ENVIGO

WHITE PAPER // SEPTEMBER 2018

The Wistar Hannover rat for carcinogenicity studies

A more effective model

Introduction

Gaining regulatory approval of investigational new drugs and chemicals requires extensive safety analyses, including, where appropriate, two-year carcinogenicity testing in two rodent species for pharmaceuticals intended for chronic (i.e., ≥ 6 months) or chronic intermittent use, crop protection products, biocides, veterinary medicines, and industrial chemicals (produced at high tonnage levels (i.e., ≥ 1,000 tons/year)).

Since regulatory guidelines across the globe do not mandate a standard rat strain for carcinogenicity studies, a variety of strains are commonly used, with the most prominent being the Wistar Hannover (e.g., RccHan®:WIST, HsdHan®:WIST) and Sprague Dawley (e.g., Hsd:Sprague Dawley® SD®).

The Wistar Hannover rat is a widely used toxicology model in Europe and Japan and also used in the US by various multinational companies. This is based on numerous advantages that this strain offers relative to other strains, including *smaller body size, longer survival*, and *lower overall tumor incidence*. Indeed, the Wistar Hannover rat is used for the nonclinical safety assessment of many types of substances across many sectors, including medicines (e.g., Healing, 2016; Maliver, 2017; York, 2007), industrial chemicals (e.g., Guérard, 2018; Collí-Dulá, 2016; Fennell, 2015), foods (e.g., Auñon-Calles, 2013; Zeljenková, 2014), crop protection products (e.g., Hester, 2006; Wolf, 2006), and environmental hazards (e.g., Klose, 2014; Pothmann, 2015) among others.

The advantageous features of the strain can translate to better long-term study outcomes, better adherence to the guiding principles of the Three Rs, and improved cost-effectiveness. Notably, the cost-effective benefits arise from multiple perspectives:

- Less test article may be required
- Fewer animals required at the beginning of the study (allowing for decreased *per diem* and overall housing and husbandry cost savings)
- Potential for less time per animal for pathology due to lower overall tumor incidence (this can vary depending on the industry being served)

Over 20 years, Envigo has amassed millions of historical control data (HCD) points for the Wistar Hannover rat, including 104-week survival and growth, spontaneous neoplasm and non-neoplasm at multiple time points, and fetal development. In addition, Envigo has completed a robust North-America-based two-year study that evaluated the growth, survival rate, and tumor incidence in the Wistar Hannover rat. In our experience, this vast database of baseline information is a key decision factor when researchers are considering which rat strain to use for their carcinogenicity studies, since HCD are critical to distinguishing possible test compound effects from background effects that might be inherent to the animal model itself (Blankenship, 2013).

The purpose of this paper is to provide key information and insights on the Wistar Hannover rat, including the following:

+ A summary of its origin and applications
+ A summary of the most salient HCD
+ Regulatory precedent for using this strain in carcinogenicity studies
+ Factors to consider when selecting a rat strain for carcinogenicity studies

Selecting the optimal rat strain for carcinogenicity testing can translate to improved study outcomes, conserved money and resources, better overall animal welfare, and, ultimately, potential for expedited drug development and new chemical authorization.

