# HRN™:
# Hepatic Cytochrome P450 Reductase Null Mouse Model

***The Hepatic Reductase Null mouse provides a unique model for drug discovery/development and ADMET studies.***

## Applications of the HRN™ Mouse Model

Taconic's HRN™ Targeted Mutation/Microinjected Mice are homozygous for the tissue-specific targeted disruption of the *Por* gene in the liver. This genetic modification results in the ablation of hepatic cytochrome P450 activity. As the cytochrome P450 system plays a major role in drug metabolism and disposition, this model is very useful for efficacy, bioavailability and other ADMET studies. The lack of first pass metabolism can increase the circulating levels of compounds normally metabolized by the P450 system, thereby increasing the chances of demonstrating *in vivo* efficacy.

**Applications include:**

- **Efficacy studies**: allows use of smaller amounts of lead compounds in early efficacy studies.
- **Metabolism studies**: evaluation of the role of P450 metabolism on drug bioavailability as well as evaluation of the role of hepatic vs. extrahepatic metabolism.
- **Metabolite profiling**: the HRN™ mouse allows true dosing of parent compounds, which may not be otherwise possible. Evaluate the contribution of metabolites vs. parent compounds to efficacy or toxicity.
- **Toxicokinetic studies**: identify the optimal route and method of administration of drugs.
- **Reduced dosing**: high clearance compounds can be easily dosed in the HRN™ mouse, compared to wild type mice which might require dosing multiple times daily or the use of constant infusion pumps.
- **Basic research**: study of the role of P450 metabolism in normal homeostasis.

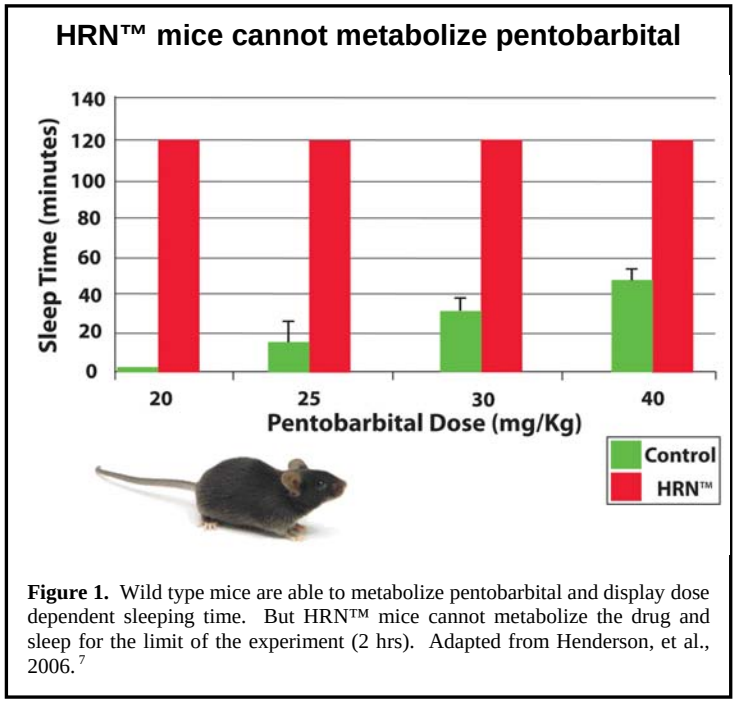


**Figure 1.** Wild type mice are able to metabolize pentobarbital and display dose dependent sleeping time. But HRN™ mice cannot metabolize the drug and sleep for the limit of the experiment (2 hrs). Adapted from Henderson, et al., 2006. [7]

## Features of the HRN™ mouse (models 007293 and 007353)

- Mice are homozygous for a liver-specific knockout of the *Por* gene.
- Animals are healthy and develop normally, but display enlarged and hyperlipidemic livers.[1]
- HRN™ mice have lowered serum cholesterol and triglycerides.

