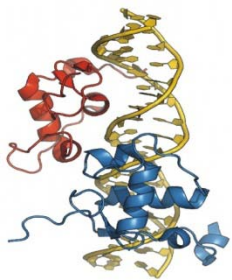


Report of a Workshop on Dose-Response Approaches for **Nuclear Receptor-** Mediated Modes of Action

The 2010 Society of Risk Analysis Meeting
Salt Lake City, Utah

Robert Budinsky
The Dow Chemical Company

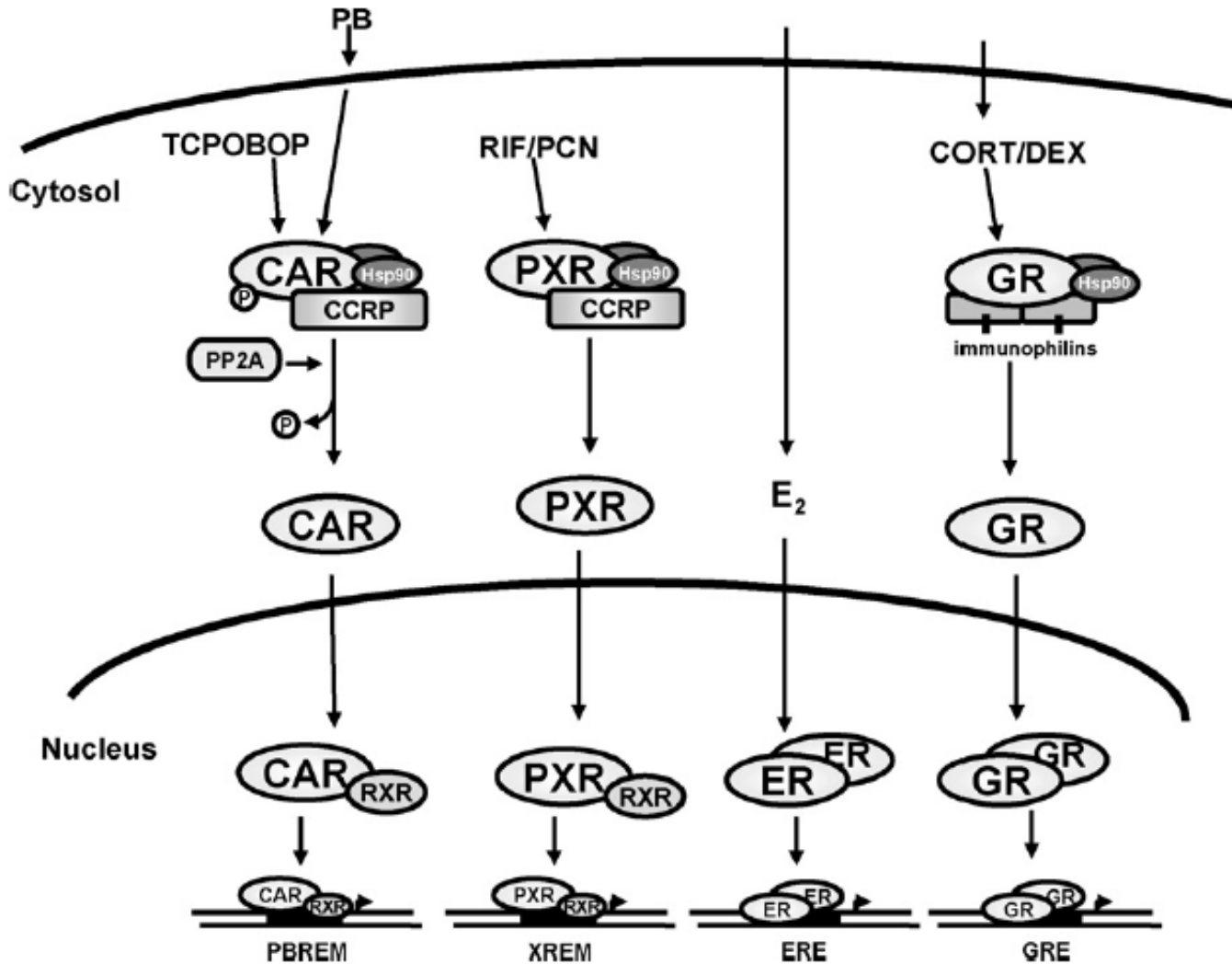


Outline

- **Nuclear Receptor Background**
 - CAR/PXR, PPAR α and AHR Rodent Liver Tumors
- **Nuclear Receptor Workshop**
 - Government, Academic, Consulting and Industry Scientists
 - NIEHS facility
 - Case studies guided by charge questions, human relevance framework and mode-of-action key events assessment
- **Dose-Response Modeling**

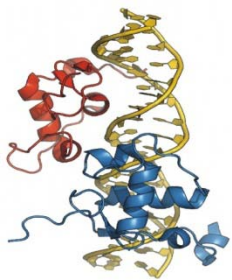


Ligand-Dependent Activation



Summary of signaling pathways for CAR and PXR, and the nuclear steroid receptors ER and GR.

Timsit and Negishi, 2007



Nuclear Receptor-Promoted Liver Tumors

- **Rodent Liver Tumors**
 - Phenobarbital: CAR/PXR
 - Cholesterol-lowering drugs: PPAR α
 - TCDD: AHR
- **Human Liver Cancer Evidence**
 - Negative for phenobarbital
 - Negative for statins
