SESSION 3: Assimilating and Using Mechanistic Information to Support Evidence Synthesis and Integration

8:40 – 9:10 Experiences with the Mode-of-Action Framework as an Organizing Framework for Mechanistic Data

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The Mode of Action /Human Relevance Framework

History of Cancer MOA/HR framework

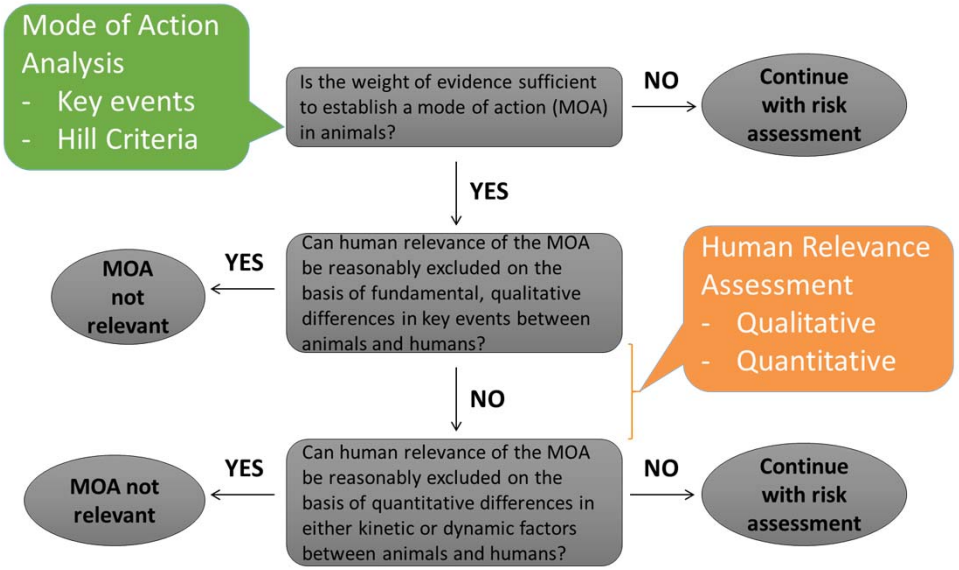
- **US EPA - Guidelines for Carcinogen Risk Assessment**
  - 1986, 1999 and 2005
- **IPCS - Mode of action of chemical carcinogenesis**
  - Sonich-Mullin et al., Regul. Toxicol. Pharmacol. 34:146-152, 2001
- **ILSI/RSI, EPA, Health Canada - Human relevance framework for chemical carcinogens**
  - Meek et al., Crit. Rev. Toxicol. 33:591-653, 2003; Cohen et al., Crit. Rev. Toxicol, 33: 581-9, 2003; Cohen et al., Tox Sci, 78: 181-186, 2004.
- **ILSI, EPA, Health Canada - Human Relevance of mode of action and life stage information of animal toxicity data**
  - Seed et al., Crit. Rev. Toxicol, 35:663-672, 2005
- **IPCS-Human relevance framework for chemical carcinogens**
  - Boobis et al., Crit. Rev. Toxicol. 36: 781-792, 2006
- **New developments of the WHO/IPCS framework on MOA and species concordance analysis**
  - Meek et al., J Appl Toxicol 34(1): 1-18, 2014

- A systematic evaluation of carcinogenic data available for a certain chemical, a postulated MOA in animals for this chemical, and its relevance to humans

Importance:

- Harmonize risk assessment.
- Provide a guideline for data analysis.
- Transparently presenting data.
- Identify data needs.
- Help in decision-making policies.

The Mode of Action /Human Relevance Framework



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1. Postulated MOA in animals
   - Key events; and associated critical parameters
2. Experimental support (Bradford Hill Criteria)
   - Dose-response relationships
   - Temporal association
   - Strength, consistency & specificity of key events and tumor response
   - Biological plausibility & coherence
   - Alternative MOAs
   - Uncertainties, inconsistencies and data gaps
3. Conclusion

Mode of Action Framework - Approach My Experience

- Process (needs to be transparent)
- Define the problem/ issue
  - Mechanism vs chemical (ie PPARa versus DEHP)
- Develop questions (mode of action diagram)
- Form a review committee
  - members should have a basic understanding of the science needed to evaluate the issue
  - should represent diverse sectors and disciplines
- Prior to the panel meeting face to face compile and share all existing data on the chemical.
  - (concentrating on the target tissue)
  - (including but not limited to ADME, metabolism, pathology, clinical chem. Or biological data) (in vivo, in vitro)
- The panel members should review data sent to them and identify (and share) other available data
- At the face to face meeting a (or several) strawmen should be proposed
  - With possible Key events, associate events and modulating factors
  - Data gaps should be identified
- Structure of Panel is Critical
  - chair or co-chairs
  - support for acquiring and disseminating data
  - Representation of critical disciplines important (pathology, molecular biology, metabolism etc)