## Scientific Profiles of the HRN™ Mouse Model

**POR serves as the essential electron donor for all cytochrome P450 enzymes.**[2] Multiple P450 genes exist, and deletion of all these genes is impractical. However, the POR enzyme serves as the key electron donor for all P450 proteins, so the tissue-

specific deletion of the *Por* gene in the HRN™ mouse results in disruption of all P450 activity in the liver. Constitutive deletion of *Por* is embryonic lethal,[3] but the tissue-specific targeting found in the HRN™ mouse results in a viable and healthy animal. The cre transgene present becomes active neonatally, so most experiments with HRN™ mice should use adult mice to ensure appropriate tissue-specific disruption of *Por*.

**POR activity is significantly reduced in HRN™ mice.** POR activity in microsomal fractions derived from livers of adult HRN™ mice is reduced by approximately 90% compared to hepatic microsomes derived from wild type controls. Significant differences were not observed for POR activity in kidney microsomal fractions of HRN™ mice compared to controls, demonstrating the specificity of the tissue-specific mutation.[1]

**HRN™ mice have phenotypic changes in the liver.** Compared to wild type controls, HRN™ mice have livers almost twice as large. Livers of HRN™ mice are also hyperlipidemic. HRN™ mice display a 90% reduction in volume of bile acid in the gall bladder and have lower serum cholesterol and triglycerides.[1]

**HRN™ mice lack significant hepatic P450 activity.** Metabolite formation by a wide range of P450 enzymes, including members of the CYP2A and CYP3A families, was significantly reduced. However, these mice display greatly altered expression of P450s, with some enzymes upregulated and others downregulated.[1,4]

**The hepatic disruption of *Por* in HRN™ mice results in profoundly altered drug metabolism.** HRN™ mice are unable to metabolize the anaesthetic pentobarbital; doses that leave wild type mice unaffected cause HRN™ mice to sleep longer than two hours.[1] This experiment demonstrates that pharmacological effects can be observed at much lower concentrations in the HRN™ mouse compared to wild type mice.

**The HRN™ model allows for sophisticated toxicokinetic experiments.** Studies using HRN™ mice demonstrated that hepatic metabolism is the major route elimination and disposition of the anticancer agent cyclophosphamide.[5]

**HRN™ mice have altered lipid metabolism.** HRN™ mice display elevated diacylglycerols and extremely elevated triacylglycerols in the liver. Lesser increases in phosphatidylcholine and cholesterol esters were noted. However, minimal changes were seen to the lipid profiles of plasma and jejunum in HRN™ mice.[6]

## Origins of the Model

The HRN™ mouse was developed in the laboratory of C. Roland Wolf of the Ninewells Hospital & Medical School. The model was created by targeting the Por gene to generate a floxed allele in GK129/1 embryonic stem cells derived from 129P2 mice and injecting the targeted cells into C57BL/6 blastocysts. Resultant chimeras were backcrossed to C57BL/6 for one generation. Mice heterozygous for the floxed Por allele were intercrossed to generate mice homozygous for the floxed Por allele on a mixed B6;129P2 background. The Alb-cre transgene was developed in the laboratory of Mark A. Magnuson at Vanderbilt University School of Medicine by microinjecting Cre recombinase gene under the control of the rat albumin enhancer/promoter into B6D2F2 zygotes. Mice homozygous for the floxed Por allele were bred to carriers for the Alb-cre transgene to generate HRN mice. The HRN™ model was backcrossed to C57BL/6J a total of 6 generations (N6). Taconic received stock from CXR Biosciences in April 2007. Line 007293 was embryo transfer derived. Mice of line 007353 were backcrossed to C57BL/6NTac (N7) and embryo transfer derived. The colonies are maintained through mating of mice that are homozygous for the floxed Por allele and carriers for the Alb-cre transgene.

## Ready for Your Experiments

Taconic's HRN™ Mouse Models are produced in Isolator Barrier Unit (IBU™) facilities. Mice are shipped in Taconic Transport Cages (TTC™) and come with an up-to-date health report documenting their Murine Pathogen Free (MPF™) health status. Barrier housing conditions are recommended for maintenance of HRN™ Mice.

**References Cited:**


1. Henderson CJ, Otto DME, Carrie D, Magnuson MA, McLaren AW, Rosewell I, Wolf CR. (2003) **Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase.** *J Biol Chem* 278(15):13480-13486.

