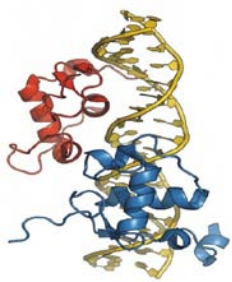




Dose-Response Approaches for Nuclear Receptor-Mediated Modes of Action Workshop Preliminary Report



Workshop Organizing Committee

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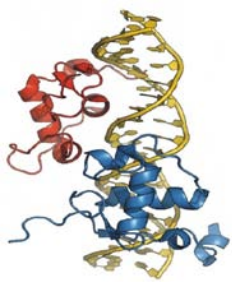


Major Goals of the Workshop

Determine whether the biology of nuclear receptors necessitates a minimum threshold of ligand to be available for activation to stimulate downstream responses including gene expression.

Determine whether a minimum threshold of receptor ligand is required for any toxicological responses.

Determine whether linear low-dose modeling of receptor ligands is appropriate, based on the underlying science of nuclear receptor signaling biology, and if not, provide insights into more appropriate low-dose modeling approaches.



Discussion Question

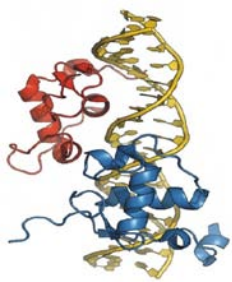
What is the mode of action (MOA) for NR-mediated rodent liver tumors for a model NR activator, as evaluated using the IPCS Framework for Human Relevance and the modified Hill Criteria applied to MOA (IPCS and EPA MOA Framework)?

Which model compound used was dependent on which NR was being considered.