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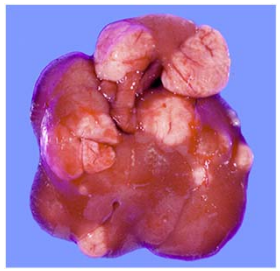
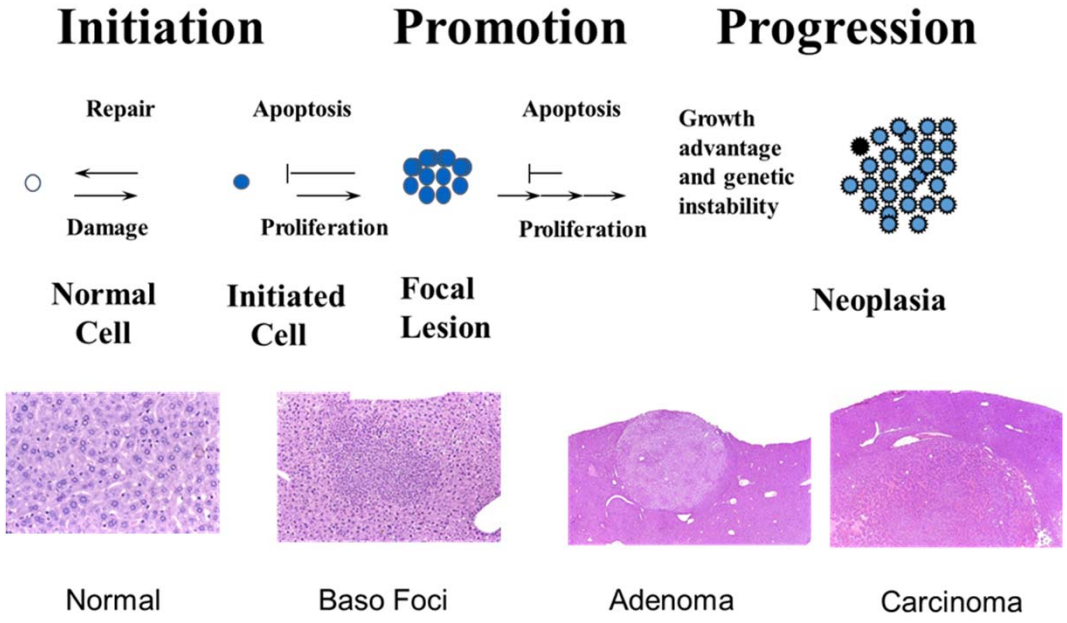
Mode of Action Framework - Approach

Key Events	An empirically observable ***causal*** precursor step to the adverse outcome that is itself a ***necessary*** element of the MOA. 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.
Associate Events	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 indictors of exposure to a xenobiotic that has stimulated the molecular initiating event or a key event.
Modulating Factors	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.

Rodent Liver Tumors

- Most common endpoint in cancer bioassays
- Liver is often the most sensitive target in 2-yr bioassays
  - Mouse: 45% of all chemicals
  - Rat: 37% of all chemicals
- Strain-dependent background levels
  - C3H/HeJ – 100%
  - B6C3F1 – 10-50%
  - C57Bl/6 – close to 0-2 %





Modes Of Action (MOU) Of Hepatic Carcinogens

A. **Genotoxic/DNA reactive** (AFB1)

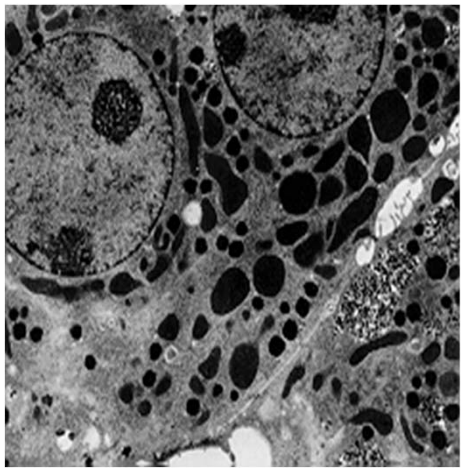
B. **Nongenotoxic/non-DNA reactive**
   - Receptor Mediated
     - CAR (Phenobarbital-like)
     - AhR (Dioxin-like)
     - Peroxisome proliferators (PPARa-mediated)
     - Steroid Hormone (ER mediated)
     - Other
   - Non Receptor Mediated
     - Cytotoxicity ($CHCl_3$, CCl_4)
     - Oxidative stress (Fe, Cu overload)
     - Infection (HBV/HCV)
     - Fatty Liver
     - Other

