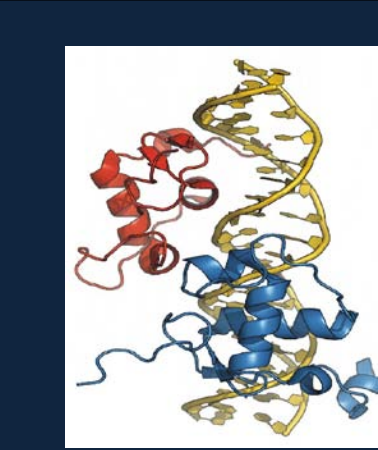




MODE OF ACTION (MOA) AND DOSE-RESPONSE APPROACHES FOR NUCLEAR RECEPTORS



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Background

- A Steering Committee chaired by Dr. Julian Preston and Dr. Melvin Andersen organized the workshop to evaluate dose-response implications of nuclear-receptor mediated modes of action for liver tumors using a case study approach.
- Evidence for the mode of action (MOA), its human relevance, and the dose-response implications for nuclear receptor-mediated liver cancer was explored based on data for selected ligands and activators.
- Expert panels were recruited, providing a broad range of expertise and perspectives, covering molecular biology, toxicology, dose-response modeling, risk assessment, and other areas.

Methods

DISCUSSION QUESTIONS: Case study panels used discussion questions to focus the MOA analysis and dose response considerations:

- Is the existing biological knowledge for liver tumors induced through the receptor sufficiently understood to identify the MOA and its component key events, associative events, and modulating factors? What are the key data gaps?
- Is the existing biological knowledge of the MOA sufficiently understood to reasonably exclude, on a qualitative or quantitative basis, the human relevance of rodent liver tumors induced through this receptor? What are the key data gaps?
- Are the data sufficient to identify a MOA? What is the relevance to humans? What are the dose-response implications of the key events in the MOA, including associative events and modulating factors? Are the data adequate to develop biologically based dose-response or other biological-informed models for this receptor? Is linear low-dose modeling appropriate based on the underlying science of nuclear receptor biology?

Mode Of Action

Approach/Results:

To reflect the availability of significant mechanistic understanding in nuclear receptor biology, the workshop panels agreed on the following definitions for use in evaluating the related biological steps in a proposed MOA to follow the IPCS (2007) framework:

- Key Event:** An empirically observable causal precursor step to the adverse outcome that is itself a necessary element of the mode of action. Key events are required events for the MOA, but often are not sufficient to induce the adverse outcome in the absence of other key events.
- Associative Event:** Biological processes that are themselves not causal necessary key events for the MOA, but are reliable indicators or markers for key events. Associative events can often be used as surrogate markers for a key event in a MOA evaluation or as indicators of exposure to a xenobiotic that has stimulated the molecular initiating event or a key event.
- Modulating Factor:** There are many factors or biological responses that are not necessary to induce the adverse outcome, but could modulate the dose-response behavior or probability of inducing one or more key events or the adverse outcome. Such biological factors are considered modulating factors

CAR/PXR

- Constitutive Androstane Receptor (CAR)/ Pregnane X Receptor (PXR)
- Case Study Approach – the CAR/PXR panel used phenobarbital as the model receptor-activator.
- The CAR expert panel identified relevant data and applied the framework with emphasis on the qualitative and quantitative aspects of human relevance. Overall the panel concluded that the mode of action is not relevant to humans based on qualitative considerations.

MOA - Rodent Liver Tumorigenicity of CAR Activator (Phenobarbital)