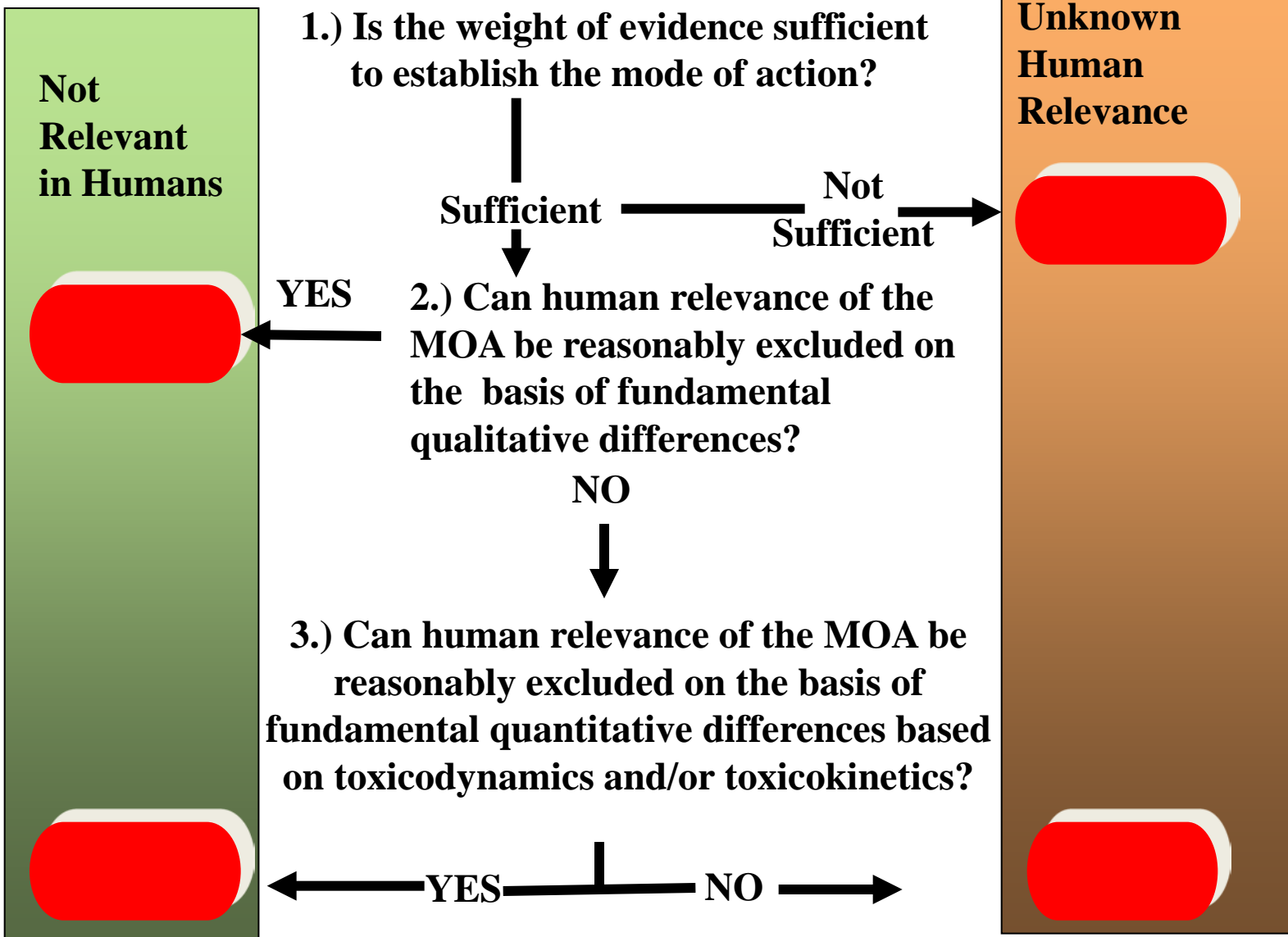
AHR – TCDD

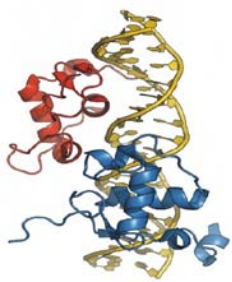
CAR/PXR – Phenobarbital

PPAR-alpha – DEHP



WHO/IPCS Human Relevance Framework





Using Hill Criteria to Determine Key Events for Mouse Liver Tumor MOA

Possible Key Events

Strength

Consistency

Specificity

Temporality

Biological Gradient

Biological Plausibility

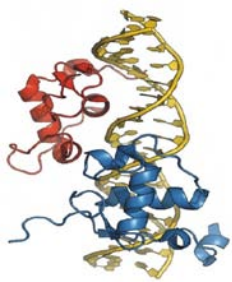
Coherence

Causal Key Event

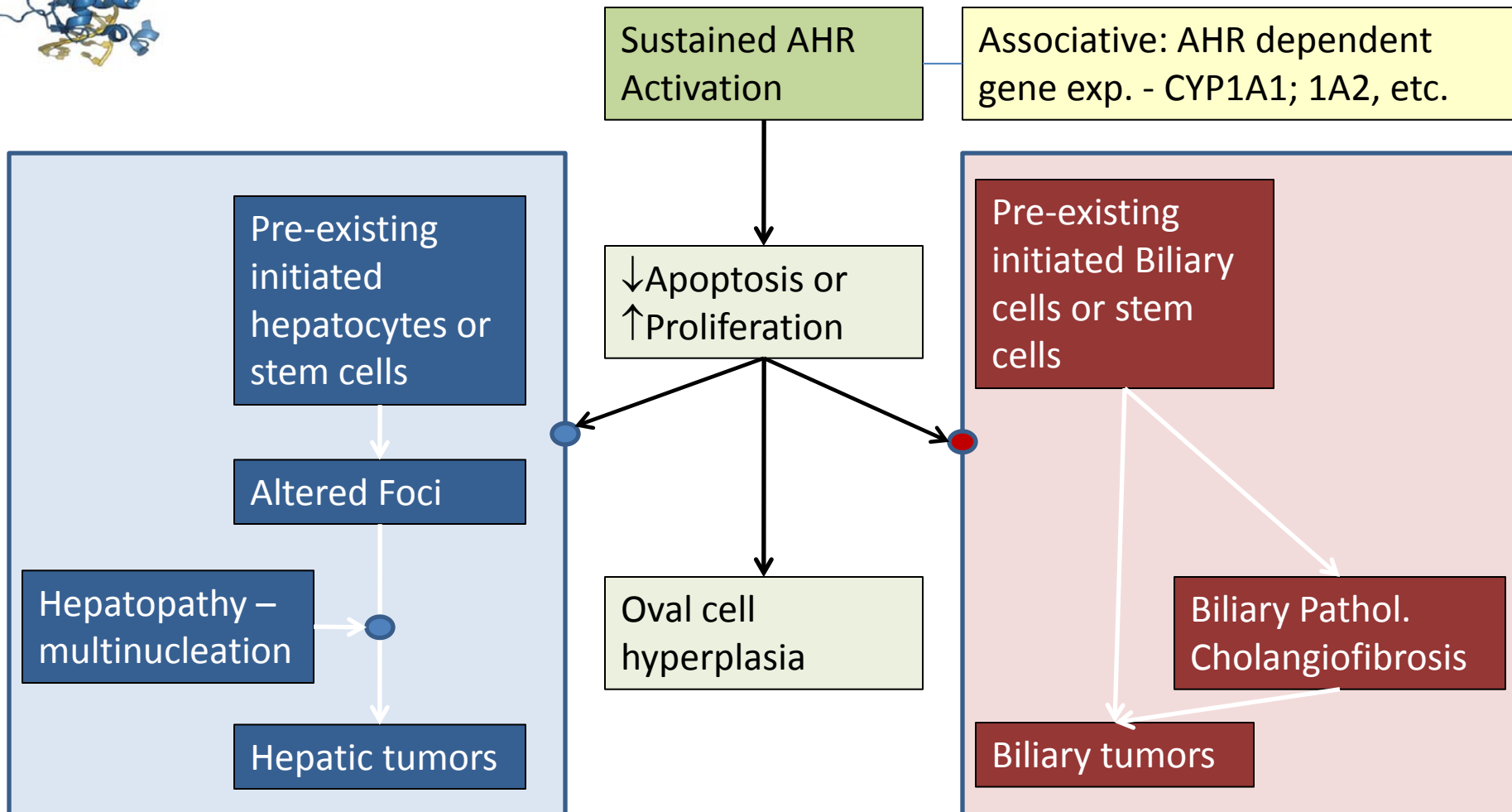
Associated Event (marker)