Peroxisome Proliferators (PPs) (PPAR alpha activators)

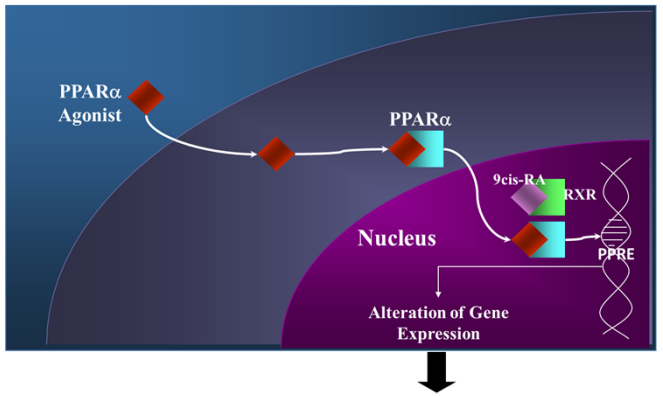
- A chemically diverse group of rodent carcinogens
  - phthalate plasticizers: DEHP
  - herbicides
  - hypolipidaemic drugs: nafenopin, fenafibrate
  - Organic solvents : TCE PCE, TCA
- Liver tumors (rats and mice)

- Response to peroxisome proliferators is mediated by PPAR alpha
- PPREs in PP responsive genes (ACO, CYPs)
- PPAR a null mouse is nonresponsive
  - To Peroxisomes
  - To DNA synthesis/apoptosis
  - To tumor formation

- 1954; 1965 Peroxisomes
  - (Rhodin; de Duve)
- 1980; Peroxisome proliferators induce cancer
  - (Reddy et al)
- 1990; PPAR alpha - Cloned
  - (Isseman and Green)
- 1995; PPAR alpha Knock Out
  - (Gonzalez et al)



ROLE OF PPARα IN TRANSCRIPTIONAL REGULATION



Phenotypic Responses

Several Workshops

- Purpose
- Describe the state of the science on the rodent MOA of liver tumor induction and human relevance by PPARa activators

- Particular importance: identification of studies with information on dose-dependent effects in the liver

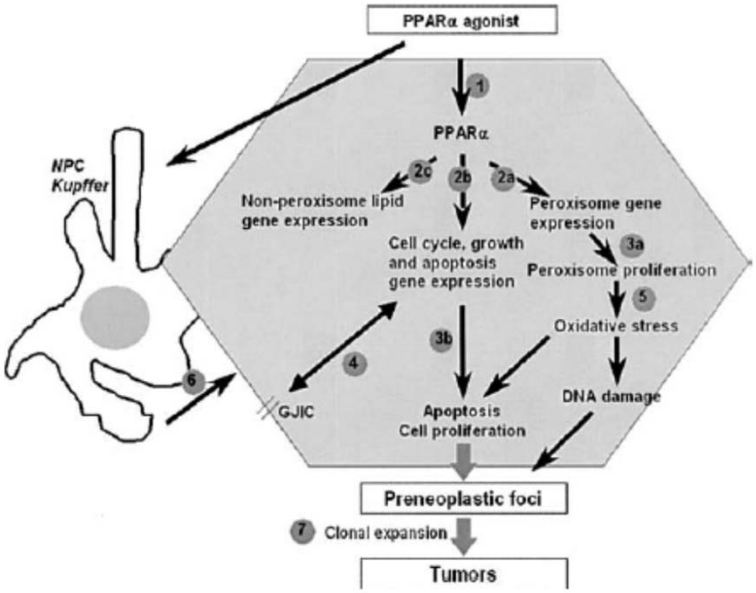
- Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A., 2003. PPARα agonist-induced rodent tumors: modes of action and human relevance. Crit. Rev. Toxicol. 33, 655– 780.
- Corton, J.C., Cunningham, M.L., Hummer, B.T., Lau, C., Meek, B., Peters, J.M., Popp, J.A., Rhomberg, L., Seed, J., Klaunig, J.E., 2014. Mode of action framework analysis for receptor-mediated toxicity: the peroxisome proliferator-activated receptor alpha (PPAR α) as a case study. Crit. Rev. Toxicol. 44, 1 – 49.
- Felter SP[1], Foreman JE[2], Boobis A[3], Corton JC[4], Doi AM[5], Flowers L[6], Goodman J[7], Haber LT[8], Jacobs A[9], Klaunig JE[10], Lynch AM[11], Moggs J[12], Pandiri 2017 **Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action.** Regul Toxicol Pharmacol. 2018 Feb;92:1-7.
- Corton, J.C., Peters, Jeffrey M., Klaunig, James E., 2018. The PPAR α -dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. Archives Toxicol

