

Developments & Good Practice in Mode of Action/Human Relevance Analysis

SOT RASS Teleseminar June 30th/2010

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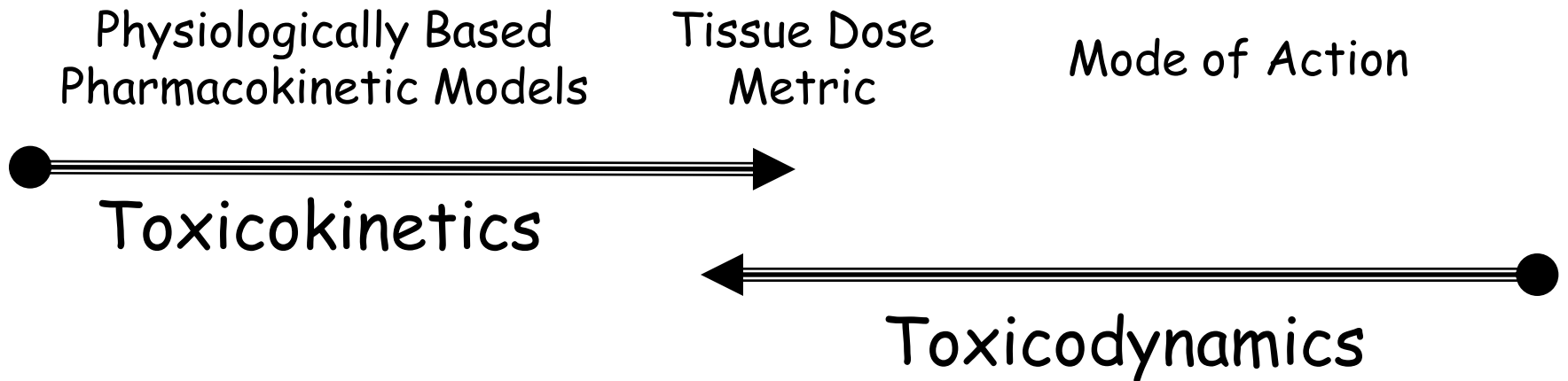
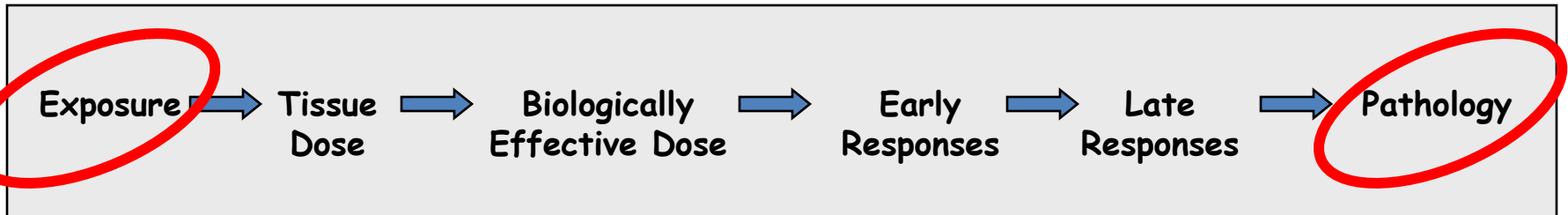


Outline

- Evolution of Mode of Action/Human Relevance (MOA/HR) Analysis
 - How it contributes/What we've learned
- Misconceptions in MOA/HR Terminology & Analysis
- Good MOA/HR Assessment Practice
 - Including observations of NAS
- Implications for PPAR α MOA

Exposure-Response Continuum (Source to Outcome Pathway)

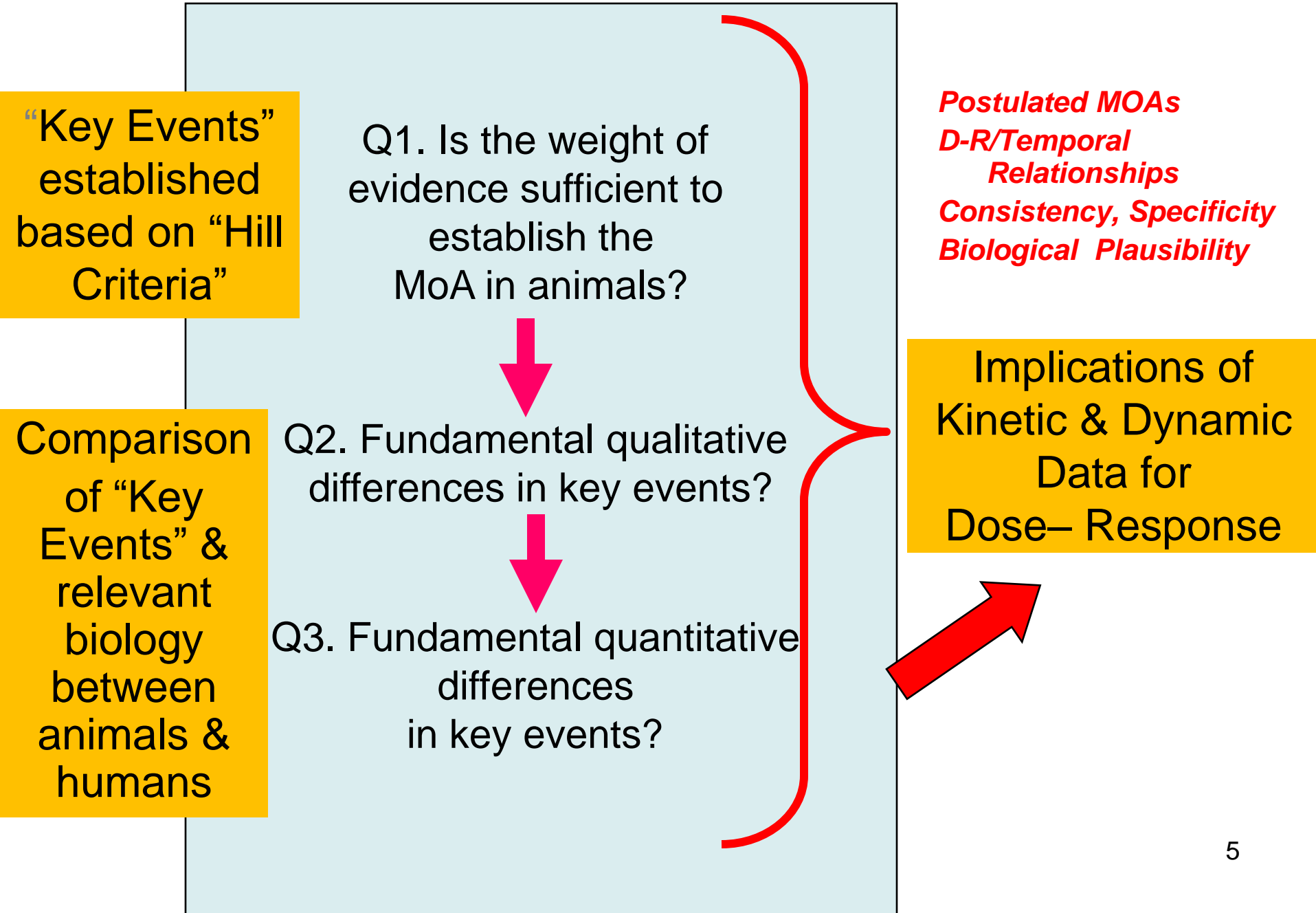
Mode of Action involves identification of several **key events** between exposure and effect