 - Negative to equivocal for TCDD (lung and all cancer mortality in slight excess)



Conference Co-Chairs: J. Preston and M. Andersen

Steering Committee Members: M. Cunningham, M. Dourson
R. Becker, M. Honeycutt, R. Budinsky, C. Elcombe, J. Klaunig

CAR/PXR

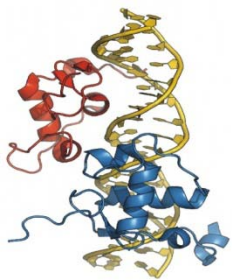
D. Wolf/C. Elcombe
R. Barrs
D. Bell
R. Cattley
R. Conolly
K. Crump
S. Ferguson
D. Geter
A. Goetz
J. Goodman
S. Hester
A. Jacobs
B. Lake
C. Omnicinski
R. Pepper
J. Ross
R. Schoeny
A. Vardy
W. Xie

PPAR α

C. Corton/J. Klaunig
P. Bentley
M. Cunningham
Y. Dragan
T. Hummer
B. Meek
J. Peters
J. Popp
L. Rhomberg
J. Seed

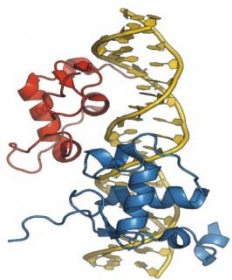
AHR

D. Schrenk/B. Budinsky
N. Walker
A. Brix
T. Simon
A. Aylward
B. Allen
T. Starr
G. Perdew
T. Gasiewicz
M. van den Berg
N. Kaminski
M. Andersen
R. Thomas
C. Rowlands
A. Maier



Structure of Workshop

- Day 1
 - Morning Plenary Sessions
 - Begin Afternoon Case Study Discussion (Background review of key events)
- Day 2
 - Continuation of Case Study Discussions (Charge Questions)
- Day 3
 - Report of Case Study Discussion to the Workshop Attendees