Workshop 1

HISTORY

- As part of the ILSI/RSI, EPA, Health Canada - Human relevance framework for chemical carcinogens workshops Peroxisome proliferation was consider as a case study
- Assembled individuals from academia, industry (chemical and pharmaceutical), EPA, ILSI-RSI and FDA
- Multiple face to face meetings over two years
- Initial meeting, changed term peroxisome proliferator to PPAR alpha agonist to reflect the common mechanism
- Based on Data available (rodent and some human (pharmaceutical)

- Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A., 2003. PPARα agonist-induced rodent tumors: modes of action and human relevance. Crit. Rev. Toxicol. 33, 655– 780.



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3. Conclusion

Conclusions from 2001-03 workshop

Mode Of Action And Human Relevance Of PPARα agonist Rodent Liver Carcinogens

Key Event	Evidence in Rodents	Evidence in Humans (Primates)
Activation of PPARα in liver	Yes	Yes
↑ Cell proliferation gene expression	Yes	Unknown/No
↑ Peroxisome proliferation gene expression	Yes	No
↑ Non Peroxisome proliferation lipid lowering	Yes	Yes
↑ Hepatocyte proliferation	Yes	No
↑ Selective increase in focal liver lesion growth (cell proliferation/apoptosis)	Yes	No
Formation of neoplastic lesion	Yes	No

Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A., 2003. PPARα agonist-induced rodent tumors: modes of action and human relevance. Crit. Rev. Toxicol. 33, 655– 780.

Workshop 2

HISTORY

- A second workshop 2013-2014 was formed to examine specifically receptor mediated MOA in liver.
- Peroxisome proliferators were examined along with CAR and AhR
- Workshop individuals came academia, industry (chemical and pharmaceutical), EPA, Health Canada, and consultants
- Available new data were included and evaluated on PPARa
- The overall conclusions were the same as the first workshop in 2001-2003

	Strawman1: Corton (2010)	Strawman2:	Strawman3: Klaunig (2003)
KE1	PPARa activation	PPARa activation	PPARa activation
KE2	Increases in oxidative stress	Altered expression of genes involved in cell growth	a. Expression of peroxisomal genes b. PPARa mediated expression of cell cycle, growth and apoptosis c. Non-peroxisomal lipid gene expression
KE3	NF-kB activation	Increased cell proliferation/decreased apoptosis	Increase in cell proliferation
KE4	Increased cell proliferation/decreased apoptosis	Selective clonal expansion of preneoplastic foci cells	Clonal expansion of preneoplastic foci
KE5	Increases in preneoplastic foci cells	Liver tumors	Liver tumors
KE6	Liver tumors		

- Corton, J.C., Cunningham, M.L., Hummer, B.T., Lau, C., Meek, B., Peters, J.M., Popp, J.A., Rhomberg, L., Seed, J., Klaunig, J.E., 2014. Mode of action framework analysis for receptor-mediated toxicity: the peroxisome proliferator-activated receptor alpha (PPAR α) as a case study. Crit. Rev. Toxicol. 44, 1 – 49.