Key Event

- An empirically **observable**, precursor step that is a **necessary** element of the mode of action, or is a **marker** for such an element
 - Key events are **necessary** but not always **sufficient**
- Early key events often chemical-related; later ones MOA-related (“tripped”)
- Examples
 - Specific metabolic transformation
 - Chemically induced direct and indirect reaction with genetic material (DNA)
 - Cytotoxicity, regenerative cell proliferation
 - Hormonal perturbations
 - Increased cell growth and organ weight

IPCS/ILSI MOA/HR (WOE) Framework

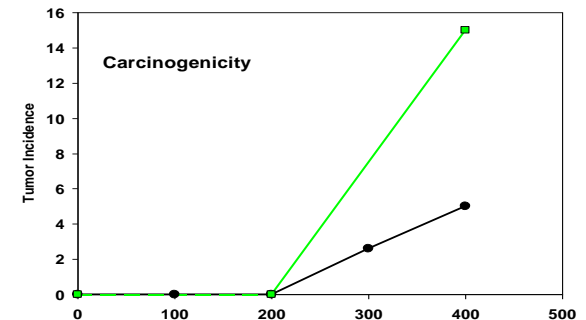
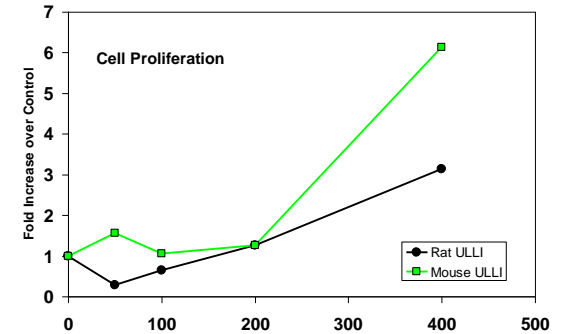
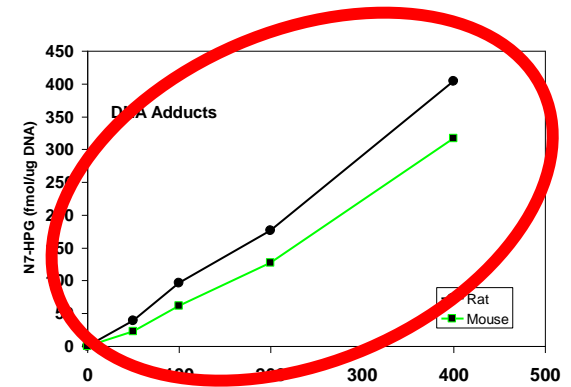
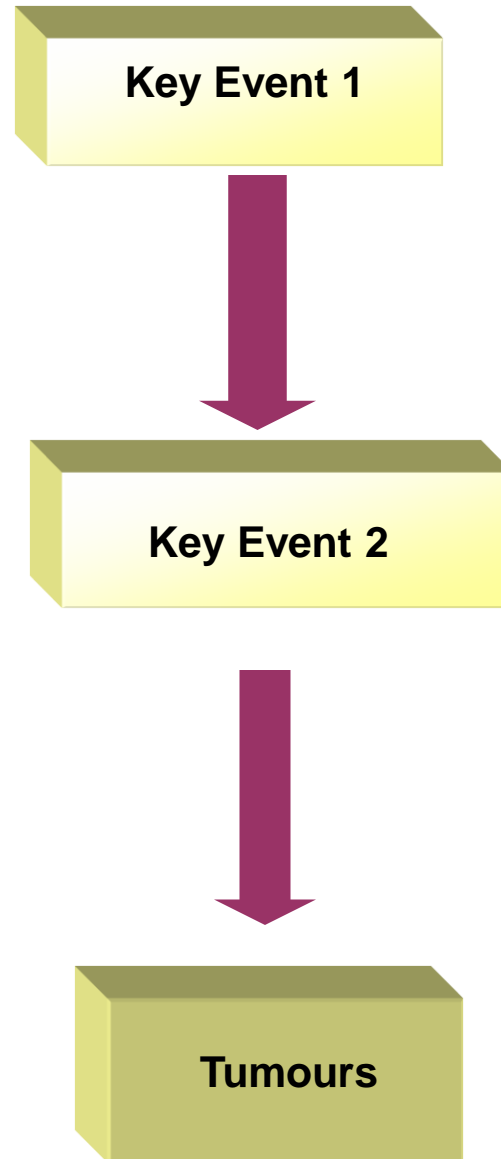


2. & 3. The Concordance Analysis

Key Event	Qualitative Concordance			Quantitative Concordance
	Animals	Humans	Strength	Humans
Metabolism by CYP2E1	Correlation with binding of metabolites	Relevant enzyme in kidney and liver	Considerable In animals; limited but relevant to humans	PBPK model incorporating metabolic rates, enzyme affinities and distribution based on <i>in vitro</i> human data supported by <i>in vivo</i> data
Sustained cell damage and repair (cytotoxicity; proliferation)	In all cases at doses that induce tumours	Liver and kidney target organs in humans	Considerable in animals, possible in humans but limited data	No data
Liver & kidney tumours	Mice & rats	Possible	Considerable in animals,; highly plausible in humans	No data

Implications for Dose-Response Analysis

What is the shape of the dose-response curve in the range of both observation and inference for the rate limiting **key events**, based on an understanding of MOA?



