Humanized Models in Toxicology and Their Application to Hazard Characterization and Risk Assessment

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The Dow Chemical Company

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Overview

- Background
  - What are humanized models?
  - Why use humanized models?
- Examples of humanized models in toxicology
  - Receptor Models
  - Metabolizing Enzyme Models
- Conclusions and Outlook
  - SWOT

Background

What are humanized models?



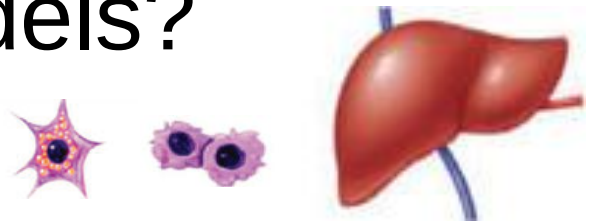



- Animal models that carry functioning human genes, cells, tissues, and/or organs.

Background

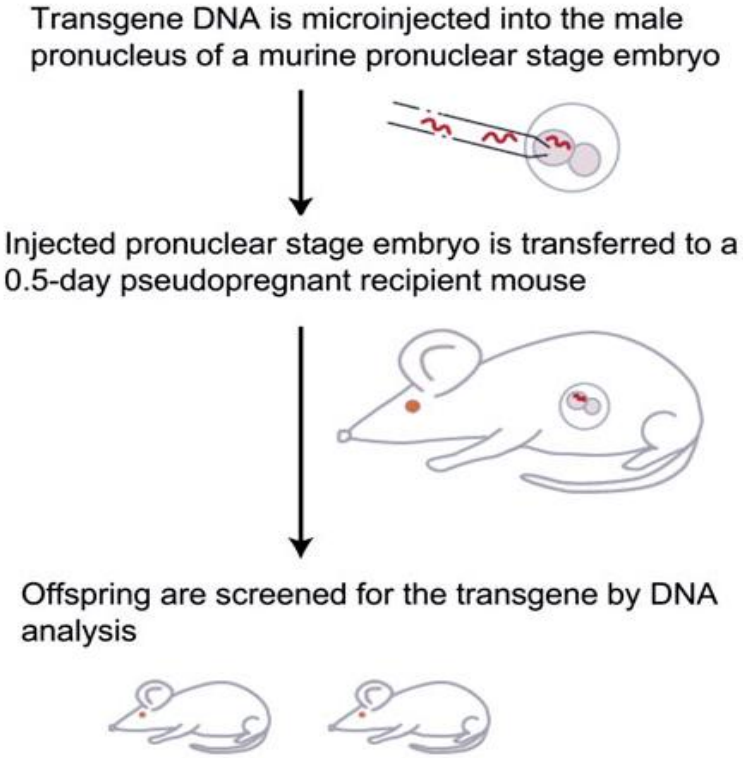
What are humanized models?

Genes

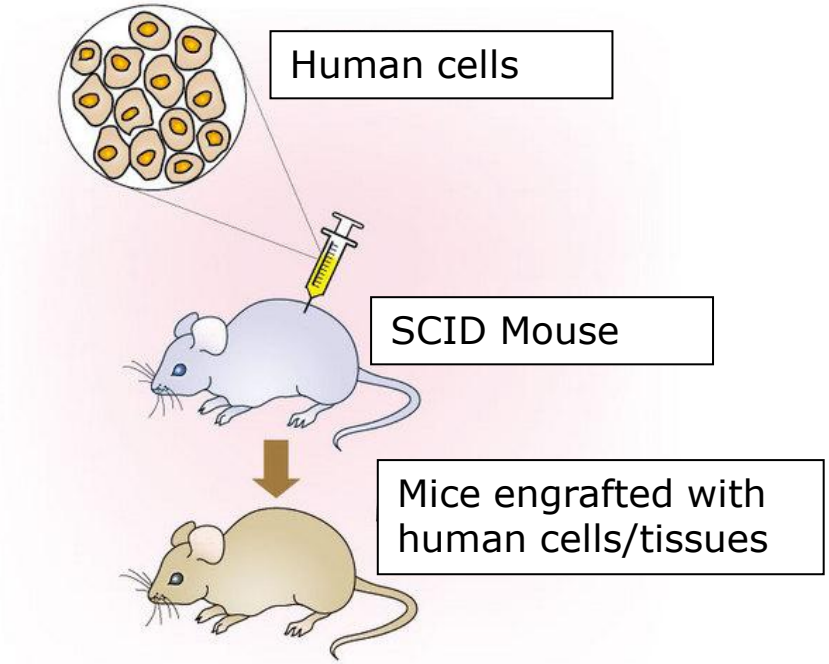


Cells, Tissues

Mice that express human genes (transgenic)



Immunodeficient mice engrafted with human cells/tissues



Background

Why use humanized models?

- Humanized models can provide insights into in vivo human biology that would otherwise not be possible due to ethical, logistical and/or technical constraints
- Broad range of humanized models/approaches available that have been used to research:
  - Human haematopoiesis
  - Innate and Adaptive Immunity
  - Autoimmunity
  - Infectious Diseases
  - Cancer Biology
  - Regenerative Medicine
  - Pharmacology/Toxicology

Background

Why use humanized models?