Origin and advantages of the Wistar Hannover rat

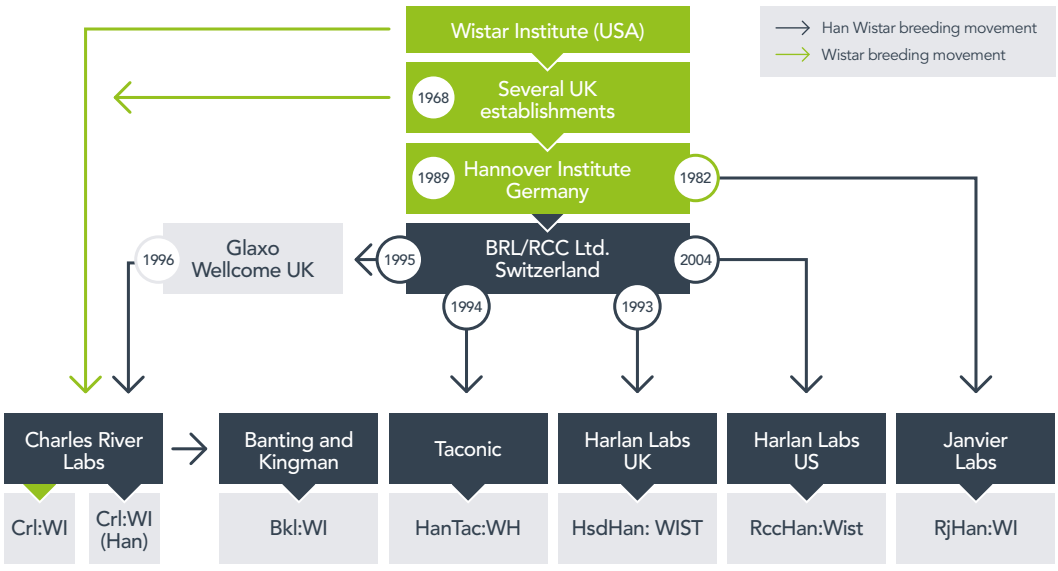
Origin

The Wistar Hannover source colony was originally obtained by the Institute for Biomedical Research and received and maintained by Harlan Laboratories via acquisition of RCC (formerly Biological Research Laboratories Limited (BRL) of Füllinsdorf, Switzerland).[1]

This source colony was established with the original 156 breeder pairs from BRL. All current global colonies of this model were derived from this original Füllinsdorf colony. Envigo employs a global breeding program for the stock of this Wistar Hannover strain using a Poiley rotational system, which reduces inbreeding and maintains maximum heterogeneity between animals in the colony. All colonies are genetically tested annually using a custom 96 single nucleotide polymorphism (SNP) marker panel to assess allelic frequencies within and between colonies worldwide (Wistar Han Outbred Rat, 2017).

While several commercial entities have established colonies derived from the original Wistar Hannover strain (e.g., RccHan®:WIST, Crl:WI(Han) and RjHan:WI) (see Figure 1), only Envigo provides the original Wistar Hannover stock maintained by strict breeding principles and backed up by over 20 years of HC data (Wistar Han Outbred Rat, 2017).

Figure 1: Simplified scheme depicting the derivation of commercial strains of Wistar Hannover and Wistar rats. Adapted from Wood et al. (2017).



[1]Harlan became Envigo in 2015

Advantages

The benefits of rodents in carcinogenicity testing have long been recognized, going back at least 100 years (*See* reviews by Kemp, 2016 and Kacew, 1996). There are general features shared by many rat strains that make them advantageous for nonclinical safety studies, including anatomical, physiological, and metabolic pathway similarities to humans and the ease of breeding and maintenance at low cost (Kacew, 1996).

However, as indicated earlier, specific attributes (including smaller body size, longer survival, and lower overall tumor incidence) that are unique to Wistar Hannover rats make it an exceptional strain for carcinogenicity testing, relative to other rat strains commonly chosen for such studies. These attractive characteristics have been documented in the peer-reviewed literature, which is consistent with Envigo's proprietary HCD. For instance, as in Envigo's data, it was shown that relative to Sprague Dawley (and Fischer F344) animals, Wistar Hannover rats have a lower body weight, longer survival, and lower overall occurrence of spontaneous neoplastic and non-neoplastic lesions (Weber, 2017; Weber, 2011).

Lower body weight is beneficial since less of the test article can be used, which allows for lower *per diem* and overall cost savings related to housing and husbandry. Survival rate is also a key feature that must be considered prior to initiating a carcinogenicity study. Enhanced survival is important because it can provide confidence that the study will be successfully completed and may reduce the initial number of animals enrolled by minimizing attrition during the study. This can also help researchers adhere to the guiding principles of the Three Rs. Lastly, the lower overall spontaneous tumor incidence provides not only the potential for greater certainty of the measure of carcinogenicity attributable to the test article but also less time per animal for pathology. Taken together, these properties have helped position the Wistar Hannover rat as the primary choice for carcinogenicity testing since it is a model that meets all the technical and scientific criteria for effective study execution.