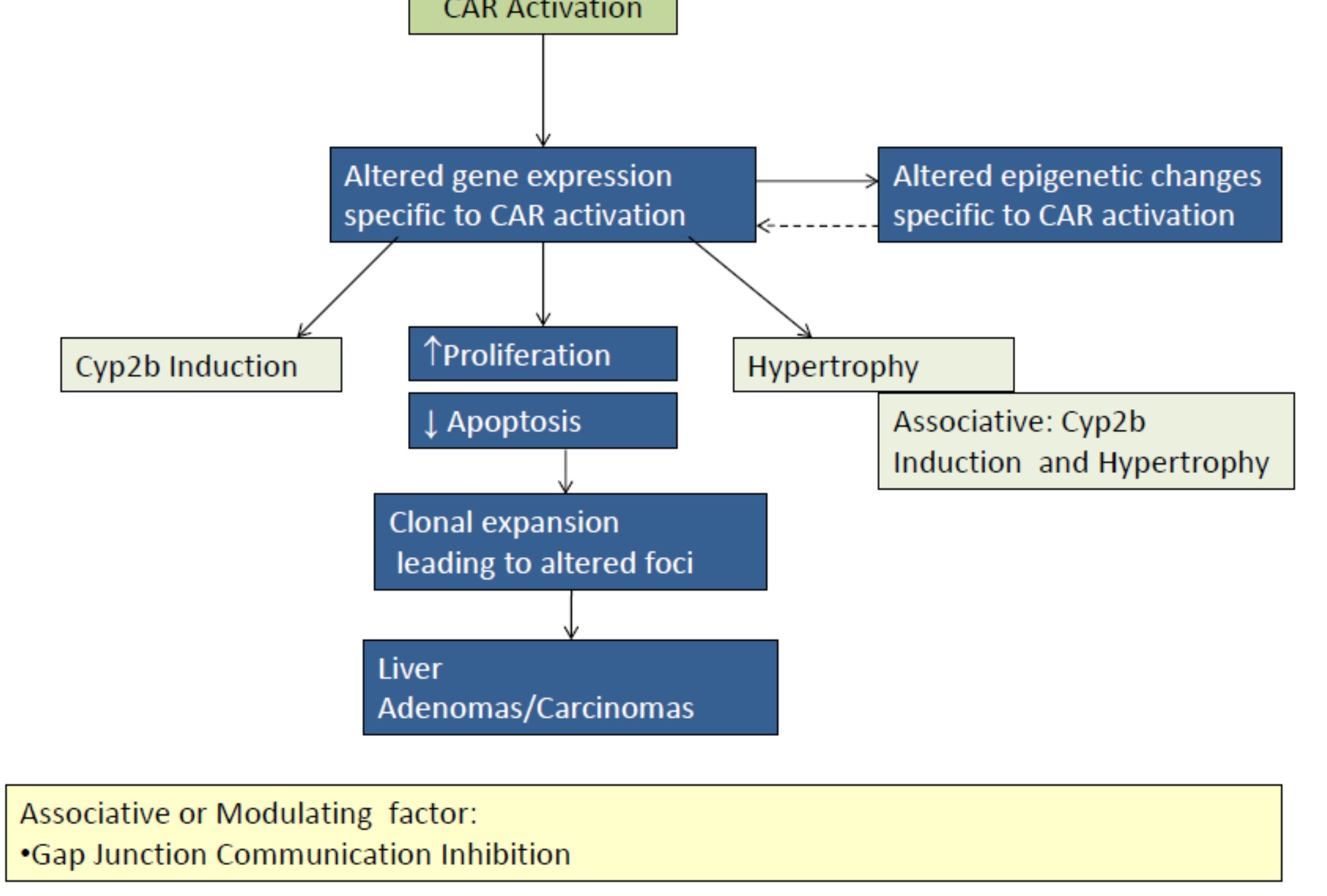


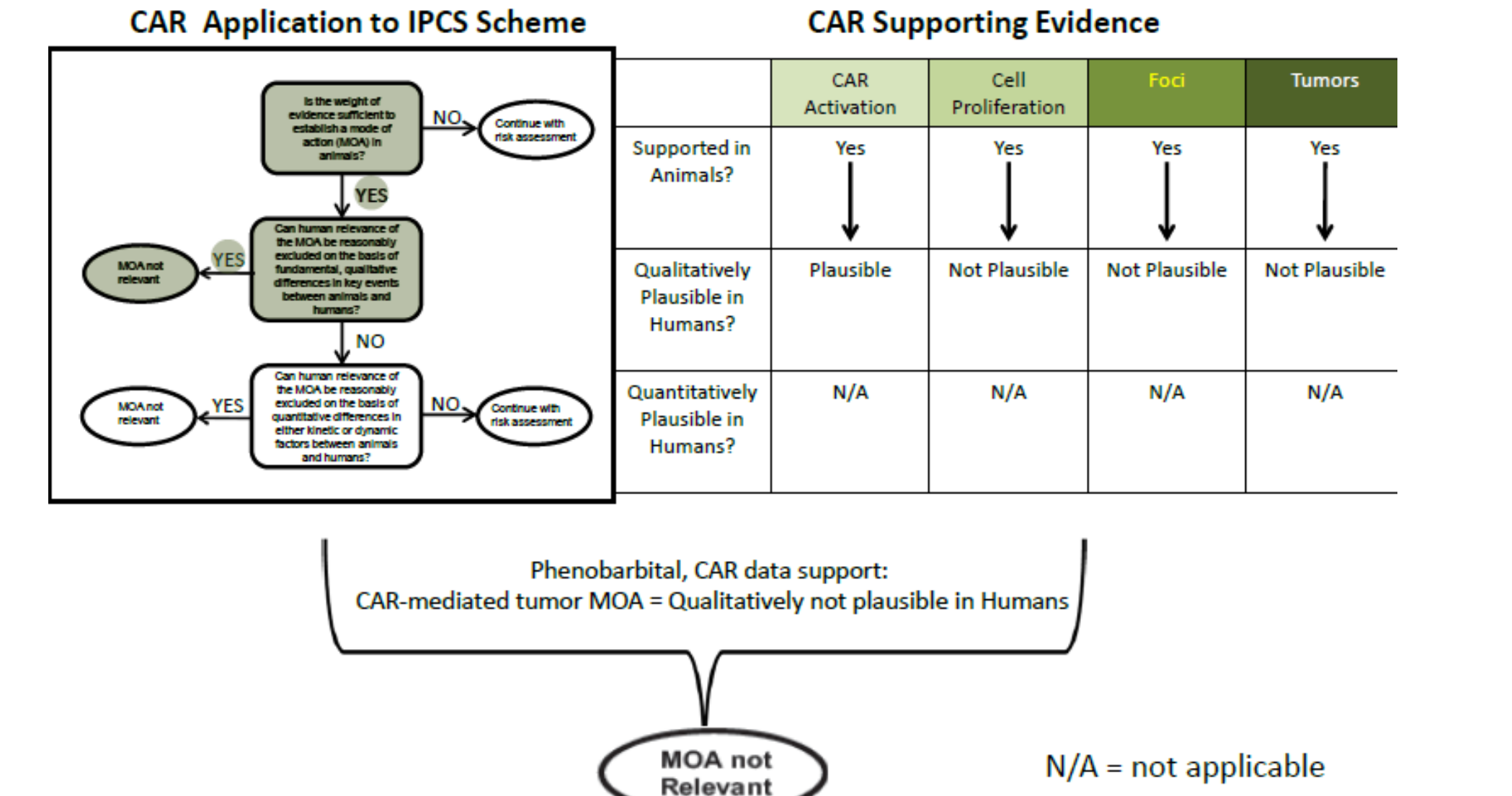
Table 1: Dose-Response, Temporality Concordance Table (Male CD1 or Male C57BL/10J Mice)

Dose (mg/kg/day)	Key Event 1 (CAR activation)	Key Event 2 (Altered gene expression)	Key Event 3 (↑ Cell Proliferation)	Key Event 4 (Clonal expansion leading to altered foci)	Liver Adenomas/Carcinomas
0.15	-	-	-	nd	nd
1.5 - 10	+	+	-	nd	-
15	+	+	+	nd	nd
75-150	+	+	+	nd	+
22 - 56	nd	nd	+	-	-
98-113	nd	nd	+	+	+

1. CAR Activation inferred in vivo based on surrogate markers: 5-β PREG-3β-OH activity, 7-α-OH-PREG activity, CYP2B10 mRNA. CAR activation demonstrated in other in vivo studies at single dose levels in in vitro studies. 2. Altered gene expression specific to CAR inferred based on 5-β PREG-3β-OH activity, 7-α-OH-PREG activity, CYP2B10 mRNA (1.3 mg/kg/d), hepatocellular hypertrophy (15 mg/kg/d) & associative events not determined. No dose response data available for epigenetic changes. Data are from CD-1 mouse studies (1, 16) [grey shading] or from C57BL/10J mouse studies (15) [white shading].