Humanized mice in translational biomedical research

Leonard D. Shultz, Fumihiko Ishikawa‡ and Dale L. Greiner§*

Nat Rev Immunol. 2007 Feb;7(2):118-30

Transgenic Animal Models in Toxicology: Historical Perspectives and Future Outlook

Darrell R. Boverhof,*,[1] Mark P Chamberlain,† Clifford R. Elcombe,† Frank J. Gonzalez,‡ Robert H. Heflich,§ Lya G. Hernández,¶ Abigail C. Jacobs,|| David Jacobson-Kram,|| Mirjam Luijten,¶ Adriana Maggi,||| Mugimane G. Manjanatha,§ Jan van Benthem,¶ and B. Bhaskar Gollapudi*

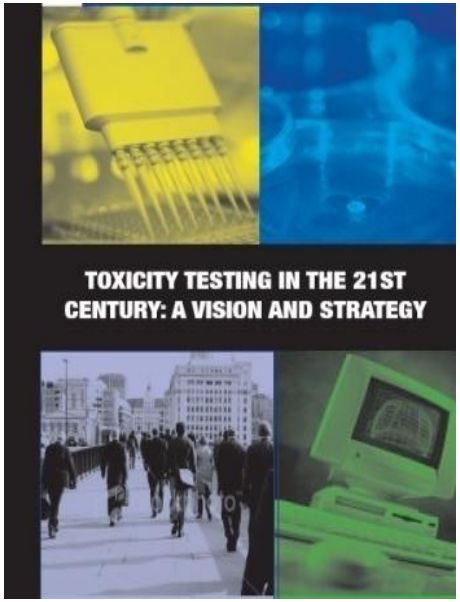
Tox Sci. 121(2), 207–233 (2011)

Background

Why use humanized models…in Toxicology?

- Rodents are not humans
- Help to better predict human responses and/or understand the human relevance of observed rodent responses
- Provide insight into mode/mechanism of action

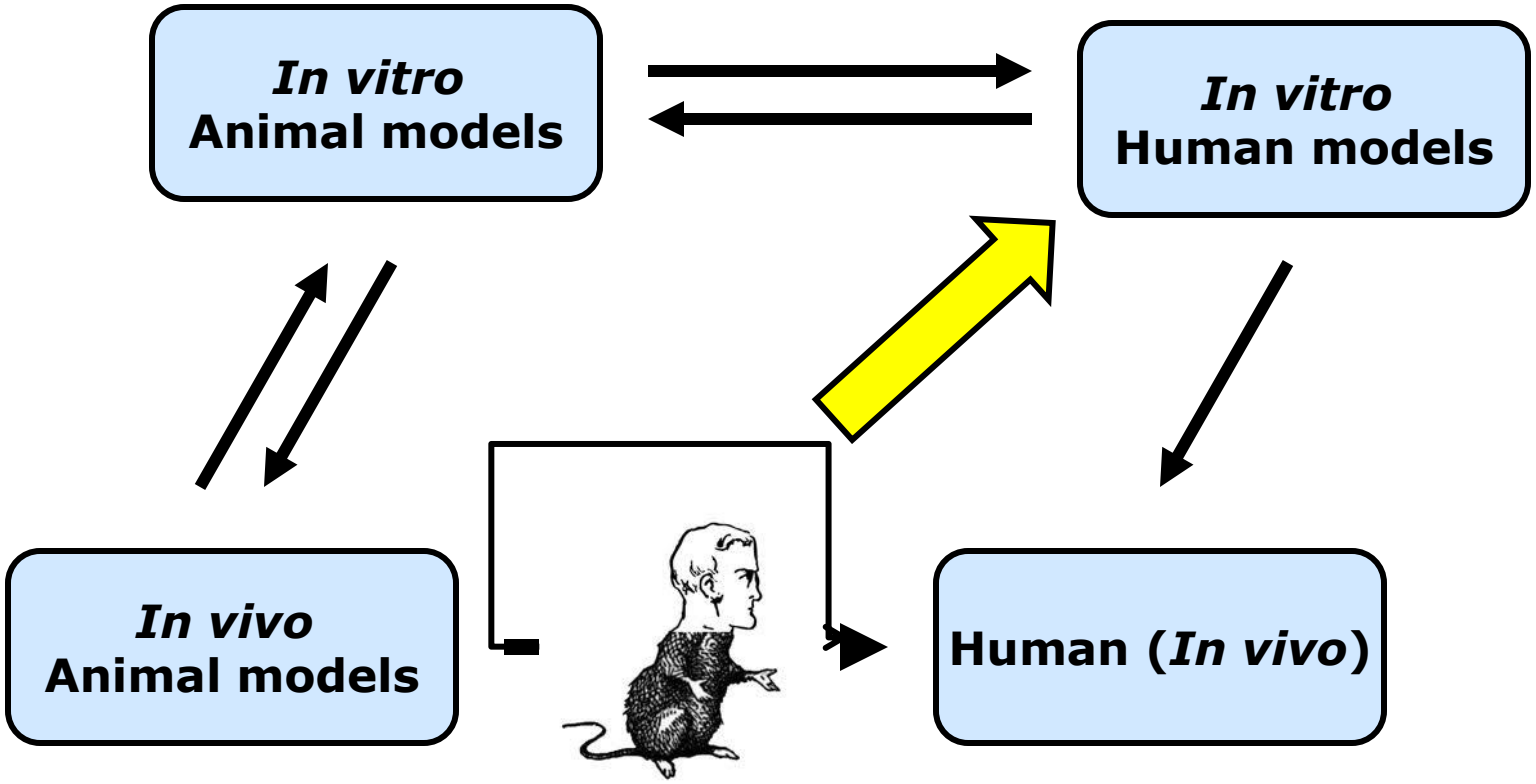
- Toxicity Testing in the 21st Century- A Vision and a Strategy
- Recommends moving away from high dose animal studies and apical endpoints to “toxicity” pathways analysis in human cells/cell lines
- Challenging us to apply new technologies to toxicity testing