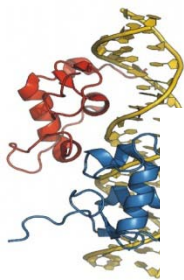
Major Goals of the Workshop

Establish a mode of action (MOA) for NR-mediated rodent liver tumors

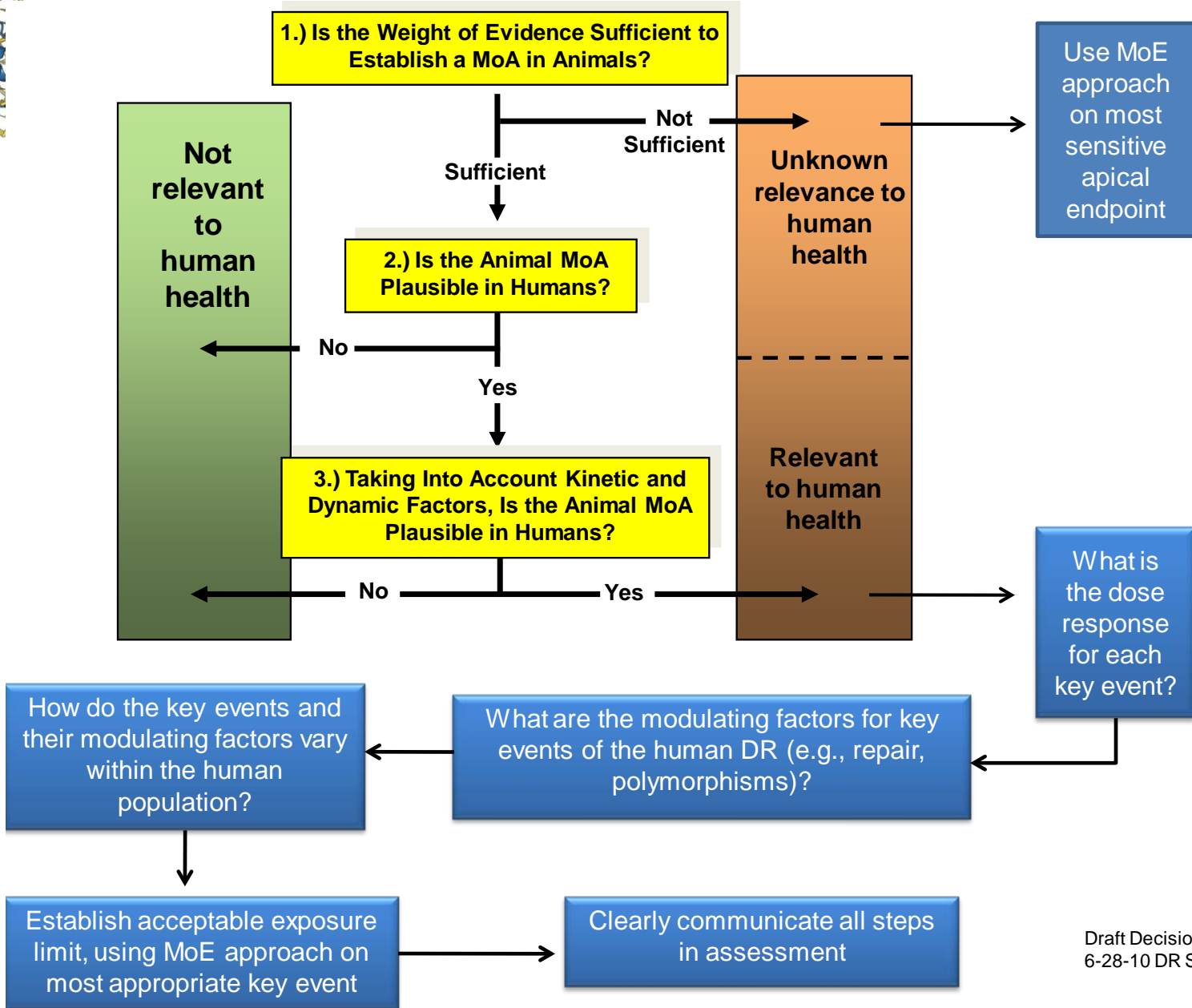
- Apply the IPCS Framework for Human Relevance and the modified Hill Criteria applied to MOA

General Charge Questions

1. Is a minimum threshold of receptor ligand required for
 - gene transcription?
 - biochemical, cellular and tissue responses?
2. Is linear low-dose modeling of receptor ligands appropriate, based on the underlying science of nuclear receptor signaling biology, and if not, provide insights into more appropriate low-dose modeling approaches?