2. Goeptar AR, Scheerens H, Vermeulen NP. (1995) **Oxygen and xenobiotic reductase activities of cytochrome P450**. *Crit Rev Toxicol* 25(1):25-65.
3. Shen AL, O'Leary KA, Kasper CB. (2002) **Association of multiple developmental defects and embryonic lethality with loss of microsomal NADPH-cytochrome P450 oxidoreductase**. ***J Biol Chem*** 277(8):6536-6541.
4. Wang XJ, Chamberlain M, Vassieva O, Henderson CJ, Wolf CR. (2005) **Relationship between hepatic phenotype and changes in gene expression in the cytochrome P450 reductase (POR) null mice**. *Biochem J* 388:857-867.
5. Pass GJ, Carrie D, Boylan M, Lorimore S, Wright E, Houston B, Henderson CJ, Wolf CR. (2005) **Role of hepatic cytochrome P450s in the pharmacokinetics and toxicity of cyclophosphamide: studies with the hepatic cytochrome P450 reductase null mouse**. *Cancer Res* 65(10): 4211-4217.
6. Mutch DM, Klocke B, Morrison P, Murray CA, Henderson CJ, Siefert M, Williamson G. (2007) **The disruption of hepatic cytochrome P450 reductase alters mouse lipid metabolism. *J Proteome Res*** 6(10):3976-84.
7. Henderson CJ, Pass GJ, Wolf CR. (2006) **The hepatic cytochrome P450 reductase null mouse as a tool to identify a successful candidate entity.** *Cancer Res* 162:111-117.



***For more information or to place an order contact:***

**TACONIC**
One Hudson City Centre
Hudson, NY 12534
Toll Free: 1-888-TACONIC
Phone: 518-537-6208
Fax: 518-537-7287
e-mail: custserv@taconic.com
**Internet: http://www.taconic.com**

**in Europe:** Taconic Europe
Bomholtvej 10 P.O. Box 39
DK 8680 Ry DENMARK
Phone: +45 70 23 04 05
Fax: +45 86 84 16 99
e-mail: TaconicEurope@taconic.com
**Internet: http://www.taconic.com**

**in Japan:** CLEA Japan, Inc.
Phone: 03-5704-7063
Fax: 03-3792-5298
e-mail: ad-import@clea-japan.com
**Internet: http://clea-japan.com**

**Rev. 3/08**

Please Note: e-mail transmission of this document may result in the loss of formatting or symbols, i.e., Greek letters or symbols for trademark, degrees, etc.

## Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to ADME research. Call or fax for information about these additional models:

- **Bcrp Targeted Mutation Mouse (model 002767)** – carrying a disrupted *Abcg2* gene. Associated with multi-drug resistance. Useful for studies of drug uptake and cellular transport.
- **Mdr1a Targeted Mutation Mouse (model MDR1A)** – carrying a disrupted *Abcb1a* gene, a multi-drug resistance-associated transport protein, conferring a deficiency in the blood-brain barrier; useful in neurotoxicology and in studies of drug design, cellular transport and testing
- **Mdr1a/b Targeted Mutation Mouse (model 001487)** – carrying disruptions of two genes, *Abcb1a* and *Abcb1b* and lacking cellular transport mechanisms by their two multi-drug resistance-associated protein products, conferring a deficiency in the blood-brain barrier; useful in neurotoxicology and in studies of drug design, cellular transport and testing.
- **Mdr1a/b-Bcrp Targeted Mutation Mouse (model 003998)** – carries disruptions of three genes; *Abcb1a*,*Abcb1b*, and *Abcg2*, that encode for three drug-extruding transporters.
- **Mrp1 Targeted Mutation Mouse (model 001486)** – carrying a disruption of the *Abcc1a* (multi-drug

resistant associated protein gene), an ATP dependent drug-extruding transporter. This mouse exhibits impaired inflammatory stimulus response and is useful for studying the role of MRP1 in mediating inflammation responses and testing drug disposition *in vivo*.

- **Oct1/2 Targeted Mutation Mouse (model 006622)** – carrying a disruption of the *Slc22a1* and *Slc22a2* genes, which encode the organic cation transporters 1 and 2. This model is important for a wide range of ADME-tox and oncology studies.
- **PXR Targeted Mutation Mouse (model 001414)** – carrying a disruption of the *N11i2* gene, which encodes the pregnane X receptor. PXR controls the expression of various drug metabolizing enzymes in response to xenobiotic exposure.