Background

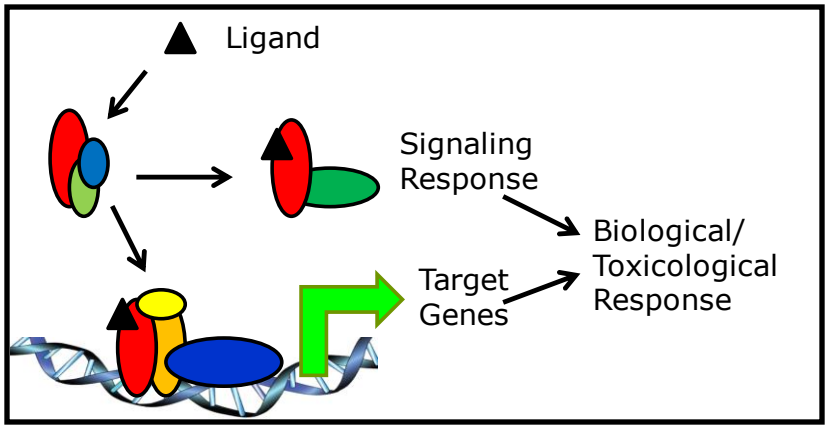
Why use humanized models…in Toxicology?

Approaches to better predict human responses/ decrease uncertainty in human hazard assessment

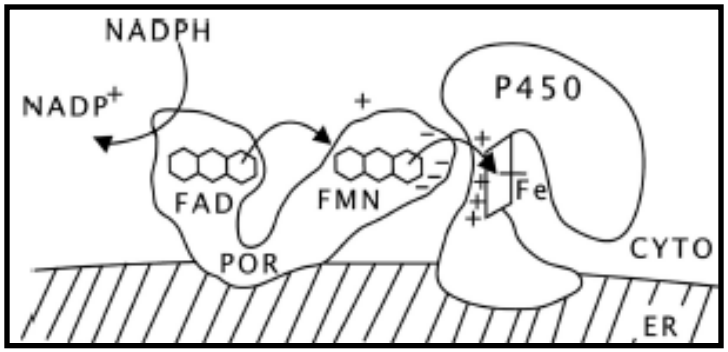


Examples of humanized models in toxicology

- Receptor Models

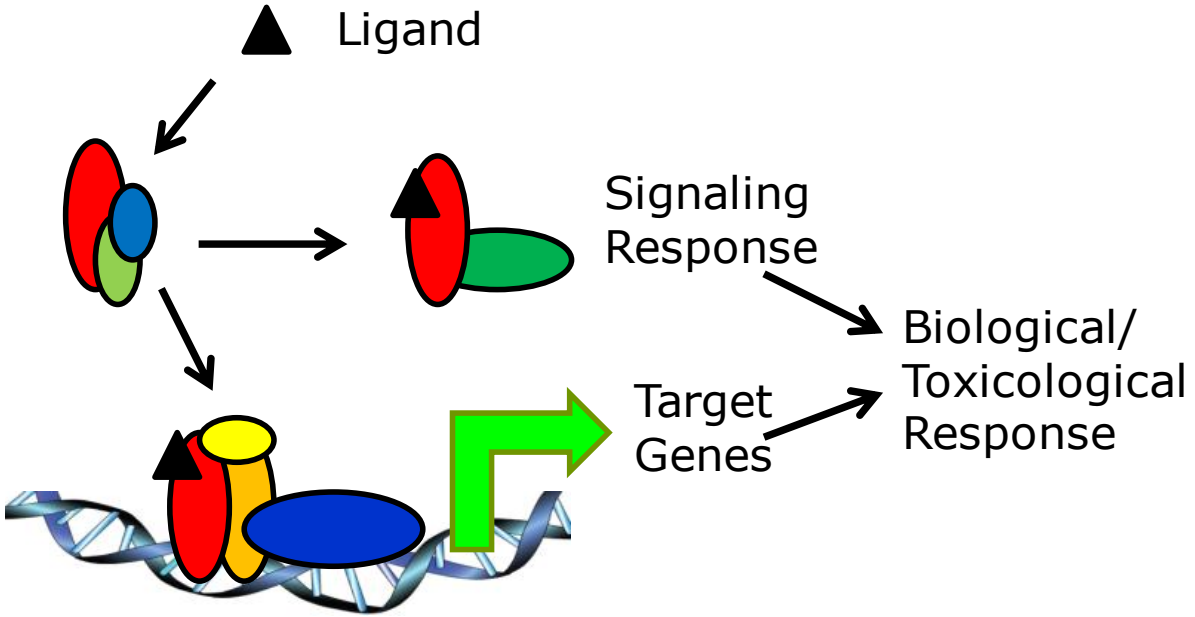


- Metabolizing Enzyme Models



Examples of humanized models in toxicology- Receptor Models

- Receptors that are ligand-activated transcription factors can respond to xenobiotics
  - Nuclear Receptors- PPARα, CAR, PXR, FXR
    - Capable of being activated by structurally diverse ligands
    - Several points for species differences in these pathways that may dictate species specific responses



Examples of humanized models in toxicology- Receptor Models

- Knock-out and Humanized Ligand Activated Receptor Models

Biological Function

Receptor-knockout	Reference
Ahr	(Fernandez-Salguero *et al.*, 1995)
Car	(Ueda *et al.*, 2002)
Fxr	(Sinal *et al.*, 2000b)
Pxr	(Kliewer *et al.*, 1998; Xie *et al.*, 2000)
Ppara	(Lee *et al.*, 1995)