Draft Human Relevance Framework (ILSI-HESI risk 21)



Draft Decision Logic
6-28-10 DR Subteam Meeting



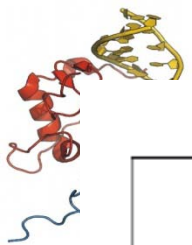
Using Hill Considerations to Determine Key Events for Rodent Liver Tumor MOA

Evaluate

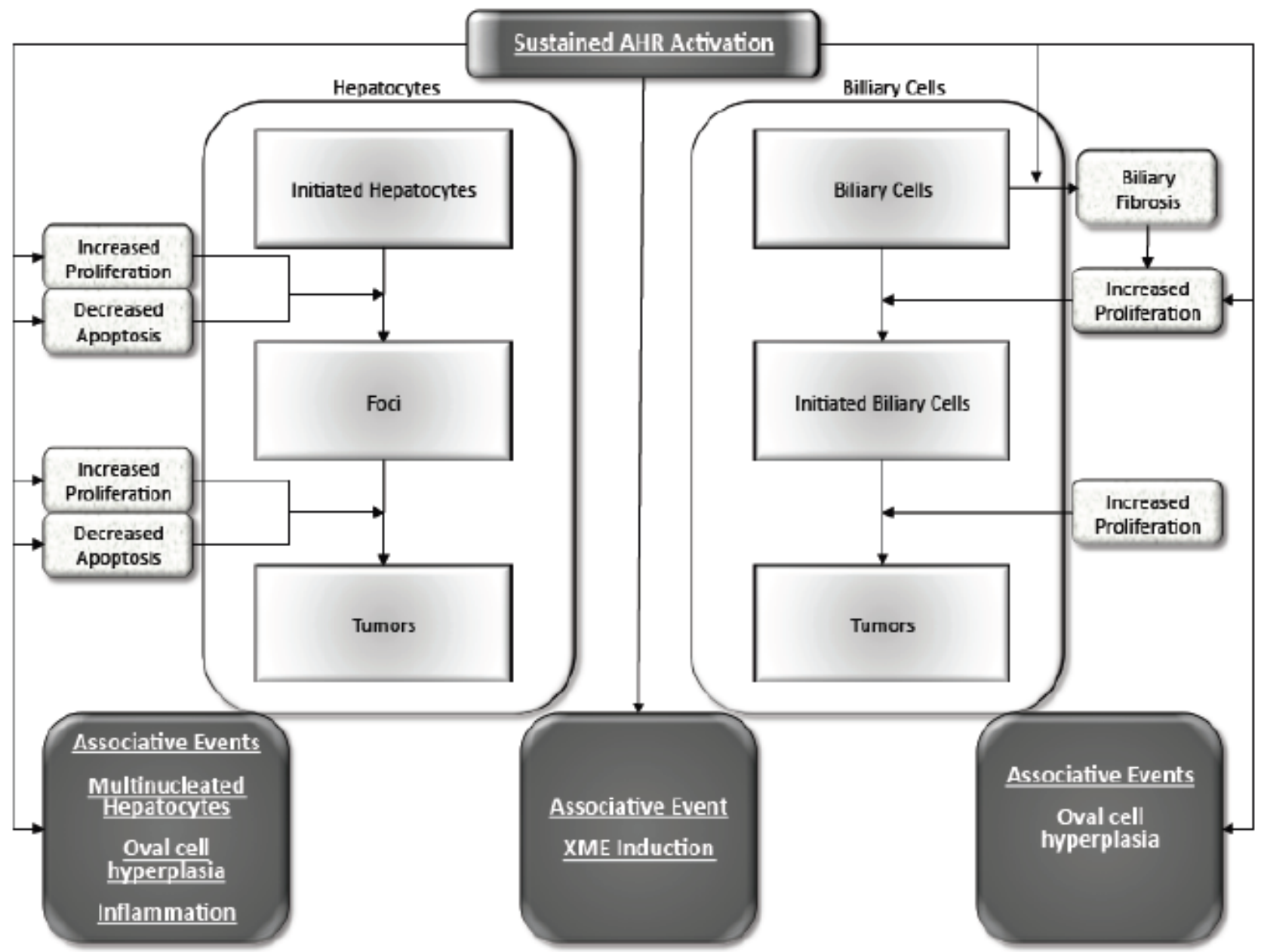
Possible Key Events

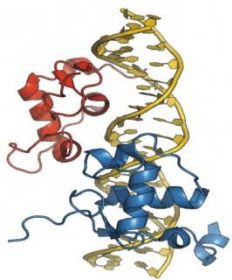
- Strength
- Consistency
- Specificity
- Temporality
- Biological Gradient
- Biological Plausibility
- Coherence

- Causal Key Event
- Associated Event (marker)
- Modulating Factor
- None of the above



AHR-Key Events: Rodent Liver Tumorigenicity





CAR MOA-Key Events: Rodent Liver Tumorigenicity

CAR Over Activation

Altered gene expression
specific to CAR activation

Altered epigenetic changes specific
to CAR activation

Cyp2b Induction

Hypertrophy

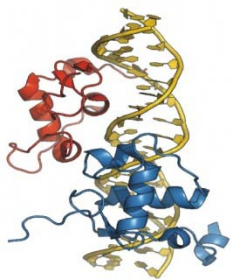
Increased cell
proliferation

Decreased
apoptosis

Gap Junction
Communication Inhibition

Clonal expansion
leading to altered foci

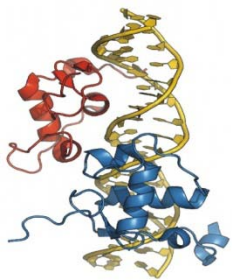
Liver Adenomas/Carcinomas



PPAR α -Key Events: Rodent Liver Tumorigenicity