The following section provides a sample of the HC data available for Envigo's Wistar Hannover rat.

Historical control data

As noted previously, over 20 years, Envigo has amassed a huge number of HCD points for the Wistar Hannover rat.

Survival

Figure 2 shows survival data for control animals collected during 50 carcinogenicity studies. Overall, at 104 weeks, the mean survival rate is 72% and 65% for males and females, respectively.

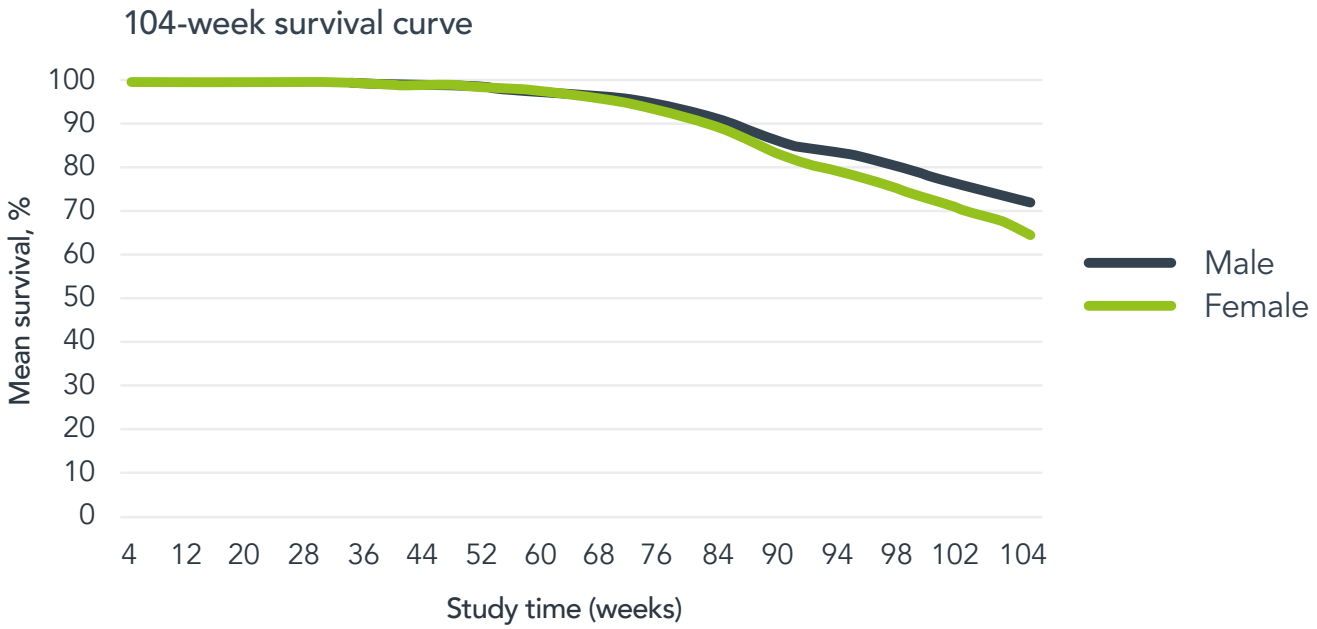


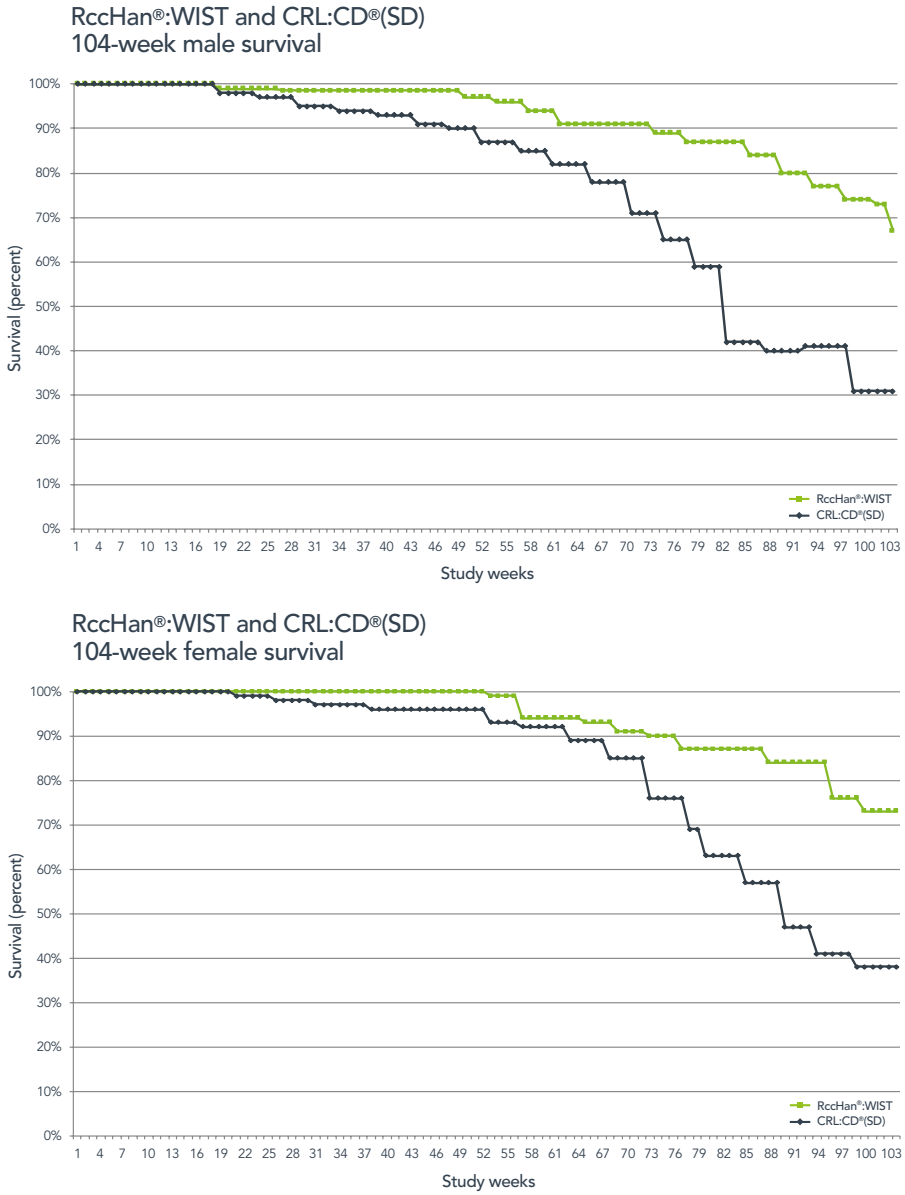
Figure 2: Survival at 104 study weeks of both male and female Wistar Hannover rats.
Data are based on 50 carcinogenicity studies at UK sites of Envigo for the period 1996–2018.

Additional data continue to be generated, and recently, Envigo completed a North-America-based two-year study to evaluate the growth, survival rate, and tumor incidence of the Wistar Hannover rat. These data were compared to body weight and survival data previously collected for a Sprague Dawley strain (CRL:CD®(SD)).

As shown in Figure 3 (top), the survival advantage offered by Wistar Hannover rats is striking. By week 104, the survival rate of Wistar Hannover males was almost 70%, compared to only 31% for the CRL:CD®(SD) rat. Similarly, Figure 3 (bottom) shows a 104-week survival rate of 73% for Wistar Hannover females, compared to only 38% for the CRL:CD®(SD) rat.

Survival (cont.)

Figure 3: Survival curves for Wistar Hannover and CRL:CD®(SD) rats over 104 weeks. (Top) Survival curves for male Wistar Hannover and CRL:CD®(SD) rats, and (Bottom) survival curves for female Wistar Hannover and CRL:CD®(SD) rats.



Overall, based on Envigo's analysis of all HCD, the North American data are consistent with the larger body of data collected over 20 years.