Human Response

Receptor-humanized	Reference
AHR-humanized	(Moriguchi *et al.,* 2003; Flaveny and Perdew, 2009)
CAR-humanized	(Zhang *et al.*, 2002; Scheer *et al.*, 2010)
PXR-humanized	(Xie *et al.*, 2000; Ma *et al.*, 2007; Scheer *et al.*, 2010)
PPARA-humanized	(Cheung *et al.*, 2004; Yang *et al.*, 2008)

Examples of humanized models in toxicology- CAR/PXR

- CAR and PXR- Promiscuous receptors- activated by a range of xenobiotics
- Activation results in induction of a range of xenobiotic metabolizing enzymes- important in xenobiotic metabolism and drug/drug interactions
- Known species-specific (mouse vs human) ligands
- The CAR-ligand Phenobarbital causes hypertrophy and hyperplasia and hepatocarcinogenesis in mice (non-genotoxic)
- In humans, Phenobarbital causes liver enlargement but not the hyperplastic responses (in vitro and in vivo) and does not appear to result in hepatocarcinogeneis.

Examples of humanized models in toxicology- CAR/PXR

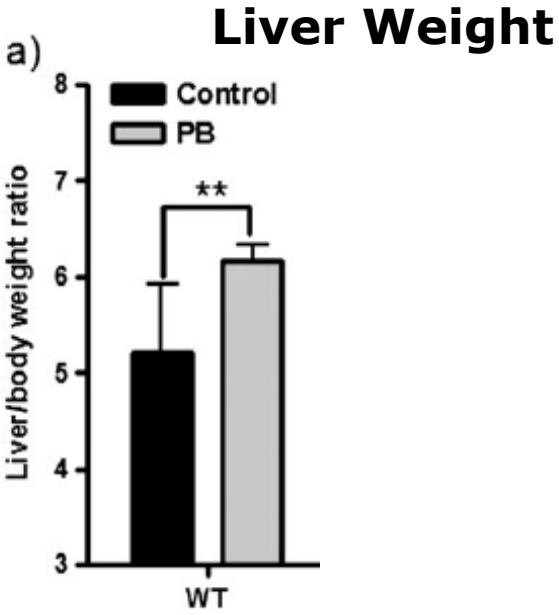
Human Constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) Support the Hypertrophic but not the Hyperplastic Response to the Murine Nongenotoxic Hepatocarcinogens Phenobarbital and Chlordane *In Vivo*

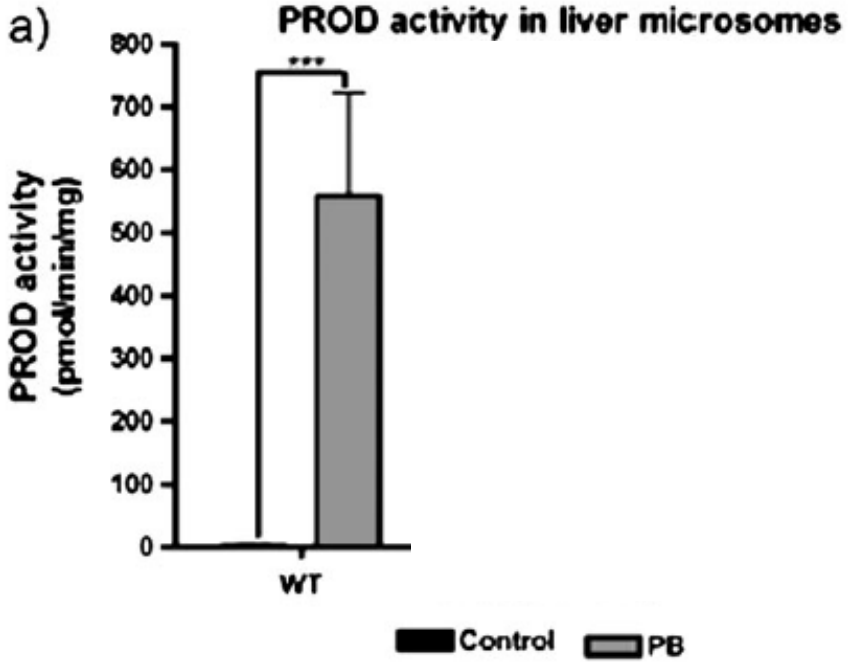
Jillian Ross,* Simon M. Plummer,* Anja Rode,† Nico Scheer,† Conrad C. Bower,‡ Ortwin Vogel,§ Colin J. Henderson,‡ C. Roland Wolf,* and Clifford R. Elcombe*,[1]

Tox Sci. 116(2), 452–466 (2010)

- Compared hepatic responses to Phenobarbital
  - Wild Type (WT) mice (mouse CAR/PXR)
  - Knockout mice (CARKO/PXRKO)
  - Humanized mice (huCAR/huPXR)