Key Events: 2010 version

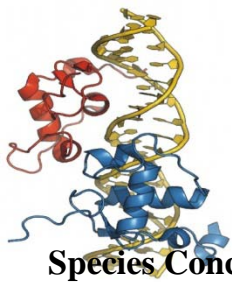
1. Metabolic activation – *if necessary*
2. Activation of the PPAR α
3. Increased hepatocellular proliferation with or without decrease apoptosis
4. Selective expansion of preneoplastic hepatocytes
5. Neoplasm formation



AHR Species Concordance

| Key Event | Rats | Humans |
|--------------------------|--|--|
| Sustained AHR Activation | Yes (in vitro and in vivo based on associative events – XME gene expression) | Yes (in vitro and in vivo based on associative events – XME gene expression) |
| Inhibition of Apoptosis | Yes (in vitro and in vivo data) – mechanism not yet clear | Yes (based on in vitro data in human cells); no in vivo data |
| Altered Hepatic Foci | Yes (observed in rat bioassays) | Inadequate data |
| Liver tumors | Yes | Negative to equivocal for liver and bile duct tumors |

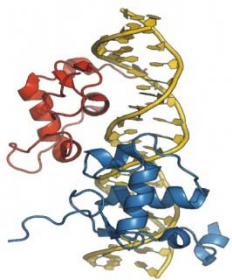
Note: There are data for early key events that suggest quantitative differences – but magnitude is likely to be endpoint specific.