Table 2: Species Concordance, Human Relevance Table

Key Event or Associative Event	Mouse	Rat	Hamsters	Primates	Human
CAR activation	Yes (1,2) (in vitro and in vivo)	Yes	Yes	Yes	Yes (157) (in vitro)
Altered gene expression	Yes (16, 97, 107)	Yes	Yes	Yes	Yes (in vitro)
Altered DNA methylation/epigenetic changes	DNA methylation altered (29-43)	DNA methylation altered (24)	No Data	No Data	Possible but no data
Cyp 2B induction	Yes (15, 16)	Yes	Yes	Yes	Yes (154) (in vitro)
Hypertrophy	Yes (15, 16)	Yes	Yes	Yes	Yes (121, 165, 169) (in vivo)
Increased cell proliferation	Yes (15, 16)	Yes (28)	No	No	No (171) (in vitro and hCAR/hPXR mice in vivo)
Decreased apoptosis	Yes – but mixed results (44, 47, 145-5)	Yes	No	?	No (137) (in vitro)
Gap junction communication inhibition	Yes (187-192)	Yes (187-189)	No (187-189)	No (189)	No (189) (in vitro)
Clonal expansion (foci)	Yes (15)	Yes	No	No Data	Possible but no data
Tumors	Yes – most strains (9-10, 15)	Yes – certain strains (9-10)	No (131)	No Data	No (9-10) (in vivo)



PPARα

- Peroxisome Proliferator-Activated Receptor Alpha (PPARα)
- Case Study Approach – the PPARα panel used di-(2-ethylhexyl) phthalate (DEHP) and clofibrate as the model receptor-activators.
- For PPARα, the expert panel built upon previous applications of the MOA/HRF framework using significant new data that allowed for refinement of the key event descriptions and updated considerations related to human relevance. Overall the panel concluded that the mode of action is not relevant to humans based on qualitative and quantitative considerations.