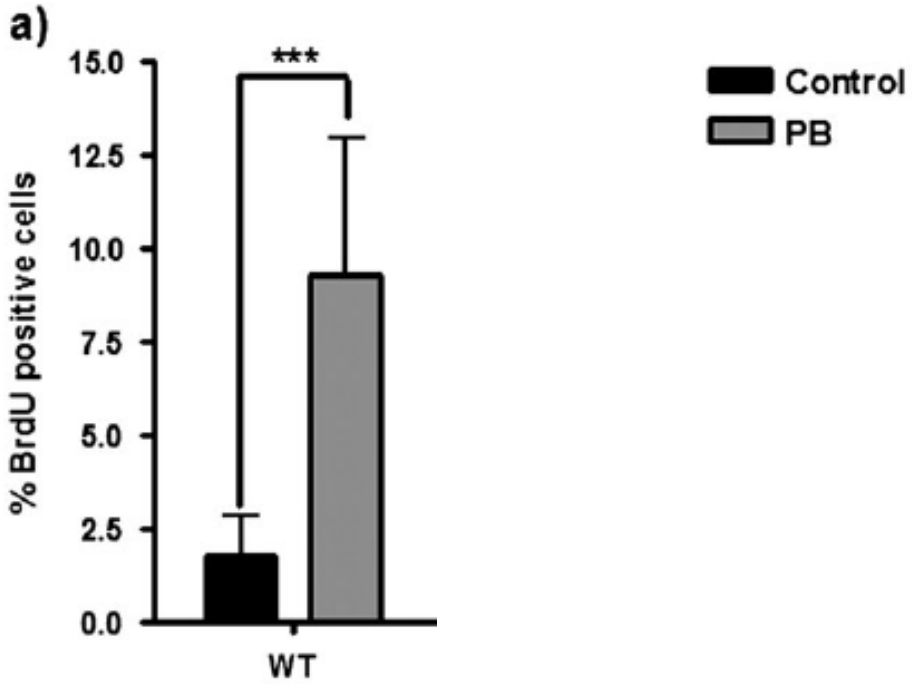
Examples of humanized models in toxicology- CAR/PXR





- PB increased liver weight and hypertrophy in WT and humanized
- PROD (Cyp2b) activity induced in WT and humanized

Examples of humanized models in toxicology- CAR/PXR



- Hepatic Proliferative response was observed only in WT and not humanized mice
- Gene expression analysis-
  - Induction of proliferative genes unique to WT mouse

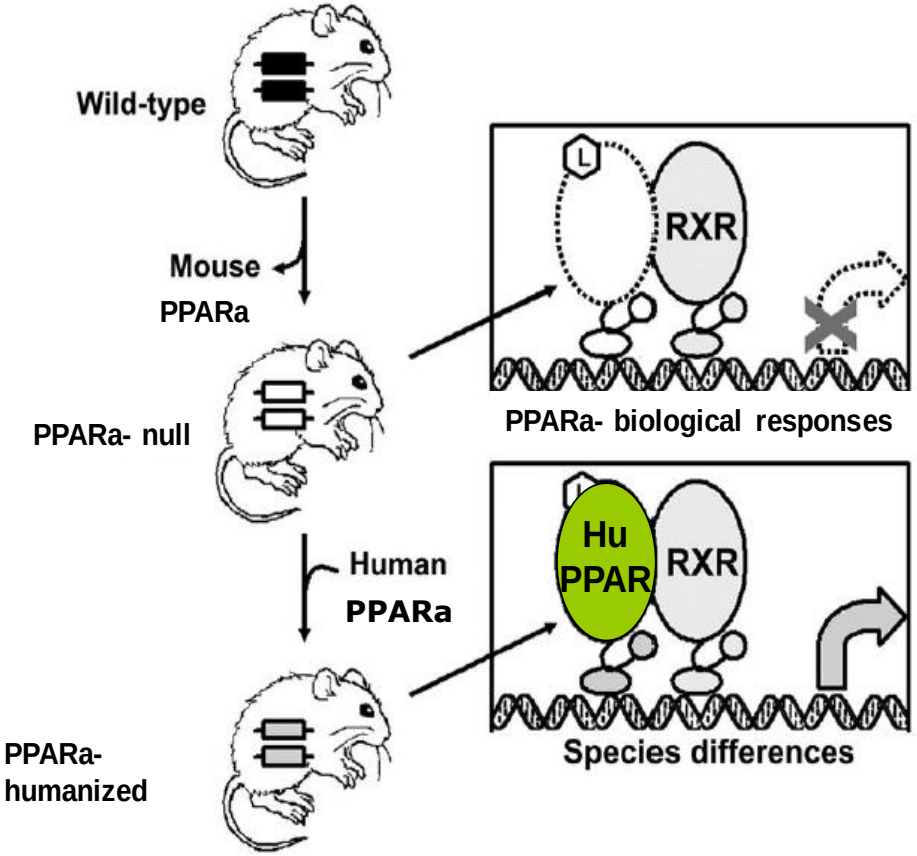
- **Conclusion**- If receptor mediated cell proliferation is a key event for the hepatocarcinogenicity of PB, then PB is unlikely to pose a hepatocarcinogenic hazard to humans

Examples of humanized models in toxicology- PPARα

- PPARα – is the target of lipid lowering fibrate drugs (e.g. fenofibrate Wy-14,643)
- Short term treatment with PPARα ligands results in hepatic hypertrophy and hyperplasia (and peroxisome proliferation)
- Activation in rodents results in liver tumors (non-genotoxic hepatocarcinogens)
- In contrast- humans appear resistant to the hepatocarcinogenic effects (Cattley et al., 1998; Klaunig et al., 2003)