Key Event

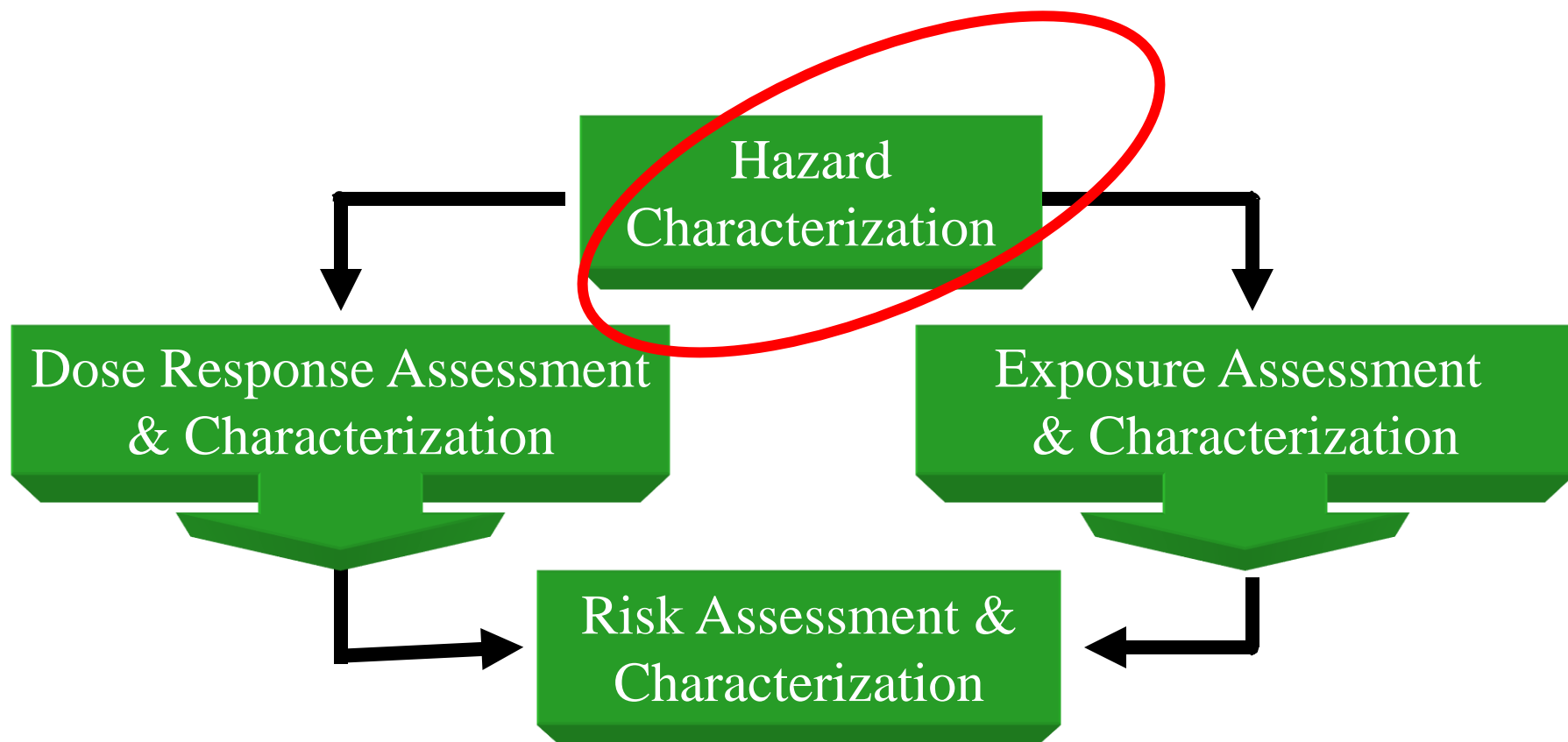
Focus on MOA in Framework Analysis

Increasing predictive capacity and utility of risk assessment

- Drawing maximally and early on the most relevant information
 - data on kinetics/dynamics and the broader biology base
- Transparency
 - Rigor & consistency of documentation
 - Explicit separation of science judgment on weight of evidence from science (public) policy considerations
- Doing the right research/testing
 - Chemical Specific: Iterative dialogue between risk assessors/researchers
 - Developing more progressive testing strategies

The NAS 4-Step Paradigm

The Need to Move On



Hazard Characterization (early focus not only on effect but how the effect is induced - mode of action)

Default

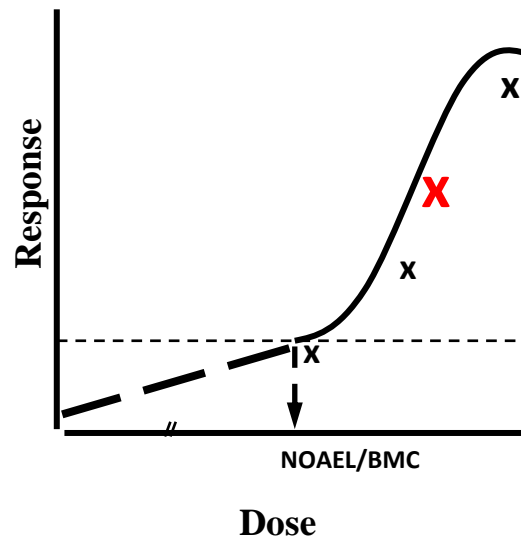
Biologically (Mode of Action) Based

- Curve fitting at high dose for point of departure for late (apical) endpoints
- Linear extrapolation or N/LO(A)EL or BMC/D
- Interspecies differences/human variability (x10)

UF



- Earlier endpoints in the most relevant species, considering early on, kinetic and dynamic data, to address extrapolations

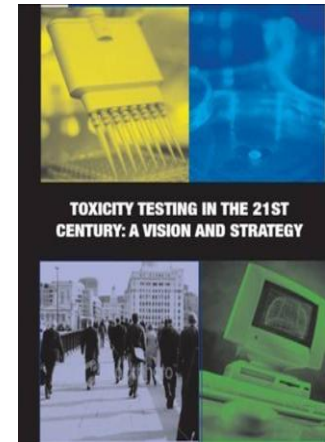


July 2007

Toxicity Testing in the 21st Century: A Vision and a Strategy

REPORT
IN BRIEF

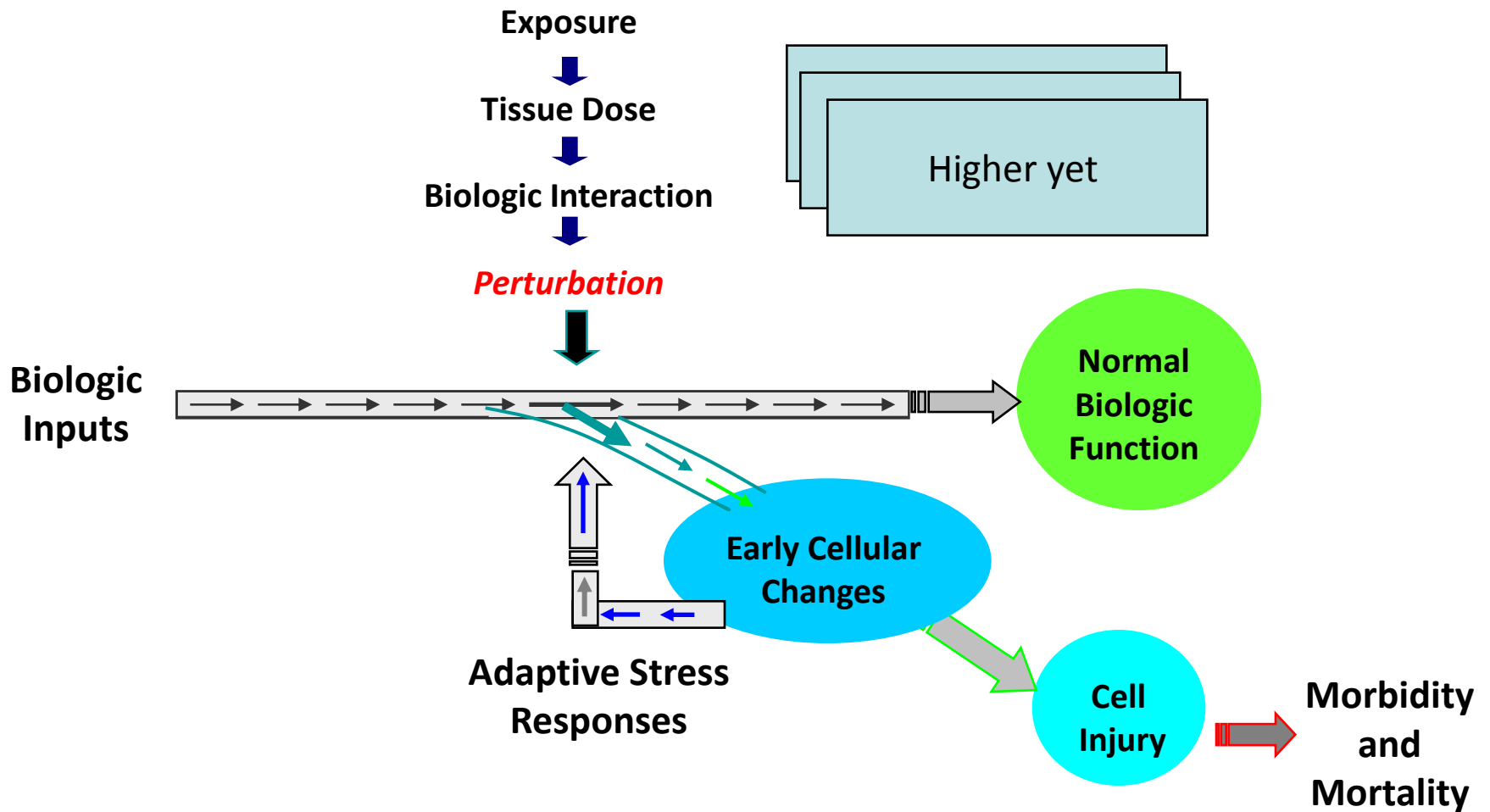
Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment. These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures. They could also reduce the need for animal testing by substituting more laboratory tests based on human cells. This National Research Council report creates a far-reaching vision for the future of toxicity testing.