MOA - Rodent Liver Tumorigenicity of PPARα Activators

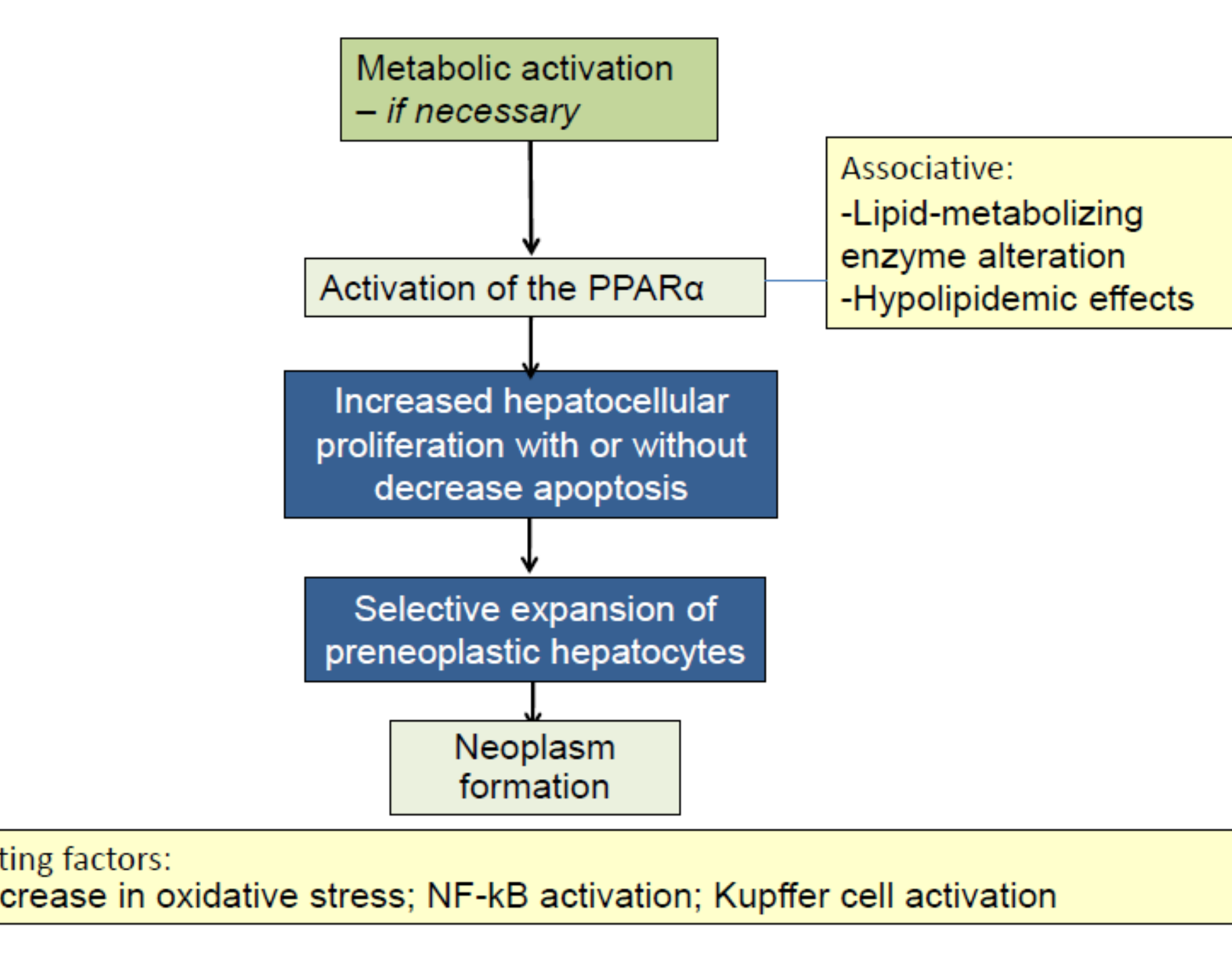
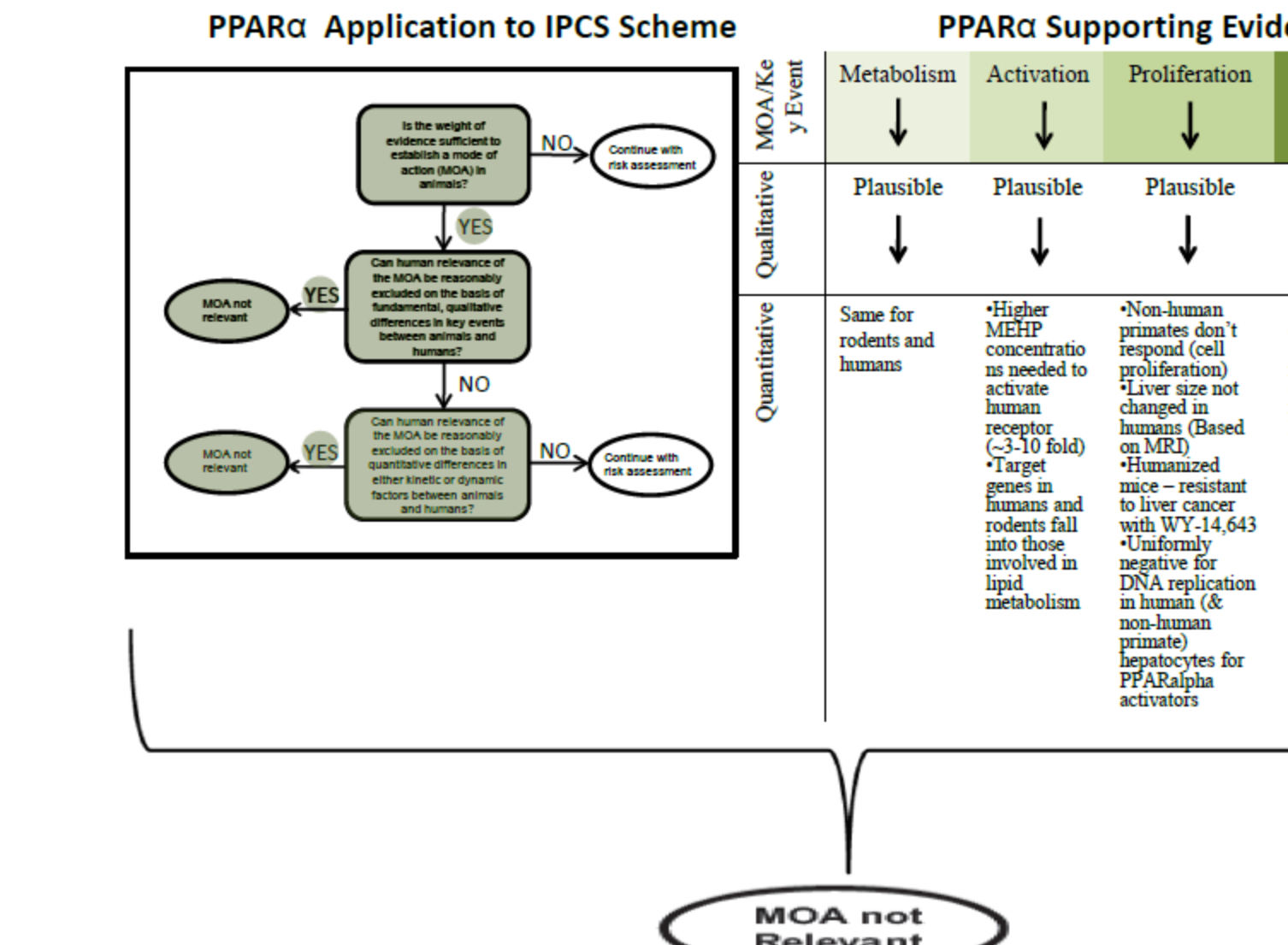