Additional available historical control data

The following HCD are available for the Wistar Hannover for 20 years:

- Incidence of neoplastic lesions
- Food consumption
- Organ weight
- Complete blood count (CBC) profiles
- Serum chemistry profiles
- Ophthalmoscopic (including slit lamp) examination results
- Urinalysis results
- Physiologic results
- Genetics and phenotypes
- Reproduction and development
- Images of neoplastic and non-neoplastic changes at 3, 6, 12, and 24 months

Data collected as part of the Wistar Hannover North American study included the following:

- Incidence of neoplastic lesions
- Food consumption
- Organ weight
- CBC profiles
- Serum chemistry profiles
- Urinalysis results

All available HCD for the Envigo Wistar Hannover rat can be downloaded from:

www.envigo.com/products-services/research-models-services/models/rcchanwist-background-data/

Regulatory precedent

As indicated earlier, the Wistar Hannover rat is the strain of choice for toxicological and carcinogenicity studies in Europe and Japan, and it has been relied upon for countless regulatory submissions in these regions for various types of agents across different commercial sectors.

Numerous multinational companies also use the Wistar Hannover in the US to form the basis of their nonclinical safety data submitted to regulatory agencies. For instance, in the pharmaceutical sector, US companies and European companies with a presence in the US have employed the Wistar Hannover for their nonclinical safety data, including carcinogenicity evaluation. Below are two examples of FDA-approved medications wherein the sponsors employed the Wistar Hannover rat for their two-year carcinogenicity studies.

Gattex (teduglutide): NPS Pharmaceuticals

Teduglutide is a peptide analog of glucagon-like peptide-2 (GLP-2). A single amino acid substitution provides resistance to *in vivo* degradation of teduglutide by dipeptidyl protease-IV (DPP-IV), resulting in an extended half-life. It is FDA approved for the treatment of adult patients with short bowel syndrome who are dependent on parenteral support.

As part of the nonclinical toxicology evaluation of teduglutide, a two-year carcinogenicity study (subcutaneous route) was conducted in Wistar Hannover rats using doses of 3, 10, and 35 mg/kg/day. According to publicly available regulatory submission documents, teduglutide caused a statistically significant increase in the incidences of adenomas in the bile duct and jejunum of male rats, while there were no drug-related tumor findings in females.[2]

After reviewing the complete submission package, the FDA approved teduglutide for marketing.

[2]https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203441orig1s000medr.pdf
[3]https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/204629orig1s000pharmr.pdf

Jardiance® (empagliflozin): Boehringer Ingelheim

Empagliflozin is a highly potent, selective, competitive inhibitor of sodium-glucose cotransporter 2 (SGLT2). It is FDA approved as a treatment for Type 2 diabetes in patients with normal kidney function.

As part of the nonclinical toxicology evaluation of empagliflozin, a two-year carcinogenicity study (oral gavage) was conducted in Wistar Hannover rats using doses of 100, 300, and 700 mg/kg. According to publicly available regulatory submission documents, empagliflozin did not increase the incidence of tumors in female rats at any dose. However, in male rats, the drug increased the incidence of whole body/cavity hemangioma, which was statistically significant only at the highest dose (700 mg/kg). Empagliflozin also increased testicular Leydig cell tumors in the 300 and 700 mg/kg groups.[3]

After reviewing the complete submission package, the FDA approved empagliflozin for marketing. Overall, there is strong regulatory precedent for the use of the Wistar Hannover rat in nonclinical toxicology assessments, including two-year carcinogenicity studies. Safety assessments relying upon this strain have led to successful drug approvals in Europe, Japan, and the US.

Key considerations when selecting a rat strain for carcinogenicity studies

In planning a rat carcinogenicity study, you must consider several factors when choosing an appropriate strain that will provide the best opportunity for robust study results and the ability to complete the study within the allocated budgets and resources.

To select the optimal model for your study, you should take the following key factors into consideration:

- HCD with a minimum of 3–5 years of data
- Selection of your strain at the earliest stage possible to avoid delays, repeated studies, or bridging studies
- Survival rate, body weight, and lesion incidence (These can have a significant impact on animal welfare and the financial cost of a study. You should consider the costs of the test article, vivarium space, and technician time.)
- Solid regulatory precedent

Conclusions

The Wistar Hannover rat is the toxicology model of choice in many parts of the world. It has successfully been used for the nonclinical safety assessment, including two-year carcinogenicity studies, of many types of substances across many sectors.