CAR Species Concordance

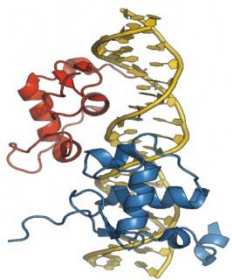
Species Concordance Table – CAR Activation MOA with Phenobarbital as One Example

| Key Event or Marker | Mouse | Rat | Hamsters | Primates | Human |
|---|--|---------------------------------------|--------------|-----------------------|---|
| CAR activation | Yes (1, 2) (in vitro and in vivo) | Yes | Yes | Yes | Yes (157) acknowledge differences (in vitro) |
| Altered gene expression | Yes (16) | Yes | Yes | Yes | Yes (in vitro) |
| Altered DNA methylation/epigenetic changes | DNA methylation altered (35-37) | DNA methylation altered ²⁴ | No data | No data | Possible but no data |
| Cyp 2B induction | Yes (16) | Yes | Yes | Yes | Yes (154) (in vitro) |
| Hypertrophy | Yes (16) | Yes | Yes | Yes | Yes (121, 168, 169) (in vivo) |
| Increased cell proliferation | Yes (16) | Yes ²⁸ | No | No? (check for refs). | No (171) (in vitro) (and hCAR/ hPXR mice in vivo) |
| Decreased apoptosis | Yes – but mixed results (44, 47, 145, 146) | Yes | No | ? | No (137) (in vitro) |
| Gap Junction Intracellular Communication inhibition | Yes | Yes | No (Klaunig) | No (Klaunig) | No (Baker, 1995) (in vitro) |
| Clonal expansion (Foci) | Yes | Yes | No | No data | Possible but No data either way |
| Tumors | Yes – most strains (9, 10, 15) | Yes – certain strains (9, 10) | No (131) | No data | No (9, 10) in vivo |



PPAR α Species Concordance

| <u>STEP</u> | <u>Qualitative</u> | <u>Quantitative</u> |
|---------------|--------------------|--|
| Metabolism | plausible | Same for rodents and humans |
| Activation | plausible | Higher MEHP concentrations needed to activate human receptor (~3-10 fold) Target genes not responding in human cells compared to rat cell line |
| Proliferation | plausible | Non-human primates don't respond (cell proliferation) Liver size not changed in humans (Based on MRI) Humanized mice – no effect at tumorigenic doses Uniformly negative for DNA replication in human (& non-human primate) hepatocytes |
| Foci | plausible | No evidence Fairly rare observation in human liver |
| Tumors | plausible | Epi data – no evidence (decades of exposures) – albeit @ lower doses than tumor production in rodents (gemfib and clofib) Usually in humans requires chronic injury, infection (Hep B,C, etc), cirrhosis (alcohol) |