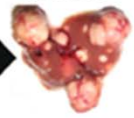
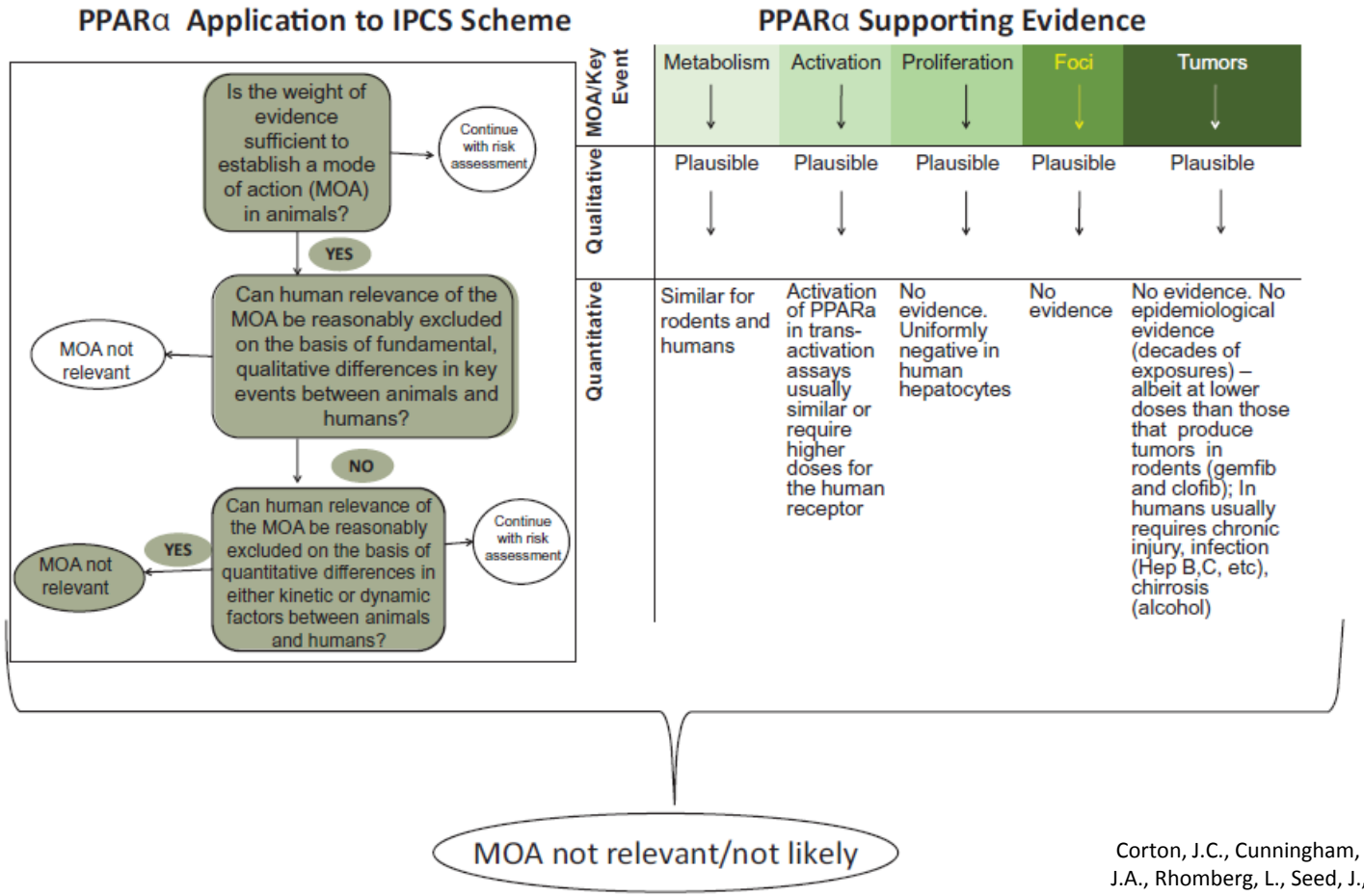
Chemical	KE1: PPARα Activation	KE2: Alteration of Cell Growth Pathways	KE3: Perturbation of Cell Growth and Survival			KE4: Clonal Expansion	Apical Endpoint: Liver Tumors
			Acute Cell Prolif.	Chronic Cell Prolif.	Apoptosis		
WY-14,643	↑[1]	↑[2,35,38,39]	↑[34]	↑[36]			↑[32]
DEHP	↑[3,5]	↑[14,40]	↑[4]	↑[37]			↑[33]
Clofibrate	↑[6]	↑[8] NC[9]	↑[7]				NC[29]
Nafenopin	↑[10]	↑[12]			NC[11]		↑[31]
Ciprofibrate	↑[13]	↑[15,41]	↑[14]				↑[28]
Methyl Clofenapate	↑[6]		↑[16]				↑[30]
Gemfibrozil (CI-718)	↑[13]					NC[17]	NC[17]
Di-n-butyl phthalate	NC[18]	↑[19,20]					
Trichloroacetate	↑[6]	↑[24,42]	↑[21]			↑[22]	↑[23]
Perfluorooctanoate	↑[25]	↑[26,39] NC[27]	↑[39]				

Table 14. Experimental evaluation of rodent liver mode of action.