Toxicity Testing in the 21st Century: A Vision and A Strategy

NAS Final Report Released June 12, 2007

Toxicity Pathway: A cellular response pathway that, when sufficiently affected (perturbed), is expected to result in an adverse health effect.



Integrating Information from Evolving Technologies

Proposed Key Events

- Nuclear receptor activation (***transcriptional profile***)
- Induction of P450 enzymes (***transcriptional profile*** confirmed by ***biochemistry***)
- Inhibition of Cyp 51 (site of action of fungicide)
- Decreased cholesterol synthesis (***transcriptional profile*** confirmed by ***clinical chemistry***)
- Mitogenesis (***histology***)
- Altered mitosis (suggested by ***inhibition of cholesterol synthesis***)
- Oxidative stress (***transcriptional profile***)

How does the MOA/HR Framework Help in Transitioning the RA Community?

The ``Joiner``?

- Enables us to relate testing results from high throughput technologies to traditional endpoints in a mode of action context
- Permits us to move away in informed fashion from hazard to more mode of action based predictive testing
- Effectively communicates this transition

Continuing Improvement of MOA/HR Analysis

- Better characterization of uncertainty vs. yes/no decisions
- Earlier/more fulsome options analysis for potential MOA; template introduced
- Templates for dose-response/temporal concordance of key events introduced
- Better integration of D-R/temporal concordance for key events with subsequent D-R analysis for risk characterization

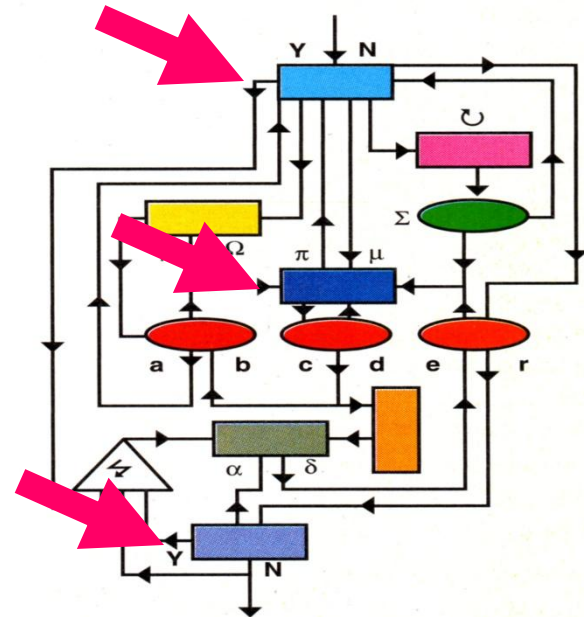
Mode vs. Mechanism

Plausible Hypothesis

Key event (e.g. biochem; histopath):

- Critical
- Can measure
- Repeatable

Detailed Molecular Description



Perturbations in toxicity “pathways” contribute to key events.

Popular Misconceptions – MOA/HR Analysis

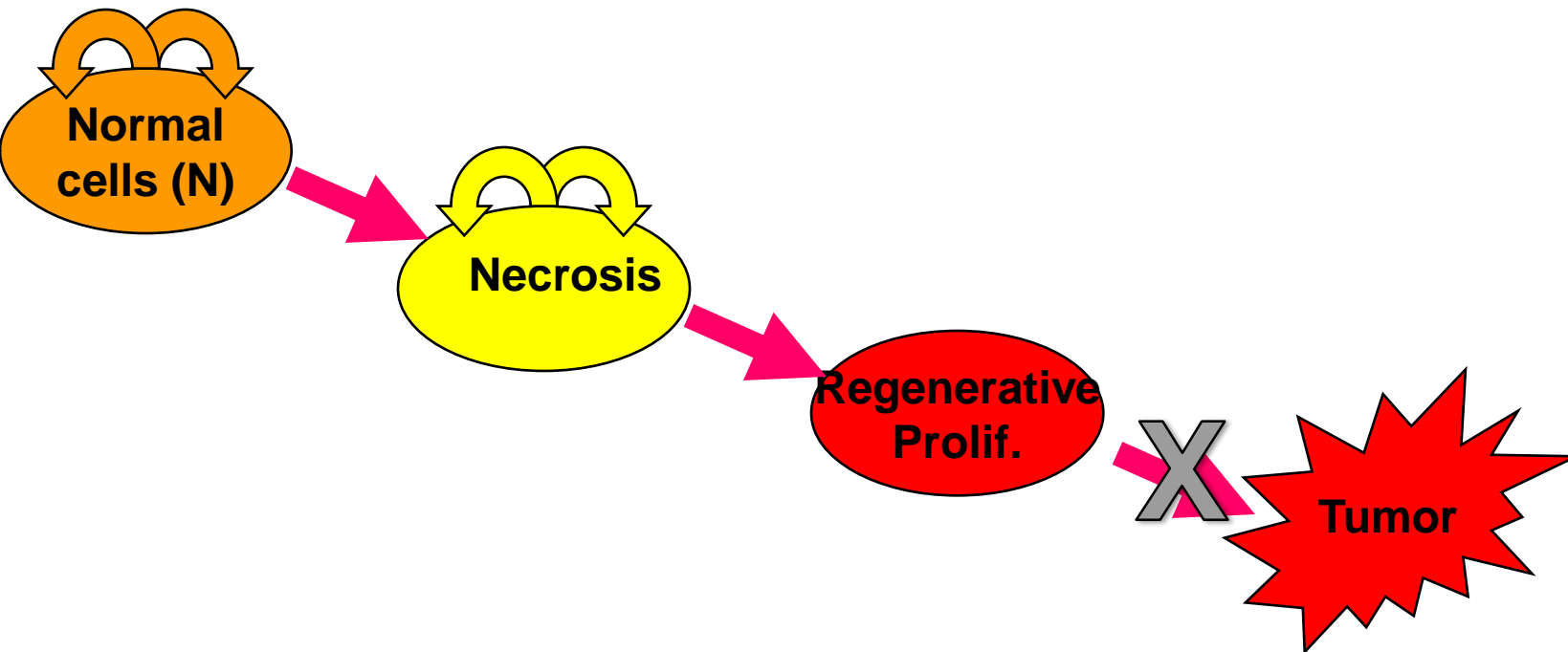
- That “multiple modes of action” contribute to the same effect
 - the “hiding in the bushes” theory (raising doubt), but without development