Table 1: Dose-Response, Temporality Concordance Table for DEHP in rats

Dose of DEHP (ppm in diet)	Key Event 1 (Metabolic Activation)	Key Event 2 (PPARα Activation)	Key Event 3 (Cell Proliferation +/- apoptosis)	Key Event 4 (Selective expansion of preneoplastic hepatocytes)	Hepatocellular Tumors
500	+	-	ND	ND	-
1000	+	+/-	-	ND	-
2500	+	+	ND	ND	+/-
6000	+	+	+	ND	+
12,000	+	+	+	ND	+

Table 2: Species Concordance, Human Relevance Table for PPARα Activators Including DEHP

Key Event or Associative Event	Mice	Rats	Hamsters	Primates	Humans
Metabolic Activation (if necessary)	yes	yes	yes	yes	yes
Lipid-metabolizing enzyme alteration	Yes	yes	yes	yes	yes
Hypolipidemic effects	yes	yes	yes	yes	yes
Activation of PPARα	yes	yes	yes	yes	yes
Increased hepatocellular proliferation with or without decrease apoptosis	Yes Both in vitro and in vivo	Yes Both in vitro and in vivo	No evidence from in vivo studies	No Not seen in vitro in primary hepatocytes or in vivo	No Not seen in primary hepatocytes in vitro
Selective expansion of preneoplastic hepatocytes	yes	yes	No evidence from in vivo studies	No data	No data
Neoplasm formation	yes	yes	no	No evidence	No evidence



AHR

- Aryl Hydrocarbon Receptor (AHR)
- Case Study Approach – the AHR panel used 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as the model receptor-activator (planar persistent activator).
- The AHR expert panel, for the first time in an expert panel format, rigorously applied the MOA/HRF framework and agreed on an MOA. Overall the panel concluded that the mode of action cannot be reasonably excluded for humans, and to continue with the risk assessment.