Experimental approach	Measurements	Outcome for PPARα agonist MOA classification
1. Alternative mode(s) of action	DNA mutagenicity assays (e.g. short-term *in vitro* and *in vivo* screening assays)	No evidence of direct DNA damage
	Liver enzymes (e.g. ALT), pathological evaluation of H&E stained liver slices	No evidence of cytotoxicity in the liver at doses that increase liver tumors
	Gene and/or protein expression of CYP family members (CYP1A, CYP1B, CYP2B, CYP3A)	Low levels or no activation compared to induction of PPARα regulated genes; dose–response analysis indicates that genes regulated by PPARα are induced at doses below or coincident with tumor induction
2. Evidence of PPARα activation in wild-type mice or rats	Palmitoyl-CoA oxidase activity or acyl-CoA oxidase gene or protein expression	Increases
	Peroxisome proliferation or induction of a marker of peroxisome proliferation	Increases
	Hepatocyte proliferation after acute administration of compound	Increases
3. Evidence in wild-type and PPARα-null mouse comparisons	Expression of genes or proteins that are biomarkers for PPARα and other MOA	Expression changes of genes typically regulated by PPARα are abolished in the PPARα-null mouse

Application of the Human Relevance Framework to the PPARa MOA.



Corton, J.C., Cunningham, M.L., Hummer, B.T., Lau, C., Meek, B., Peters, J.M., Popp, J.A., Rhomberg, L., Seed, J., Klaunig, J.E., 2014. Mode of action framework analysis for receptor-mediated toxicity: the peroxisome proliferator-activated receptor alpha (PPAR α) as a case study. Crit. Rev. Toxicol. 44, 1 – 49.

Table 12. Comparative analysis of rodent and human data – liver tumors.

Causal key events	Plausible in humans?	Taking into account kinetic and dynamic factors, is the key event plausible in humans?	Comments
1. Activation of PPARα	Yes	Yes	PPARα is a target of human hypolipidemic drugs
2. Alteration in cell growth pathways	Yes	Unknown	Human liver has the capacity to regenerate. There is abundant evidence that a number of pathways are involved
3. Perturbation of cell growth and survival	Yes	Not likely but plausible	Not seen in independent studies of human hepatocytes *in vitro*; not measured *in vivo*; not seen in non-human primates *in vivo* or *in vitro*; not seen in guinea pigs; lack of or inconsistent effects in hamsters
4. Selective clonal expansion of preneoplastic foci	Yes	Not likely but plausible	No response in hamsters, hepatic foci are a rare finding in humans
5. Liver tumors	Yes	Not likely but plausible	Not measured in livers of humans exposed to PPARα activators; no tumors in hamsters with expression of PPARα intermediate between mice/rats and humans; no evidence of liver tumors in people exposed to hypolipidemic PPARα activators for up to 13 years

Conclusions from Workshop 2

- Hill's modified considerations

- Consistency of the MOA between chemicals
- Species concordance
- Strength, consistency, specificity of association
- Dose–response concordance
- Temporal relationship
- Biological plausibility and coherence

- The workgroup agreed that the weight of evidence for the hypothesized MOA for PPARa-mediated liver cancer in mice and rats is substantial, consistent and cohesive.

- Dose-response of Key Events

- Only robust DEHP and gemfibrozil datasets were used by the panel for dose-response modeling:

- In general the doses at which key or associative events are activated by a compound are at or below those that cause increases in liver tumors.

- The existing description of concentration/dose-response data for these key events sufficient for dose-response modeling.

- A threshold dose-response approach is supported by the data examined by the panel.

Mode Of Action Of Peroxisome Proliferators MOA In Rodent Liver Carcinogenesis: Required Data

Required Data

- Non-mutagenic
- Other MOAs excluded
- Dose response, temporal response
- Activation of PPAR alpha receptor
- Induction of hepatocyte cell growth
  - (cell proliferation/ apoptosis)
- Induction of Peroxisome proliferation
- Increase in selective preneoplastic hepatoctye cell growth
  - (cell proliferation/ apoptosis)
- Hepatic tumors