- Rather, “mode of action” is the most likely set of key events contributing to an adverse effect
 - based on hypothesis generation which necessarily requires multidisciplinary input
 - risk assessment/research

Popular Misconceptions – MOA/HR Analysis

- Dose response **concordance** between key/end events
- That observation of early but not late key events in some of the experimental studies detracts from the weight of evidence for consistency
 - Lack of understanding that early key events are **essential** but not necessarily **sufficient**

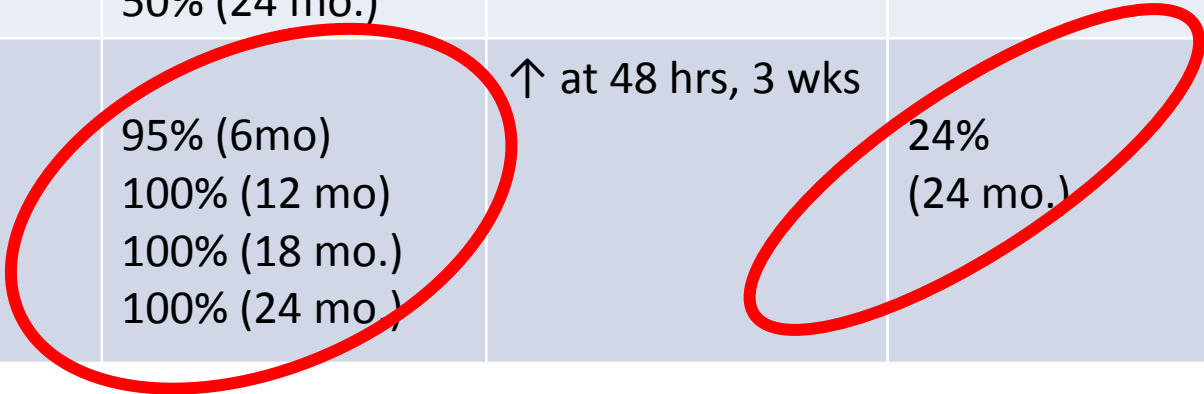
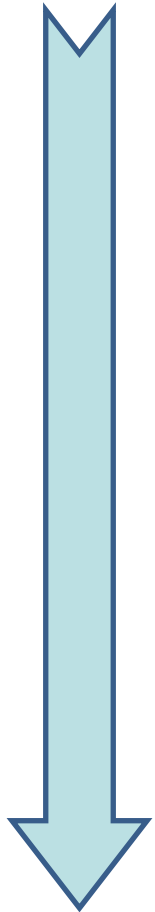


Dose – Response and Temporality

Temporal



Dose (mg/kg bw/day)	Sustained Cytotoxicity Key event 1	Regenerative Proliferation Key event 2	Renal Tumours in Rats Key event 3
38	0% (6mo) 5% (12 mo) 15% (18 mo.) 15% (24 mo.)	↑ at 48 hrs	3% (24 mo.)
81	25% (6mo) 33% (12 mo) 58% (18 mo.) 50% (24 mo.)	↑ at 48 hrs	6% (24 mo.)
160	95% (6mo) 100% (12 mo) 100% (18 mo.) 100% (24 mo.)	↑ at 48 hrs, 3 wks	24% (24 mo.)



Dose-Response

Common Barriers to the Use of Mode of Action in Risk Assessment

- Limited Early Interdisciplinary Communication
 - risk assessors/research community
- Limited Expertise/Understanding
 - accessing adequate expertise and training
- Limited Transparency
 - Lack of discipline in supporting (i.e., research) /documenting case
- That we shouldn't incorporate partial mode of action data, now
 - Awaiting the “holy grail” of future testing strategies
- Why fix what isn't broken (***“institutional inertia”***)?
 - Traditional “default” approach is:
 - Easy (requires less justification)
 - simple to explain
 - “believed” to be protective



NAS Advice to EPA re PPAR α

MOA (May 12th RASS)

- a viable possibility [*Trichloroethylene, 2006*]
- phthalate-associated cancers mediated by mechanisms independent of PPAR α ?
[*Phthalates and Cumulative Risk Assessment, 2008*]
- Ito et al. (2007) calls into question conclusions regarding DEHP's carcinogenicity to humans
[*Science and Decisions, 2009*]
- the committee is not yet convinced of the proof of the hypothesis that the PPAR α MOA is the sole MOA [*Tetrachloroethylene, 2010*]

About Advisory Groups

- They bring extraordinary amounts of multidisciplinary expertise to the table
- They work extraordinarily hard, and
- They offer insights that might not have otherwise been realized

However: They don't really conduct mode of action or risk assessments

About Advisory Groups (Cont'd)

- ***For risk assessment:***
 - Much expertise resides in Governments who commission the input
 - They (& others) cannot contribute directly
 - Time for delivery of products is limited
 - Sometimes leading to partial analysis, contradictions
 - It's often easier to make recommendations than to implement them
 - though sometimes visionary, don't offer pragmatic operational plan; constraints may not be well recognized
 - There isn't continuity
 - Who explains and develops?

The committee is not yet convinced of the proof of the hypothesis that the PPAR α MOA is the sole MOA [Tetrachloroethylene, 2010]

- The committee didn't conduct an MOA analysis for perc
- ***Rather***, they made recommendations to EPA to conduct a more thorough and systematic analysis of MOA ***for liver tumors, particularly***
 - EPA analysis for the liver considered inadequate, requiring revision for focus and integrated analysis
- The committee “generally supported” the comprehensive analysis of a dissenting member (PPAR α mediated liver tumors)
 - Felt that this provided an example to EPA of how to perform

Observations of the NAS Perc Committee on Assessment Practice

- Recognition of the importance of both content and process for robust assessment
- Identified need for preassessment problem formulation/issue identification, to (among other things):
 - Solicit multidisciplinary input at an early stage in such critical matters as mode of action.....
- This early phase needs also address:
 - a priori delineation and weighting of criteria for evidence of hazard, &
 - options analysis for dose-response assessment/associated uncertainties

“Expected to contribute considerably to transparency in the separation of science judgment from science-policy choices”

Ito et al. (2007) calls into question conclusions regarding DEHP's carcinogenicity to humans [Science and Decisions, 2009]



Science and Decisions: Advancing Risk Assessment

NAS Final Report Released, 2008

NAS Committee: Advancing Risk Assessment

Focus

- Improving the ***utility*** & ***technical analysis*** that supports risk assessment

Outcome

- A number of controversial recommendations which have been helpful to stimulate discussion
 - Problem formulation
 - Mode of action (though seemingly not well developed)/harmonized dose-response methodology

Given the extent of their charge, how much effort was dedicated to a mode of action analysis on PPAR α ?