Modulating Factor

None of the above

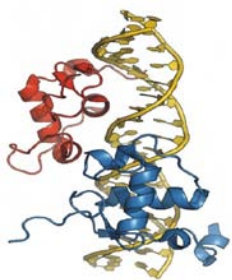


MOA - Rodent Liver Tumorigenicity of Planar AHR Ligands



Modulating factors:

- Estradiol effects; oxidative Stress; cell communication; mitoinhibition



CAR MOA: Key Events

CAR Over Activation

Altered gene expression
specific to CAR activation

Altered epigenetic changes specific
to CAR activation

Cyp2b Induction

Hypertrophy

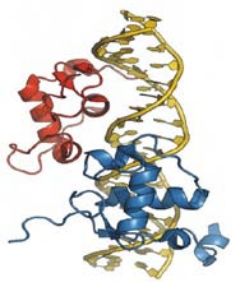
Increased cell
proliferation

Decreased
apoptosis

Gap Junction
Communication Inhibition

Clonal expansion
leading to altered foci

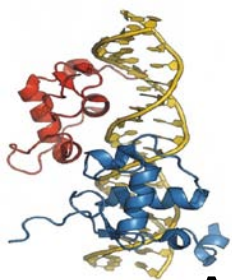
Liver Adenomas/Carcinomas



Mode of Action of Rodent Liver Carcinogens: PPAR α involvement

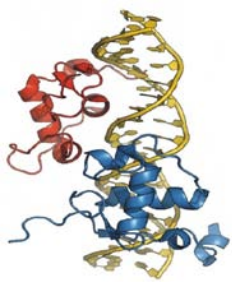
Key Events: 2010 version

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



Modulating Factors

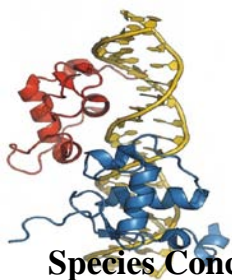
- A number of associative events in Klaunig et al., 2003 model were initially nominated as potential key events based on new data
 - Increase in oxidative stress
 - NF-kB activation
 - Kupffer cell activation
- Consideration of evidence led to designation of these events as modulating factors
- Inhibition of gap junction was considered to be a generic (nonspecific) marker of many biological processes and therefore left out of model



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	Inadequate data for liver tumors; possible for tumors at all sites.

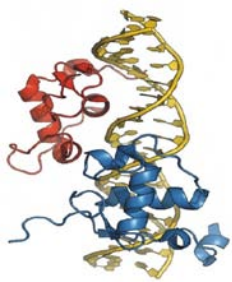
Note: There are data for early key events that suggest quantitative differences – but magnitude is likely to be endpoint specific.



MOA Human Relevance

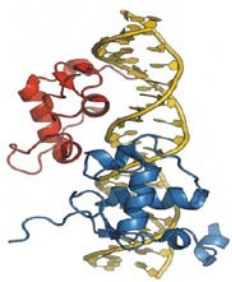
Species Concordance Table – CAR Activation MOA with Phenobarbital as One Example

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



Human Relevance to Rodent Models for PPAR α : Biological Plausibility/Concordance Analysis

<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)



Ideas for generation of desired data set

- **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.
 - NR null mouse study to show lack of effects, if possible.
 - NR activation/nuclear translocation assays – if suitable models are available and valid.
- **Confirmation of lack of human relevance via NR MOA**
 - e.g. use of primary human hepatocytes and when appropriate humanized models



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.