The wide-spread use of this strain is based on several advantages that it provides in comparison to other strains, including *smaller body size, longer survival*, and *lower overall tumor incidence*. These advantages can translate to better long-term study outcomes, better adherence to the guiding principles of the Three Rs, and improved cost-effectiveness.

Envigo has amassed millions of HCD points for the Wistar Hannover rat, including 104-week survival and growth, spontaneous neoplasm and non-neoplasm at multiple time points, and fetal development. In addition, Envigo has completed a North-America-based two-year study that evaluated the growth, survival rate, and tumor incidence in the Wistar Hannover rat.

Selecting the Wistar Hannover rat for two-year carcinogenicity studies is a solid choice that can generate robust study results and increase the likelihood of completing a study within budget.

To learn more about Envigo's Wistar Hannover rat or to begin a conversation about your project with Envigo experts, please visit envigo.com/WistarHan.

Authors

Alan Broadmeadow – Senior Toxicologist, Envigo

BTech (Hons), DipRCPath (Toxicology), Eurotox Registered Toxicologist

Alan is originally from Beverley in the East Riding of Yorkshire and joined Envigo in 1977 after graduating with a BTech honours degree at the University of Bradford, West Yorkshire, England. He holds the Diploma of the Royal College of Pathologists in Toxicology, is a Eurotox Registered Toxicologist and a member of the British Toxicology Society.

Alan is based at the Envigo laboratories at Eye, Suffolk, England and has over 41 years of experience in general toxicology studies in multiple species, by a range of routes and with study durations up two years (carcinogenicity studies). Alan is a Toxicologist for a variety of animal studies and projects conducted within the Division of Safety Assessment at the Eye and Huntingdon sites of Envigo and on either of these two sites, and co-ordinates toxicology programmes for a number of sponsors, particularly those in the crop protection sector. Alan provides a scientific advisory and problem solving service to Study Directors throughout the Toxicology Division, and assists the Business Development and Programme Management functions with the scientific aspects of enquiry handling and also in the provision of consultancy for projects not involving the running of studies at Envigo.

He interacts with clients in the pharmaceutical, agrochemical and food industries in Europe, Japan and the U.S.A. and has expertise in the design of studies for regulatory submission for all of these business sectors and for all of these regions. He specialises in carcinogenicity and dermal toxicity studies and studies conducted in the common marmoset.

Alan comments on, and/or co-ordinates responses from within Envigo, to any OECD guideline change relating to the Testing of Chemical guidelines. Alan is the named deputy for the UK Home Office Project Licence for toxicity and tumourigenicity studies in rodents.

Authors (cont.)

Mandy Horn – Scientist Veterinary Science, Research and Support, Envigo

MS – Animal Sciences

Mandy Horn is a Scientist working in the North America Veterinary Science, Research and Support department at Envigo, and also serves on the IACUC.

She received her Master of Science degree in Animal Sciences from Purdue University in 2008 with a focus on ruminant nutrition and reproduction, and began her career in the laboratory animal industry shortly thereafter. At Envigo, she is responsible for managing the North America VSRS team, including internal and external project management, model-related technical support, and health monitoring of internal colonies.

During her tenure at Envigo, she has presented on several topics including Envigo small animal models, biosecurity, health monitoring, and animal husbandry at local and national scientific meetings, further supporting Envigo's scientific excellence.

References

1. Auñon-Calles, D., Canut, L., & Visioli, F. (2013). **Toxicological evaluation of pure hydroxytyrosol.** Food and chemical toxicology, 55, 498-504.

2. Blankenship, B., and H. Skaggs. 2013. **'Findings in Historical Control Harlan RCCHanTM: WIST Rats from 4-, 13-, 26-Week Studies'**, Toxicol Pathol, 41: 537-47.

3. Collí-Dulá, R. C., Friedman, M. A., Hansen, B., & Denslow, N. D. (2016). **Transcriptomics analysis and hormonal changes of male and female neonatal rats treated chronically with a low dose of acrylamide in their drinking water.** Toxicology reports, 3, 414-426.