MOA - Rodent Liver Tumorigenicity of Planar AHR Ligands

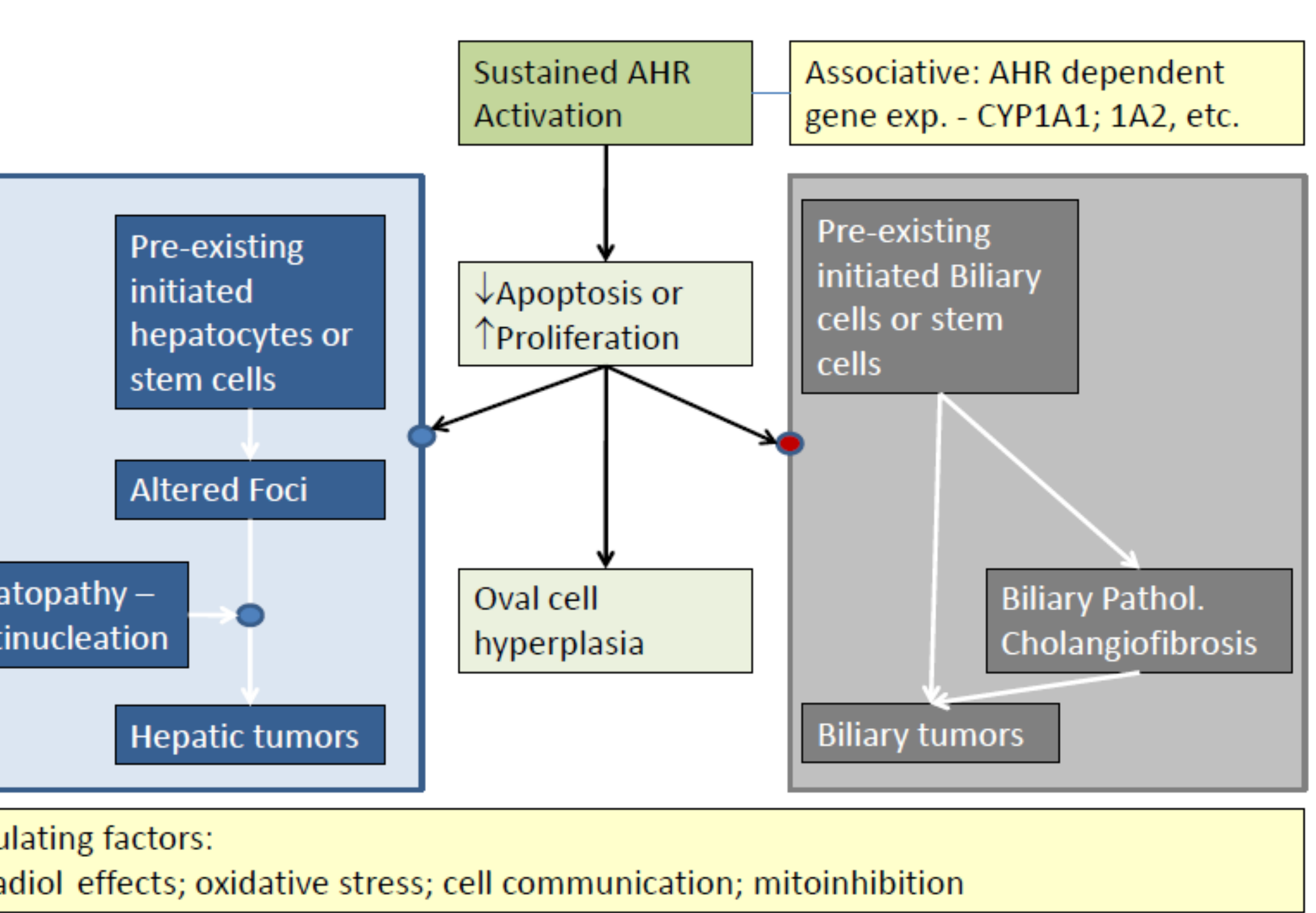
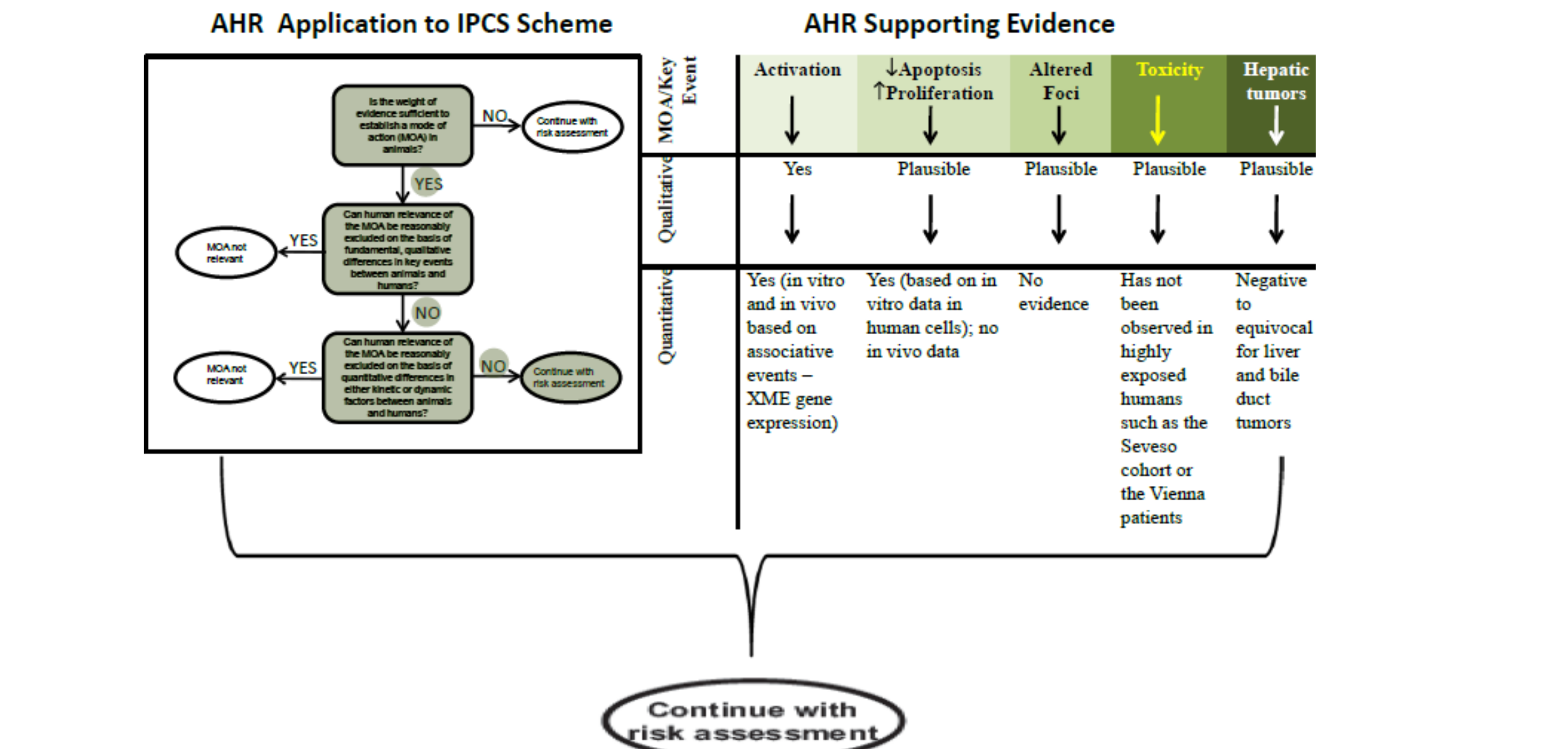