Examples of humanized models in toxicology- PPARα

Series of papers from Gonzalez Lab

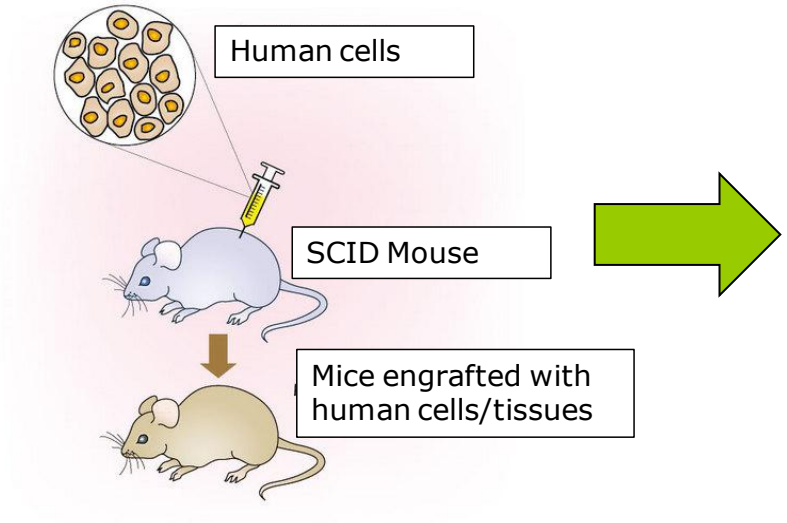


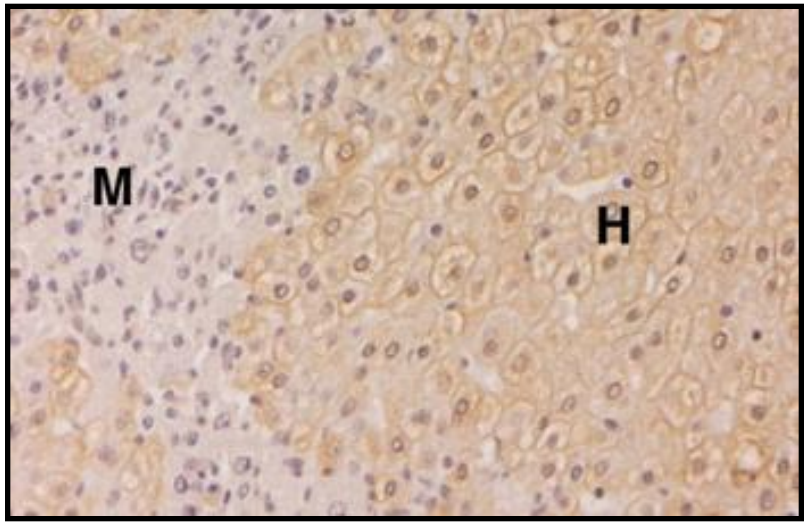
- WT mice- induction of genes and enzymes associated with FA metabolism and transport
  - hepatic hypertrophy and hyperplasia and hepatocarcinogenesis
- PPARa null mice- No WT responses including a lack of hepatocarcinogenic effect
- PPARa humanized mice
  - Induction of genes and enzymes associated with FA metabolism and transport
  - No proliferative response including a lack of hepatocarcinogenic effect

- Further molecular assessment revealed that activation of PPARa in WT mice induces c-myc while this is not observed in humanized mice

Examples of humanized models in toxicology- PPARα

Mice with Humanized Liver

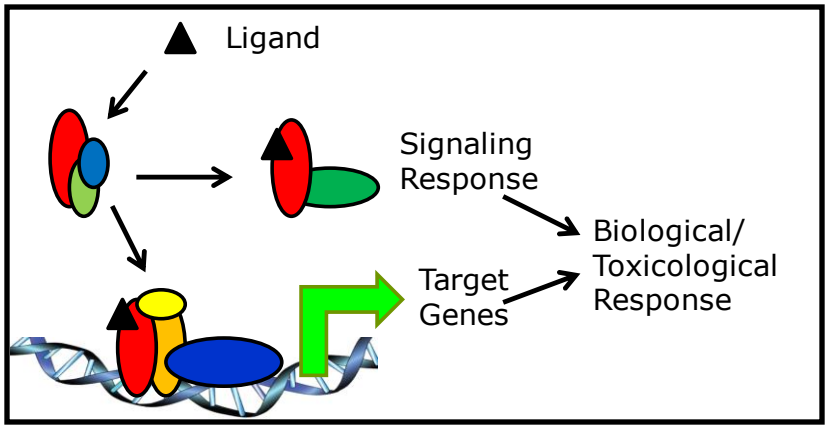




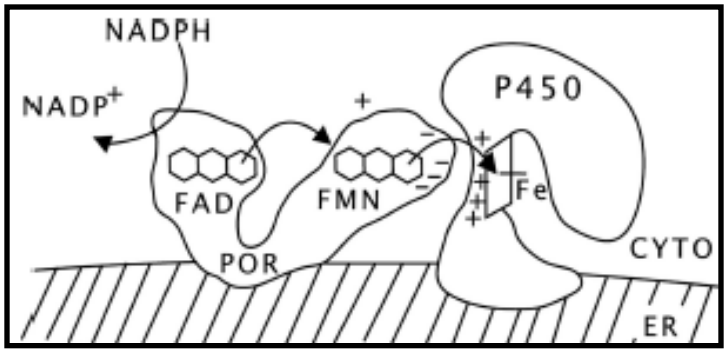
- Treatment of chimeric mice with humanized liver with PPARa ligands resulted in proliferation in mouse hepatocytes but not human hepatocytes
- **Conclusion**- If receptor mediated cell proliferation is a key event for the hepatocarcinogenicity, then PPAR ligands are unlikely to pose a hepatocarcinogenic hazard to humans