Other considerations

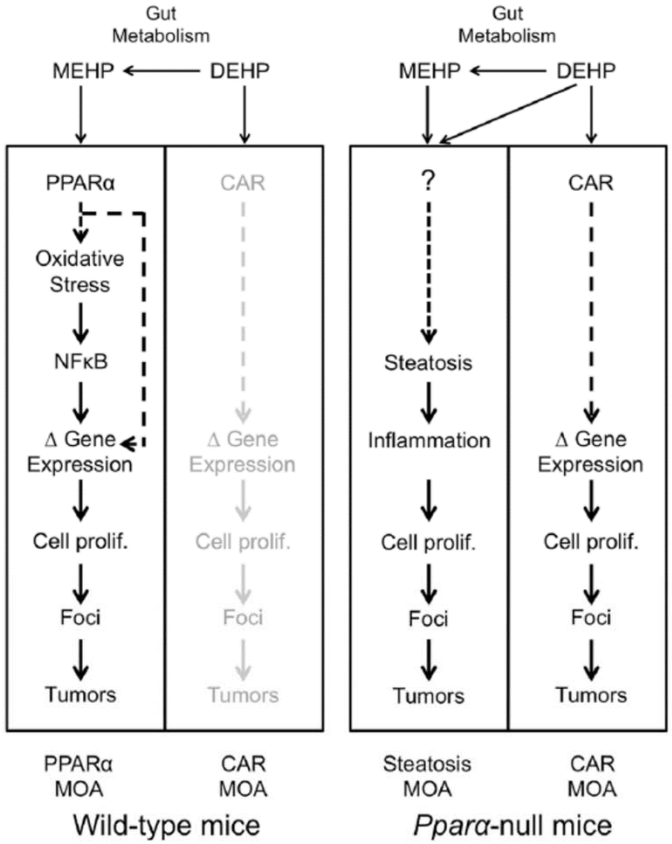
- Dual modes of action
  - dose dependent
  - Cytotoxicity vs receptor mediated
- Dose response characteristics
  - Adaptive versus adverse effects
- Associated Events and Modulating Factors
- Results from
  - genetically modified mice
  - Tissue / cell culture
- Quality of Data considered

- Thank you

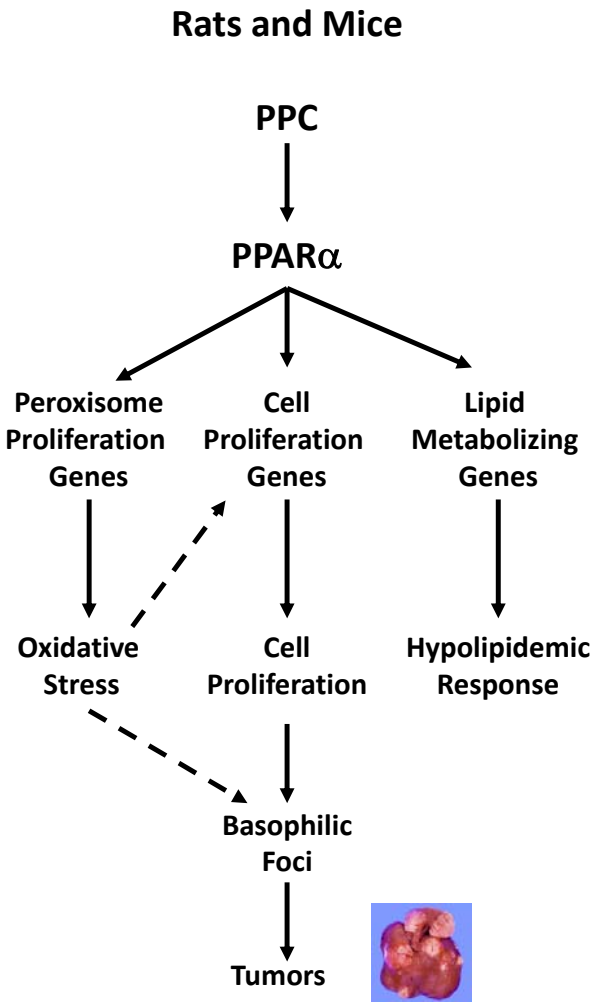
- EXTRA

Genetically modified mice

Chemical	KE1: PPARα Activation	KE2: Alteration of Cell Growth Pathways	KE3: Perturbation of Cell Growth and Survival		KE4: Clonal Expansion	Apical Endpoint: Liver Tumors
			Acute Cell Prolif.	Chronic Cell Prolif.		
LAP-VP16PPARα	↑[1]	NM	↑[1]	↑[1]	NC[1]	NC[1]
Humanized PPARα (TRE-hPPARα)	↑[2]	NC[6,8]		NC[5,6]	NC[3]	NC[3]
Humanized PPARα (TRE-hPPARα^{PAC})	↑[4]	NC[4]	↑[7]			