Principles of Good Assessment Practice

- Early Issue Identification
- Inclusiveness
- Sound Science and Science Advice
- Uncertainty and Risk
- Transparency and Openness
- Review

The Role of Formal Issue Identification More than a Statement of the Issue; A Process

- ***Early Consideration*** of All Relevant (assimilated) information/expertise
 - Relying as much as possible on existing assessments, “peers” (e.g., research community)
- Determining ***need for risk assessment*** based on consideration of factors such as nature and feasibility of risk management
- Determining ***focus and scope*** of risk assessment, based on potential options for management
- Ensuring that any assessment ***meets the considered need***
- ***Communication*** and formal engagement
 - Stakeholders/risk managers/public

Principles of Good (MOA) Assessment Practice

- Early Issue Identification
- Inclusiveness
- Sound Science and Science Advice
- Uncertainty and Risk
- **Transparency and Openness**
- Review

Checklist/ Questions for Transparency in Assessments/MOA Analysis

- What was the timeframe for and extent of consideration of relevant data?
 - E.g., Selected without bias from the totality of the data available
- What was the objective/focus? (i.e., where were efforts focused and why?)
 - E.g., Extent of reliance on previous assessments
- What was the specific nature of preparation and review?
 - E.g., ***Peer input***, Peer consultation, Peer review
- Approach to Assessment
 - E.g., What were the criteria for consideration of the weight of evidence
 - Specific interpretation and weighting
 - E.g., Presentation of several options for exposure estimation and dose-response analysis along with their attendant uncertainty
 - Specific interpretation and weighting?

More Information?

Evolution of the ILSI/IPCS Frameworks – Mode of Action

- Meek & Klaunig (2010) *Chemico-Biological Interactions* 184:279–285

Links for relevant information from the NAS report on Perc:

- http://books.nap.edu/openbook.php?record_id=12863&page=115
- http://books.nap.edu/openbook.php?record_id=12863&page=162

Good Assessment Practice/Peer Engagement

- Meek, M.E., Patterson, J. et al. *Risk Analysis* 28(1):1609-1621 (2007)
- Meek, M.E. *Reg. Tox. Pharm.* 53: 156–157 (2009)

MODE OF ACTION ANALYSIS AND HUMAN RELEVANCE OF LIVER TUMORS INDUCED BY PPAR α ACTIVATION

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DISCLAIMER

The views expressed in this presentation are those of Dr. Chris Corton and do not reflect policy or endorsement by the US EPA.

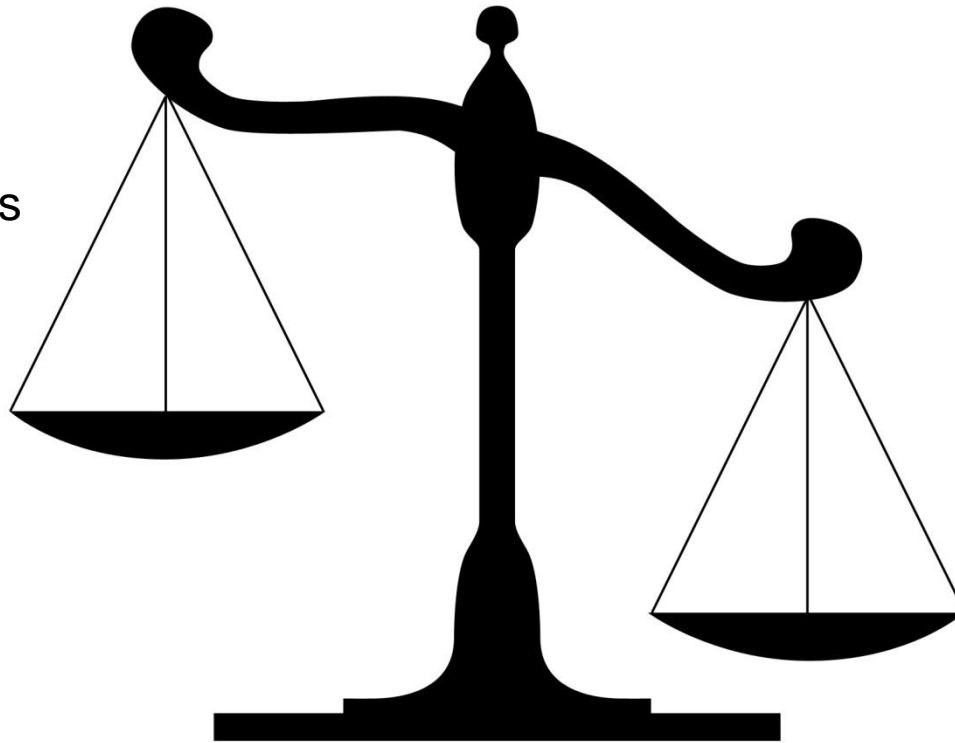
TOPICS

- A brief review of data relevant to the PPAR α MOA and human relevance
- “Tests” of the MOA by Guyton
 - PPAR α -independent tumors by DEHP (Ito et al., 2007)
 - Hepatocyte proliferation in the absence of tumor induction (Yang et al., 2007)

WEIGHT OF EVIDENCE: HOW DO WE DETERMINE THE IMPORTANCE OF RELEVANT STUDIES?

PPAR α MOA

- 40+ years of research
- Consistency across
 - Studies
 - Chemicals
 - Labs
- Established MOA accepted by all stakeholder groups



- A handful of studies that are seemingly inconsistent with the MOA
- Examples
 - Ito et al., 2007
 - Yang et al., 2007

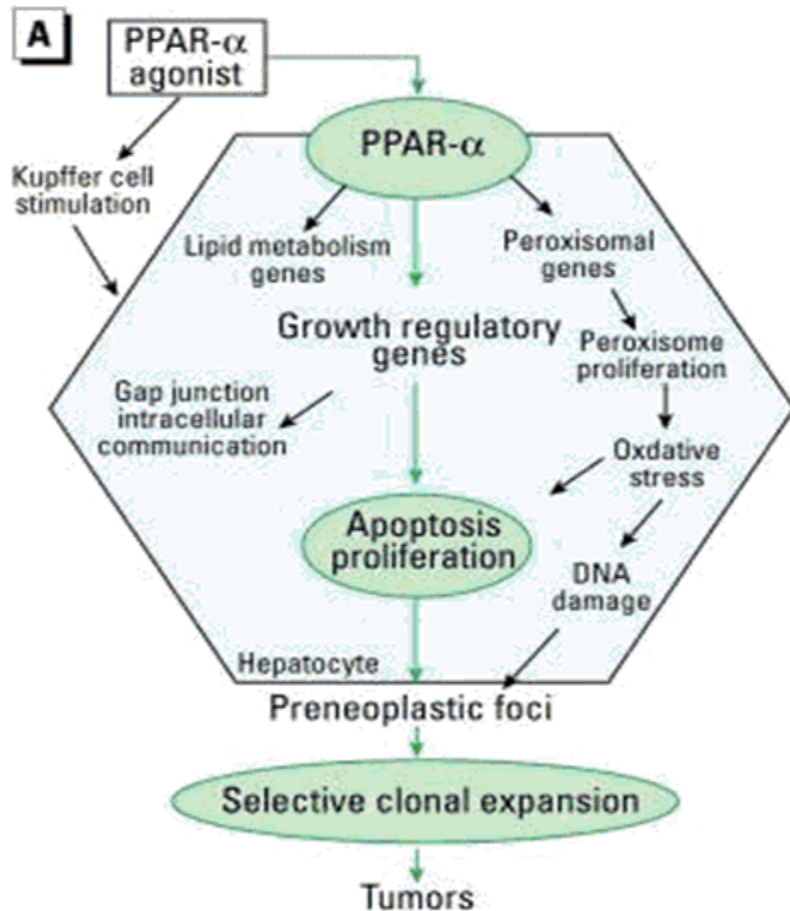
What is important?