Examples of humanized models in toxicology

- Receptor Models



- Metabolizing Enzyme Models



Examples of humanized models in toxicology- Metabolizing Enzymes

- Hepatic Phase I and II enzymes play important roles in chemical bioactivation and clearance
- Knock-out models have been used to confirm the role of these enzymes in toxicology and carcinogenesis.
- There are known species differences in the expression and/or catalytic activity these enzymes
- Humanized models have been created to more accurately reflect human metabolism and toxicology

Examples of humanized models in toxicology- Metabolizing Enzymes

- Knock-out and Humanized P450 Metabolizing Enzyme Models

Biological Function

P450 knockout	Reference
Cyp1a1	(Dalton *et al.*, 2000)
Cyp1a2	(Pineau *et al.*, 1995)
Cyp1a1/ Cyp1a2	(Dragin *et al.*, 2007)
Cyp1b1	(Buters *et al.*, 1999)
Cyp2c44	(Pozzi *et al.*, 2010)
Cyp2e1	(Lee *et al.*, 1996)
Cyp2j5	(Athirakul *et al.*, 2008)
Cyp3a cluster	(Scheer *et al.*, 2008)
Cyp4a11	(Nakagawa *et al.*, 2006)
Cyp4a14	(Holla *et al.*, 2001)

Human Response

P450-humanized mice	Reference
CYP1A2-humanized	(Cheung *et al.*, 2005a)
CYP1A1/1A2-humanized	(Dragin *et al.*, 2007)
CYP2C18/2C19 transgenic	(Lofgren *et al.*, 2008)
CYP2D6 BAC transgenic	(Corchero *et al.*, 2001)
CYP2E1-humanized	(Cheung *et al.*, 2005b)
CYP3A4 BAC transgenic	(Granvil *et al.*, 2003)

Examples of humanized models in toxicology- CYP2F2

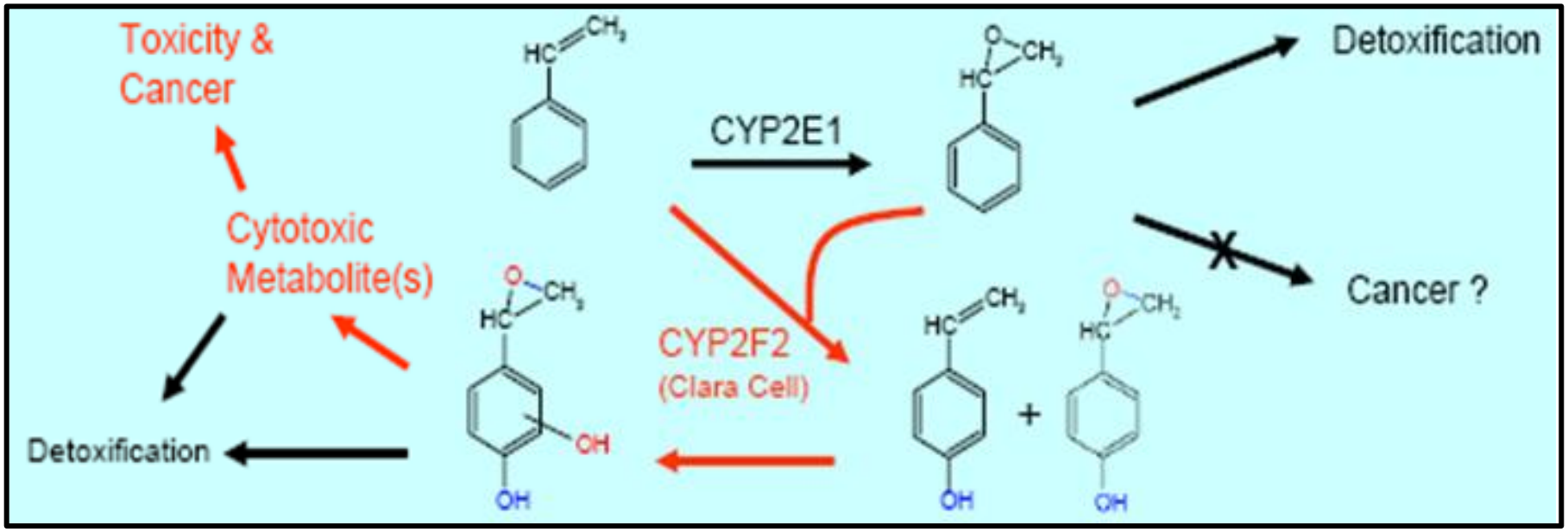
- Styrene and other chemicals produce lung toxicity and tumors in mice but not rats
- Lung toxicity in mice is dependent on Cyp2f2 conversion of styrene to cytotoxic metabolites (studies with Cyp2f2 KO mice)

Cytotoxicity → **Regenerative Proliferation** → **Tumors**

- Rats have lower catalytic activity for this enzymatic pathway- no cytotoxicity or tumors
- What about humans? Humanized mice created