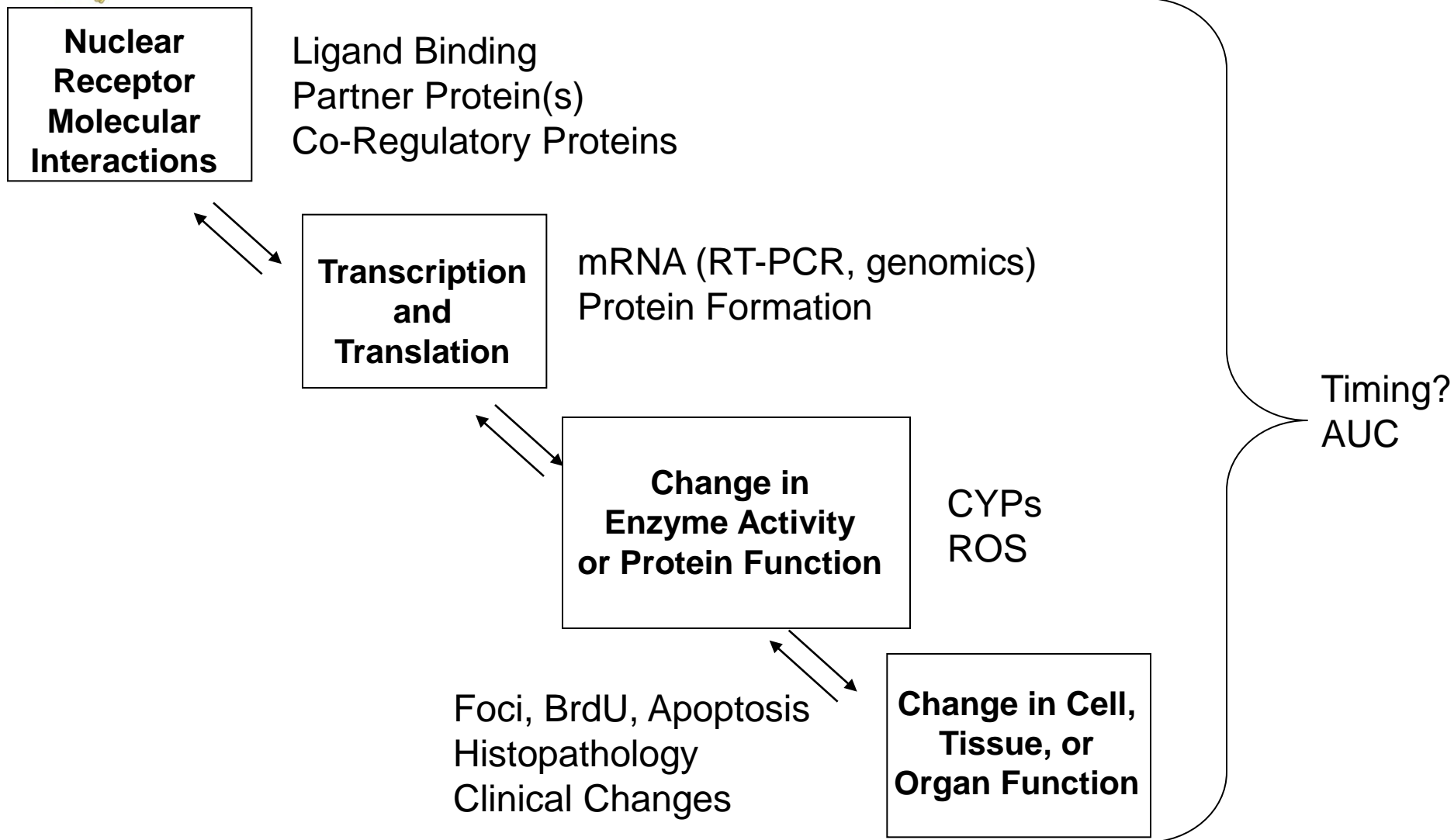


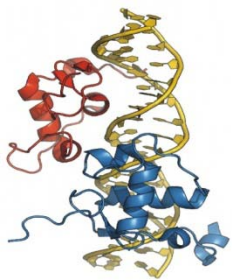
Desired MOA Data Sets

- **Exclude other MOAs** (cytotox, mutagenicity, if not already available).
- **Data to confirm the rodent MOA**
 - Appropriate rodent studies to examine endpoints including:
 - early, observable key events (e.g. Cell proliferation, CYP induction, apoptosis suppression, hypertrophy, liver wt)
 - Evaluate in a dose-response design.
 - Knockout models
 - Genomics, Proteomics
- **Confirmation of lack of human relevance via NR MOA**
 - e.g. use of primary human hepatocytes and when appropriate humanized models



