)
Chronic Progressive Nephropathy

- Incidence (%)
- Males
- Females

- Sprague Dawley
- Han Wistar
Cardiomyopathy

Incidence (%) vs. Time

- 13 wk studies
- 26 wk studies
- 104 wk studies

Males
- Sprague Dawley
- Han Wistar

Females
- Sprague Dawley
- Han Wistar
4-, 13- or 26-week studies may have already been conducted in SD rats before the carcinogenicity ‘package’ comes to Envigo.

The FDA is expecting at least a 13-week study in the same strain when carcinogenicity protocols are presented to CAC for their review.

A change of strain consequently requires an additional rat study of 13-weeks duration:

- ↑ number animals used for the full programme
- could delay the carcinogenicity study by 6 months or more (this might not be acceptable to the clinical or marketing program)
Other study types - General toxicity
28 day study

+ Total number of rats on study (8-10 wk old rats)
+ Han Wistar rats are smaller, lower blood draw per rat

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Tox</th>
<th>TK</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>80</td>
<td>42</td>
<td>122</td>
</tr>
<tr>
<td>HW</td>
<td>80</td>
<td>60</td>
<td>140</td>
</tr>
</tbody>
</table>

TK: 6 timepoints (0.5 mL/timepoint), 2 occasions
Extra 18 TK HW rats
Solution: microsampling
### Number of on-study rats*

<table>
<thead>
<tr>
<th>Study</th>
<th>SD rat</th>
<th>HW rat</th>
<th>Change strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4 week tox</td>
<td>80 Tox 42 TK</td>
<td>80 Tox 60 TK</td>
<td></td>
</tr>
<tr>
<td>13-week tox</td>
<td></td>
<td></td>
<td>80 Tox 60 TK</td>
</tr>
<tr>
<td>26-week tox</td>
<td>120 Tox 42 TK</td>
<td>120 Tox 60 TK</td>
<td></td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>560 65/sex/gp</td>
<td>440 50/sex/gp</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>844</td>
<td>760</td>
<td>900</td>
</tr>
</tbody>
</table>

* Assuming all are ‘standard’ 4-group oral gavage studies
Summary

+ **Body weights**
  + HW is 20-35% lighter in weight than SD rats at the end of a carcinogenicity study

+ **Survivability**
  + Survivability of HW is much better than in SD and so ensure completion of studies with confidence

+ **Pathology**
  + Similar pattern of background tumors in SD vs HW. But incidence higher in SD
Summary (2)

- Rat strain selection for regulatory toxicology studies is the customer’s decision
  - Prior experience with the TA or related TAs
- Switching in the middle of drug development
  - generally not advisable → addition of a bridging study will slow the process
- New program (up to carcinogenicity) starting with HW
  - Customer: cost effective
  - CRO: ↓ labour, ↑ housing efficiency, better animal welfare
Reproductive toxicity

+ No major positive or negative reasons to favour Han Wistar or Sprague Dawley

+ Han Wistar has smaller litter size because the body weight is lower
  + lower litter size could be less useful for juvenile toxicity studies as number of pups per endpoint assessed is lower
### Popularity of Han Wistar rats – toxicity / carcinogenicity studies (UK)

<table>
<thead>
<tr>
<th>Study Type</th>
<th>CD (Sprague)</th>
<th>Han Wistar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary studies</td>
<td>105</td>
<td>44</td>
</tr>
<tr>
<td>Repeat dose studies</td>
<td>145</td>
<td>65</td>
</tr>
<tr>
<td>Carcinogenicity studies</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Studies completed 2009 – 2013
# Popularity of Han Wistar rats – reproductive toxicity studies UK*

<table>
<thead>
<tr>
<th>Study Type</th>
<th>CD (Sprague)</th>
<th>Han Wistar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryofetal toxicity</td>
<td>44</td>
<td>18</td>
</tr>
<tr>
<td>Fertility and embryofetal development</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Pre/post natal</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>One or two generation</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Neonatal/juvenile</td>
<td>20</td>
<td>6</td>
</tr>
</tbody>
</table>

*Main plus preliminary studies combined count

Studies completed 2009 – 2013

*Main plus preliminary studies combined count
Excellent opportunities to assess functional roles of mGlu2 and mGlu3 receptors

+ The metabotropic glutamate 2 (mGlu2) receptor belongs to the family of G-protein coupled glutamate receptors that modulate transmission at synapses throughout the mammalian central nervous system, and that have been proposed as major targets for the development of drugs for human psychiatric and neurological diseases.