WEIGHT OF EVIDENCE (WOE) VS. “SELECTIVE” WEIGHTING OF EVIDENCE

- WOE approach
 - Consider all data
 - Systematic
 - Best way to identify key events, MOA and areas of uncertainty
- “Selective” weighting of evidence
 - Uses chemical-specific data
 - Weighting more heavily data that creates a “feeling” of doubt
 - Ignores body of evidence including
 - Multiple chemicals
 - Multiple endpoints
 - Does not consider logical explanations for inconsistencies with MOA
- Examples of selective weighting of evidence approach in literature for PPAR α MOA
 - Environ Health Perspect. (2009). 117(11):1664-72.
 - Environ Health Perspect. (2007). 114(9):1464-70.

PPAR α MODE OF ACTION

Wild-type mice



- ILSI sponsored project
- MOA panel included representatives from industry, academia and government (FDA, EPA)
- 2 year deliberation process
- 2 peer reviews
- Publication: Klaunig et al., 2003

MODE OF ACTION - EXPOSURE TO PPAR α ACTIVATORS IN RODENTS

Chemical	PPAR α activation	Oxidative stress	Increases in transient acute cell proliferation	Decreases in acute apoptosis	Increases in chronic cell proliferation	Increases in cell proliferation in preneoplastic foci	Liver tumors
WY-14,643	+26,27,29	+2,6,7,8,9,16,18, 72,75 _71,72	+6,7,31,35	+55	+6,7,35	+73,74	+35
DEHP	+26,27,28	+8,10,14,15,20,40,41,59 _8,23,40	+31,32,42-45	+43	_10		+10
Clofibrate	+28,29	+9,15,21,24,51,76, 84 _7,23,66	+7,33,39,45		+7		+47,48
Nafenopin	+28,30	+9,22-24 _25,65	+35	+54,83	+80 +/_35	+36	+35,49,62
Ciprofibrate	+30	+17,18	+34,37,38		+34	+37	+50
Methyl clofenapate	+28	+9 _51	+39,70	+53	+39		+9
Gemfibrozil (CI-719)	+30	+75				_52	+/_52
Di-n-butyl phthalate	_26,27	+19,40,75					
Trichloroacetate	+28	+56,67	+57,58			+57	+81,82 _85,86
Perfluoro-octanoate	+64, 78	+24,68 _60,61,68	+59,77				+62,63,69

•Consistency across many chemicals

SPECIES DIFFERENCES IN RESPONSES TO PPAR α ACTIVATORS

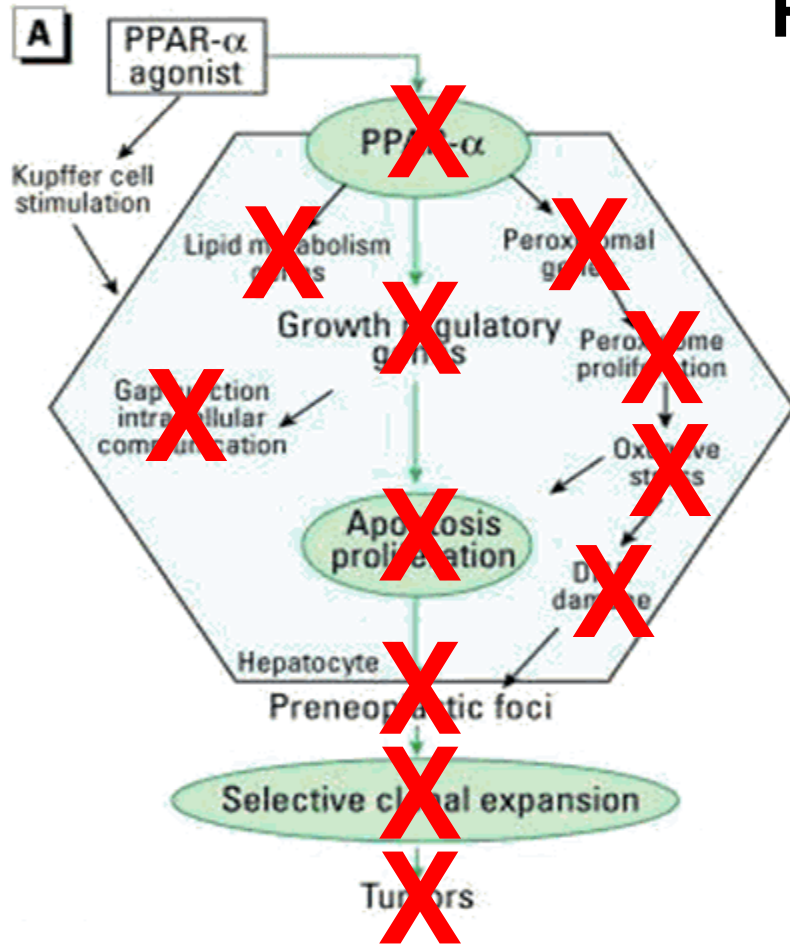
Species	Relative PPAR α expression	Chemical	Response								
			PPAR α activation	Hypolipidemic effect (decreases in triglycerides or VLDL-triglycerides)	Increases in liver weight	Oxidative stress	NF-kB activation	Increases in acute cell proliferation	Decreases in apoptosis	Liver tumors	
Rats	Likely similar to mice	See table 1 for chemical and reference	+	+	+	+	+	+	+	+	
Mice	10	See table 1 for chemical and reference	+	+	+	+	+	+	+	+	
Syrian hamster	3	Nafenopin	+1,2,23		+1,2,23				-1,2,17	+17	-1
		WY-14,643	+1,7,8	+7,9	+1,7,8,9		-5	-1,8			-1
		DEHP	(+) ^{4,27}		+4,27			(+) ⁴			-46
		Methyl clofenapate	+8	+7,9	+7,8,9			(+) ²⁸			
		Ciprofibrate	+8,22		+8,22			+25,8			
Guinea pig	1	Bezafibrate	+24		-24						
Methylclofenapate		-8,21	+9,11	-8			-8,25		-13		
Ciprofibrate		+8,18,15,22		-8,22			-8				
WY-14,643		+9,11,16,8	+9,11	-8			-8				
Nafenopin		+16,23,10,12		-12,23			-12,14,17		+32		
Fenofibrate		-19									
Perfluorodecanoic acid		-20,26		+26,20							
Bezafibrate	-24		-24								
Cynomolgus monkey	?	DEHP	-3		-3			-3			
		DINP			-3			-3			
		Clofibrate	-3		-3			-3			
		Fenofibrate	+6	-6	-6	-6		-6			
		Ciprofibrate	+6 (+) ³³		+6	-6,33		-6			
Humans ³⁰	≤ 1	See footnotes for compound used	+34 -35-39,45	+40	-31			-41-44, 45		-12,42,43,45	