Examples of humanized models in toxicology- CYP2F2

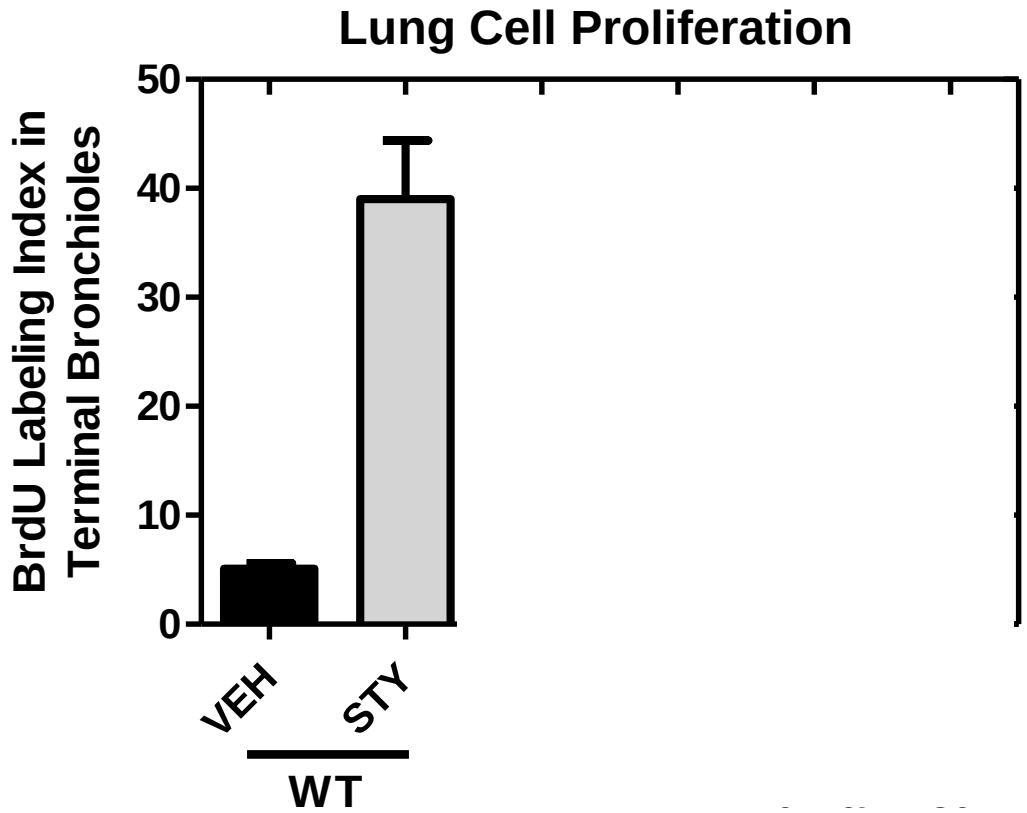
- Metabolism of Styrene and associated toxicity
- Steps in red specific to mouse



Cruzan et al., 2012. Reg. Toxicol. Pharmacol. 62: 205-220.
SIRC- Styrene Information and Research Center

Examples of humanized models in toxicology- CYP2F2

To address potential species-specific effects- generated both knockout and humanized models for CYP2F



- Humanized mice do not display the same proliferative response as mice
- These data suggest that styrene-induced lung toxicity and tumors may not be a relevant human health risk

Cruzan et al., 2012- SOT Annual Meeting Abstract.
SIRC- Styrene Information and Research Center

Examples of humanized models in toxicology- Conclusions and Outlook

- Humanized models are powerful tools to develop increased knowledge on human responses and MoA and can decrease uncertainty in human hazard evaluation and risk assessment
- Traditionally, both transgenic and engraftment approaches have been more limited to the mouse
- Newer technologies are facilitating faster model creation in mice, rats, rabbits……?
- These approaches will increase the use of transgenic models in both translational biology and toxicology research

Examples of humanized models in toxicology- Conclusions and Outlook

Knockout Rats via Embryo Microinjection of Zinc-Finger Nucleases

Aron M. Geurts,[1,2]* Gregory J. Cost,[3]* Yevgeniy Freyvert,[3] Bryan Zeitler,[3] Jeffrey C. Miller,[3] Vivian M. Choi,[3] Shirin S. Jenkins,[3] Adam Wood,[4] Xiaoxia Cui,[4] Xiangdong Meng,[3] Anna Vincent,[3] Stephen Lam,[3] Mieczyslaw Michalkiewicz,[1,2] Rebecca Schilling,[1,2] Jamie Foeckler,[3] Shawn Kalloway,[3] Hartmut Weiler,[1,2] Séverine Ménoret,[5] Ignacio Anegon,[5] Gregory D. Davis,[4] Lei Zhang,[3] Edward J. Rebar,[3] Philip D. Gregory,[3] Fyodor D. Urnov,[3] Howard J. Jacob,[1,2,6]† Roland Buelow[7]†

***Science* 325, 433 (2009)**

Targeted integration in rat and mouse embryos with zinc-finger nucleases

Xiaoxia Cui, Diana Ji, Daniel A Fisher, Yumei Wu, David M Briner & Edward J Weinstein

***Nat Biotechnol* 29, 64-67 (2011)**

"This approach enables precise genome engineering to generate point mutations, accurate insertions and deletions, and conditional knockouts and knock-ins"

Tool or assay

SWOT analysis: Humanized Animal Models

Strengths

-Allows for better characterization of human hazard and risk potential- Human relevant
-Provides additional data on mode/mechanism of action

Weaknesses

-Involves animal use
-Expensive (creation and maintenance)
-Low-throughput
-Transgenic models- human gene product in mouse environment

Opportunities

-Allow for refinement in hazard and risk assessments-
    -decreased uncertainty
    -increased human relevance
-New technologies are decreasing the cost and expanding the model species
-Can be used to further define “toxicity pathways” thereby facilitating development of in vitro assays based on MoA
-Can be used to validate in vitro hypotheses

Threats

- Models not widely available
- Models using different technologies may generate different results- may delay progress
- Lack of acceptance of this technology for advancing human health risk assessments