Quantitative Dose-Response Modeling

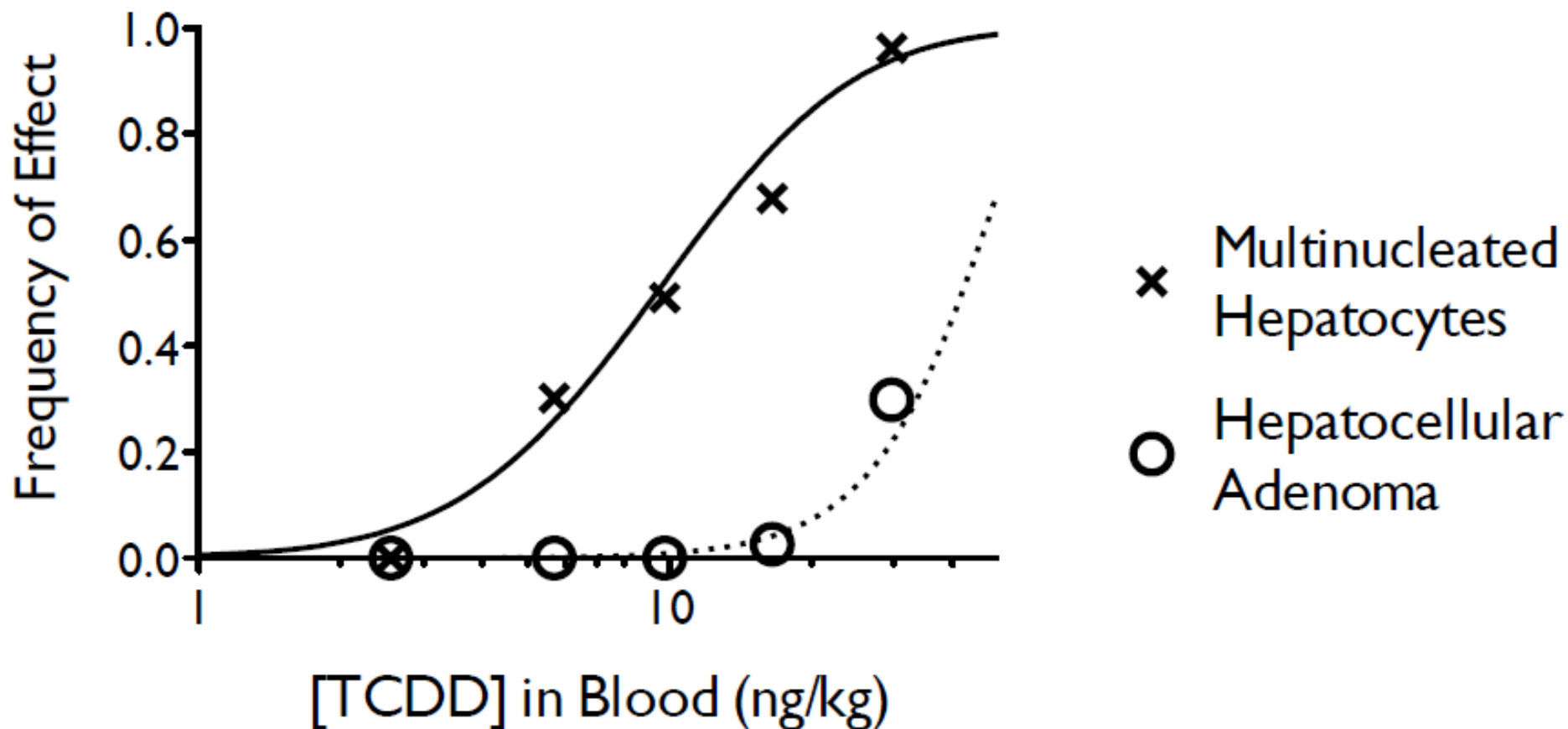




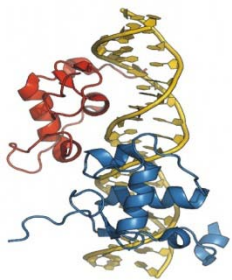
Dose Response Example for AHR: Hepatocellular Cancer Key Event

Multinucleated Hepatocyte RfD: 2 – 70 pg/kg/day (UFs: 1.0 – 30)

Hepatocellular Cancer RfD: 20 to 600 pg/kg/day (UFs: 1.0 – 30)



based on Simon et al., 2009



Summary

The AHR expert panel, for the first time in an expert panel format, rigorously applied the MOA framework and agreed on a mode of action.

The CAR expert panel identified the relevant data and rigorously applied the MOA and HRF with emphasis on the qualitative and quantitative aspects of human relevance.

The PPAR α expert panel built upon previous applications of the framework using significant new data that allowed for refinement of the key event descriptions and updated considerations related to human relevance.

Each panel identified key data needs and suggested improvements for application of the MOA/HRF.

A series of manuscripts will be forthcoming on the results of this workshop.