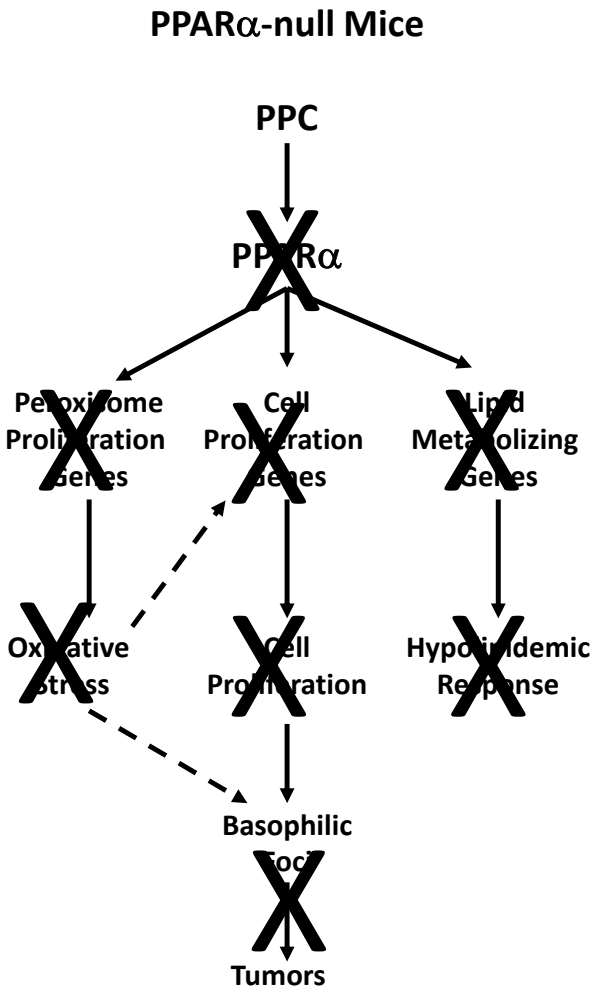


PEROXISOME PROLIFERATOR MODE OF ACTION



- Based on consensus of risk assessment panels
  - Klaunig et al., 2003
  - Corton et al 2014
  - Felter et al 2018
- PPARα activation is a key event
- PPARα regulates genes involved in
  - Peroxisome proliferation
  - Cell proliferation
  - Lipid metabolism
- Chronic exposure leads to promotion of preneoplastic foci
  - Foci from spontaneous damage or indirectly initiated through increases in oxidative stress?

PEROXISOME PROLIFERATOR CHEMICAL MODE OF ACTION



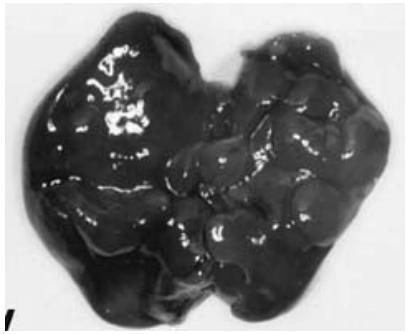
- Large number of studies showed that PPARα is required for short-term effects of peroxisome proliferator chemicals (summarized in Corton, 2009)

- Abolishment of
  - Most gene expression changes
  - Increases in liver to body weights
  - Increases in cell proliferation
  - Hypolipidemic response
  - Oxidative stress

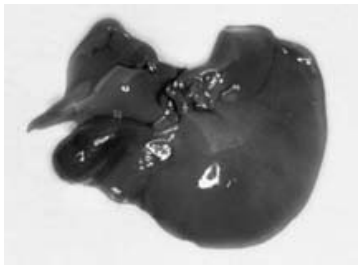
- Degree to which gene expression is abolished is chemical-dependent

PPARα IS REQUIRED FOR PPARα AGONIST-INDUCED HEPATOCARCINOGENESIS

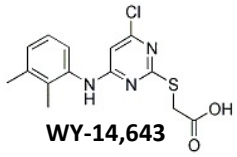
Wild-type exposed to WY



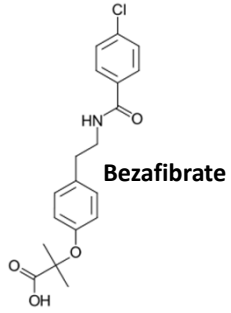
PPARα-null exposed to WY



- **Tumor studies**
  - **0.1% WY (Peters et al., 1997)**
  - **0.5% Bezafibrate (Hays et al., 2005)**
- **No evidence of tumors in PPARα-null mice**



WY-14,643



Bezafibrate

Adapted from Peters et al., 1997

Potential Questions for SESSION 3:

- What are the advantages and disadvantages of the frameworks presented in terms of supporting systematic reviews of mechanistic data?
- What elements of systematic review might have to be adapted for mechanistic data? What degree of structure is required?
- How have results from systematic reviews of mechanistic data been integrated with animal and human evidence? What was the rationale for integrating the evidence in this manner? Would the approach have to be modified for different circumstances (e.g., positive vs. negative evidence of a mechanism that is consistent with developing an outcome; inconclusive evidence)?
- How can systematic review of in vitro concentrations associated with mechanistic data be related to evidence on internal and external doses associated with health effects in vivo in animals and humans?
- How can the challenges of relating the evidence from short-term mechanistic studies to outcomes after longer term exposure in animals and humans be overcome?