Table 1: Dose Response, Temporality Concordance

Dose (ng/kg)	Key Event 1 (AHR Activation/Transcription)	Key Event 2 (↓ Apoptosis)	Key Event 3 (Proliferation/Hyperplasia)	Key Event 4 (Toxicity)	Tumors
<100		+			
100-1000	++++	+	+		
1000-2000	++++	+	++	+	
2000-5000	++++	+	+	+	
5000-10000	++++	++	+	+	
>10000	++++	++	++	++	+

Table 2: Species Concordance, Human Relevance Table

Key Event of Associative Event	Rat	Human
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)
↓ Apoptosis or ↑ Proliferation	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 Foci	Yes (observed in rat bioassays)	No evidence in humans
Toxicity	Yes (observed in rat bioassays as toxic hepatopathy)	Has not been observed in highly exposed humans such as the Seveso cohort or the Vienna patients
Hepatic tumors	Yes	Negative to equivocal for liver and bile duct tumors



Workshop Conclusions

Theme 1: Importance of Discussion Among Diverse Experts

The Peer Workshop approach featured:

- The sharing of information among a unique mix of science disciplines, leading to a greater understanding of the complexities of each area and allowing for meaningful consensus.

Theme 2: Challenges due to the current knowledge of nuclear receptor-mediated toxicity

- Mechanisms at the level of induced genes remains unclear, but this data is not required for MOA evaluation - associative events data are often used.
- There is qualitative similarity in the overall MOA for later key events for this set of nuclear receptors.

Theme 3: The application of current MOA/HRF for nuclear receptor-mediated outcomes and potential refinements to the framework

- When investigating a possible mode of action, one does not need to identify every biological step in the toxicological mechanism. In many cases, key steps in the MOA are adequately represented by associative events.
- Additional mechanistic data can inform quantitative decisions and dose-response modeling - modulating factors could influence these interpretations.
- A value of information approach could be used to better inform the dose-response implications of proposed research.
- Other refinements to the MOA approach might address the impact of multiple pathways or components within a single integrated MOA.

Workshop Sponsors

Alliance for Risk Assessment; American Chemistry Council's Center for Advancing Risk Assessment Science and Policy; CropLife America; CXR Biosciences; DuPont; The Hamner Institutes for Health; Indiana University, Dept. of Environmental Health; National Institute of Environmental Health Sciences (NIEHS); Society of Toxicology; Society for Risk Analysis; 3M Company; Toxicology Excellence for Risk Assessment; U.S. EPA, National Health and Environmental Effects Research Laboratory; U.S. EPA, Office of Chemical Safety and Pollution Prevention; U.S.EPA, Office of Water

Acknowledgements

For more information on the workshop sponsors, case study panel membership, references and other background materials see the workshop website at: <http://www.tera.org/peer/nuclearreceptor/>