4. Fennell, T. R., Snyder, R., Hansen, B., & Friedman, M. (2015). **Dosimetry of acrylamide and glycidamide over the lifespan in a 2-Year bioassay of acrylamide in Wistar Han rats.** Toxicological Sciences, 146(2), 386-394.

5. Guérard, M., Marchand, C., Funk, J., Christen, F., Winter, M., & Zeller, A. (2018). **DNA damage response of 4-chloro-ortho-toluidine in various rat tissues.** Toxicological Sciences, 163(2), 516-524.

6. Healing, G., Sulemann, T., Cotton, P., Harris, J., Hargreaves, A., Finney, R., Burdett, L. (2016). **Safety data on 19 vehicles for use in 1 month oral rodent pre-clinical studies: administration of hydroxypropyl-ß-cyclodextrin causes renal toxicity.** Journal of Applied Toxicology, 36(1), 140-150.

7. Hester, S.D., Wolf, D.C., Nesnow, S.,Thai, S. (2006) **Transcriptional Profiles in Liver from Rats Treated with Tumorigenic and Nontumorigenic Triazole Conazole Fungicides: Propiconazole, Triadimefon, and Myclobutanil.** Toxicologic Pathology, 34(7): 879-894.

8. Kacew, S., and M. F. Festing. 1996. **'Role of rat strain in the differential sensitivity to pharmaceutical agents and naturally occurring substances'**, J Toxicol Environ Health, 47: 1-30.

9. Kemp, C. J. (2015). **Animal models of chemical carcinogenesis: driving breakthroughs in cancer research for 100 years.** Cold Spring Harbor Protocols, 2015(10), pdb-top069906.

10. Klose, M., Grote, K., Spathmann, O., Streckert, J., Clemens, M., Hansen, V. W., & Lerchl, A. (2014). **Effects of early-onset radiofrequency electromagnetic field exposure (GSM 900 MHz) on behavior and memory in rats.** Radiation research, 182(4), 435-447.

11. Maliver, P., Festag, M., Bennecke, M., Christen, F., Bánfai, B., Lenz, B., & Winter, M. (2017). **Assessment of Preclinical Liver and Skeletal Muscle Biomarkers Following Clofibrate Administration in Wistar Rats.** Toxicologic pathology, 45(4), 506-525.

12. Pothmann, D., Simar, S., Schuler, D., Dony, E., Gaering, S., Net, J. L., & Nesslany, F. (2015). **Lung inflammation and lack of genotoxicity in the comet and micronucleus assays of industrial multiwalled carbon nanotubes Graphistrength© C100 after a 90-day nose-only inhalation exposure of rats.** Particle and fibre toxicology, 12(1), 21.

References (cont.)

13. Weber, K. 2017. **'Differences in Types and Incidence of Neoplasms in Wistar Han and Sprague-Dawley Rats'**, Toxicol Pathol, 45: 64-75.

14. Weber, K., T. Razinger, J. F. Hardisty, P. Mann, K. C. Martel, E. A. Frische, K. Blumbach, S. Hillen, S. Song, T. Anzai, and H. J. Chevalier. 2011. **'Differences in rat models used in routine toxicity studies'**, Int J Toxicol, 30: 162-73.

15. Wistar Hannover Outbred Rat. (2017) Envigo website, Accessed August 3, 2018. **http://www.envigo.com/products-services/research-models-services/models/research-models/rats/outbred/wistar-han-outbred-rat/hsdhanwist/**

16. York, M., Scudamore, C., Brady, S., Chen, C., Wilson, S., Curtis, M., Evans, G., Griffiths, W., Whayman, M., Williams, T., Turton, J. (2007) **Characterization of Troponin Responses in Isoproterenol-Induced Cardiac Injury in the Hanover Wistar Rat.** Toxicologic Pathology, 35(4): 606-617.

17. Zeljenková, D., Ambrušová, K., Bartušová, M., Kebis, A., Kovrižnych, J., Krivošíková, Z., Szabová, E. (2014). **Ninety-day oral toxicity studies on two genetically modified maize MON810 varieties in Wistar Han RCC rats (EU 7th Framework Programme project GRACE).** Archives of toxicology, 88(12), 2289-2314.