+ One of the issues has been that orthosteric agonists and antagonists do not separate between mGlu2 and mGlu3 receptors which have different, and possibly opposing effects. To overcome this a new selective mGlu2 receptor agonist, LY395756, and its active enantiomer, LY541850 was used to separate between the roles of mGlu2 and mGlu3 receptors in synaptic events. However it was found that many of the outbred Wistar rats studied were unresponsive to the selective mGlu2 agonist; this was traced to the lack of mGlu2 receptor expression in some Wistar rats.
cys407* mutation within the Grm2 gene and loss of mGlu2 receptor

CM Wood et al Neuropharmacology (2016)
Metabotropic glutamate 2 (mGlu2) receptors in Han Wistar rat

- Stop codon mutation at cysteine 407 in Grm2 gene
- Widespread genotypic survey shows high prevalence in some Wistar rat strains
- Particularly noted in Han Wistar
- Mutant rats lack mGlu2 receptors but retain mGlu3
- Excellent opportunities to assess functional roles of mGlu2 and mGlu3 receptors
- Provides insight into historical data interpretation
mGlu2 receptor deficiency and rodent behaviours

+ Generally lower activity but higher risk taking
+ Increase anxiety traits
+ Increased alcohol intake
+ mGlu2 receptors activation decreases dopamine release and decrease reward in self administration studies. Lack of receptor implies increased reward e.g. for alcohol
Because of the demonstrated potential of the mGlu2 receptor as a therapeutic target, this finding is of critical importance to the research community. Immediately questions arise as to:

- Why is the mGlu2 receptor missing from some rats – discussed previously
- How frequently does this occur in populations of rats used in laboratory studies
Dendrogram showing simplified scheme of derivation of commercial strains of Wistar, Han Wistar and Dark Agouti rats

CM Wood et al Neuropharmacology (2016)
### Prevelance of mutation from supplier samples

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Strain</th>
<th>Mutation</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>B &amp; K</td>
<td>Wistar</td>
<td>100% mutant</td>
<td>14</td>
</tr>
<tr>
<td>Harlan Israel</td>
<td>Wistar</td>
<td>100% mutant</td>
<td>48</td>
</tr>
<tr>
<td>Harlan USA</td>
<td>Wistar</td>
<td>100% wild type</td>
<td>48</td>
</tr>
<tr>
<td>CR UK</td>
<td>Wistar</td>
<td>100% wild type</td>
<td>50</td>
</tr>
<tr>
<td>Harlan UK USA and Israel</td>
<td>HSD Han Wistar</td>
<td>100% mutant</td>
<td>50, 50, 18</td>
</tr>
<tr>
<td>Harlan UK</td>
<td>RCC Han Wistar</td>
<td>83% mutant 17% hetero</td>
<td>6</td>
</tr>
<tr>
<td>CR UK</td>
<td>CR Han Wistar</td>
<td>35% mutant 60% hetero 5% wild type</td>
<td>20</td>
</tr>
<tr>
<td>CR France</td>
<td>CR Han Wistar</td>
<td>40% mutant 55% hetero 5% wild type</td>
<td>20</td>
</tr>
<tr>
<td>Taconic</td>
<td>T Han Wistar</td>
<td>73% mutant 27% hetero</td>
<td>15</td>
</tr>
</tbody>
</table>
Implications

+ Supplier variation important for selection of strains for certain types of study

+ Key importance to recognise the potential presence of the mutation for behavioural pharmacology studies

+ Provides a valuable tool for exploring the therapeutic potential for mGlu2 and 3 receptor ligands
Because of its low bodyweight and subsequent longevity, the HW rat is preferable for carcinogenicity studies. Lower bodyweight may reduce impact of new housing density regulations. Introduction of microsampling may reduce the number of animals used for Tk analysis. No real reason to use HW or SD, although smaller litter size for HW should be noted. mGlu2 provides a valuable tool for exploring the therapeutic potential for mGlu2 and 3 receptor ligands.
ありがとうございます
- 何か質問は？