TACONIC

# Taconic Transgenic Models
# Publication Reference List
# HRN™ Mouse


Arlt VM, Stiborová M, Henderson CJ, Thiemann M, Frei E, Aimová D, Singh R, Gamboa da Costa G, Schmitz OJ, Farmer PB, Wolf CR, Phillips DH. (2008) **Metabolic activation of benzo[a]pyrene in vitro by hepatic cytochrome P450 contrasts with detoxification in vivo: experiments with Hepatic Cytochrome P450 Reductase Null mice.** *Carcinogenesis* Epub ahead of print.

Stiborová M, Arlt VM, Henderson CJ, Wolf CR, Kotrbová V, Moserová M, Hudecek J, Phillips DH, Frei E. (2008) **Role of hepatic cytochromes P450 in bioactivation of the anticancer drug ellipticine: Studies with the hepatic NADPH:Cytochrome P450 reductase null mouse.** *Toxicol Appl* 226(3):318-27.

Finn RD, McLaren AW, Carrie D, Henderson CJ, Wolf CR. (2007) **Conditional deletion of cytochrome P450 oxidoreductase in the liver and gastrointestinal tract: a new model for studying the functions of the P450 system**. *J Pharmacol Exp Ther*. 322(1):40-7.

Mutch DM, Klocke B, Morrison P, Murray CA, Henderson CJ, Siefert M, Williamson G. (2007) **The disruption of hepatic cytochrome P450 reductase alters mouse lipid metabolism.** J Proteome Res 6(10):3976-84.

Arlt VM, Henderson CJ, Wolf CR, Schmeiser HH, Phillips DH, Stiborova M. (2006) **Bioactivation of 3-aminobenzanthrone, a human metabolite of the environmental pollutant 3-nitrobenzanthone: evidence for DNA adduct formation mediated by cytochrome P450 enzymes and peroxidases.** Cancer Lett. 234(2):220-31.

Mutch DM, Crespy V, Clough J, Henderson CJ, Lariani S, Mansourian R, Moulin J, Wolf CR, Williamson G. (2006) **Hepatic cytochrome P450 reductase null mice show reduced transcriptional response to quercetin and reveal physiological homeostasis between jejunum and liver**. Am J Physiol Gastrointest Liver Physiol 291(1):G63-72.

Arlt VM, Stiborova M, Henderson CJ, Osborne MR, Bieler CA, Frei E, Martinek V, Sopko B, Wolf CR, Schmeiser HH, Phillips DH. (2005) **Environmental pollutant and potent mutagen 3-nitrobenzanthrone forms DNA adducts after reduction by NAD(P)H: quinone oxidoreductase and conjugation by acetyltransferases and sulfotransferases in human hepatic cytosols**. Cancer Res 65(7):2644-52.

Pass GJ, Carrie D, Boylan M, Lorimore S, Wright E, Houston B, Henderson CJ, Wolf CR. (2005) **Role of hepatic cytochrome P450s in the pharmacokinetics and toxicity of cyclophosphamide: studies with the hepatic cytochrome P450 reductase null mouse**. *Cancer Res* 65(10): 4211-4217.

Stiborova M, Arlt VM, Henderson CJ, Wolf CR, Frei E, Schmeiser HH, Phillips DH. (2005) **Molecular mechanism of genotoxicity of the environmental pollutant 3-nitrobenzathrone**. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 1492(2):191-7.

Wang XJ, Chamberlain M, Vassieva O, Henderson CJ, Wolf CR. (2005) **Relationship between hepatic phenotype and changes in gene expression in the cytochrome P450 reductase (POR) null mice**. *Biochem J* 388:857-867.

Henderson CJ, Otto DME, Carrie D, Magnuson MA, McLaren AW, Rosewell I, Wolf CR. (2003) **Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase.** *J Biol Chem* 278(15):13480-13486.

Henderson CJ, Pass GJ, Wolf CR. (2006) **The hepatic cytochrome P450 reductase null mouse as a tool to identify a successful candidate entity.** *Toxicol Lett* 162(1):111-7.