PERSPECTIVE OF THE PHARMACEUTICAL INDUSTRY ON PPAR α AGONISTS

- PPAR α is a therapeutic target of hypolipidemic agents that lower blood triglycerides
 - Gemfibrozil (Lopid) (147 mg/kg/day)
 - Bezafibrate (Befizal, Bezalip, Bezatol) (9 mg/kg/day)
 - Clofibrate (Atromid) (29 mg/kg/day)
 - Fenofibrate (Antara, Fenoglide, Lipofen, Trilcor) (1.7 mg/kg/day)
 - All cause increases in rodent liver tumors
- Drugs on the market for over 40 years
- No consistent adverse effects
 - Myopathy
 - Increased serum liver enzymes
 - Cholelithiasis
 - Rare - rhabdomyolysis (severe breakdown of muscles)
- Epidemiology studies (up to 13 years of exposure to gemfibrozil or clofibrate) and a follow-up meta-analysis did not find any evidence of increases in liver disease including cancer (Community of Principal Investigators, 1980; Frick et al., 1987; Huttunen et al., 1994; Law et al., 1994)
- Monkey and guinea pig studies did not find any evidence of effects related to liver cancer (summarized in Klaunig et al., 2003)
- **Conclusion: rodent liver tumor MOA not relevant for humans**

PPAR α MODE OF ACTION

PPAR α -null mice



PPAR α -null mouse studies

•Short-term studies

- WY: Anderson et al. (2004a,b)
- DEHP, DBP: Lapinskas et al. (2005)
- DINP: Valles et al. (2003)
- TCE: Laughter et al. (2004)

•Tumor studies (PPAR α -dependent)

- 0.1% WY: Peters et al. (1997, 1998)
- 0.5% Bezafibrate: Hays et al. (2005)

PPAR α -INDEPENDENT LIVER TUMORS?

- Ito et al. (2007). Di(2-ethylhexyl)phthalate induces hepatic tumorigenesis through a peroxisome proliferator-activated receptor alpha-independent pathway. *J Occup Health.* 49(3):172-82.
- Exposed wild-type and PPAR α -null mice to 0.01% or 0.05% DEHP in diet for ~21 months
- Increases in liver tumors in PPAR α -null mice but not wild-type mice

Table 2. Body and liver weights, and neoplastic changes in livers after DEHP treatment

Genotype	Wild-type			<i>Ppar</i> α -null-type			
	Dose	0%	0.01%	0.05%	0%	0.01%	0.05%
	DEHP	DEHP	DEHP	DEHP	DEHP	DEHP	DEHP
No. necropsied ^a	24 (1)	23 (2)	20 (1)	25 (1)	25 (3)	31 (3)	
B.W. (g)	33.3 \pm 4.20	32.6 \pm 3.76	31.9 \pm 4.49	32.3 \pm 3.75	32.7 \pm 3.77	33.3 \pm 4.74	
Liver (g)	1.34 \pm 0.20	1.30 \pm 0.24	1.27 \pm 0.18	1.51 \pm 0.34	1.55 \pm 0.33	1.78 \pm 0.84	
Hepatocellular adenoma	0	2	2	0	1	6	
Hepatocellular carcinoma	0	0	0	1	0	1	
Cholangiocellular carcinoma	0	0	0	0	0	1	
Liver tumors total	0 (0%)	2 (8.7%)	2 (10.0%)	1 (4.0%)	1 (4.0%)	8 ^b (25.8%)	

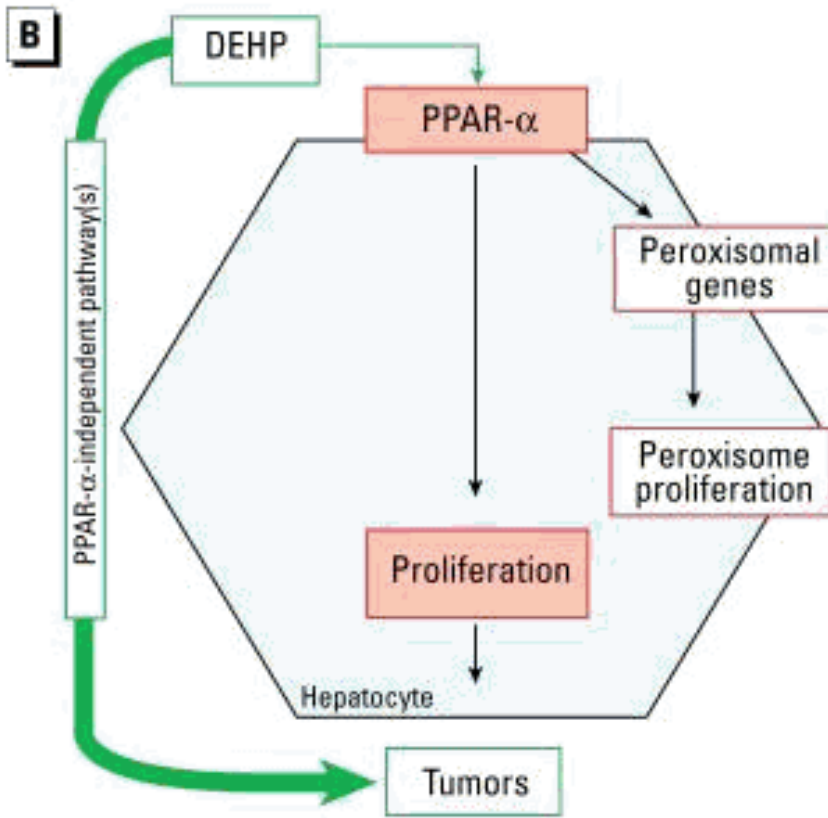
^a: Numbers of deaths before end of experiment shown in parentheses.

^b: Significant trend between control and 0.05% DEHP-treated group in *Ppar* α -null mice ($p < 0.05$).



DEHP AND PPAR α

Wild-type mice



- Guyton et al. model: DEHP causes liver tumors through a PPAR α -independent pathway in wild-type mice

- Chemical-specific response that contributes to liver tumor induction in PPAR-null mice

IS THIS LOGICAL?

DEHP may cause liver tumors in PPAR α -null mice.

Therefore,

The liver tumors in wild-type mice treated with DEHP are PPAR α -independent and the PPAR MOA cannot be valid.

PPAR α -INDEPENDENT LIVER TUMORS

- Burden of proof needed to conclude that tumors in wild-type mice are PPAR α -independent
- Need to consider all data including differences in responses to DEHP between wild-type and PPAR α -null mice
- There are data indicating that the tumors in the two strains arise by different MOA

ARE THE INCREASES IN THE DEHP-INDUCED LIVER TUMORS STATISTICALLY AND BIOLOGICALLY SIGNIFICANT?

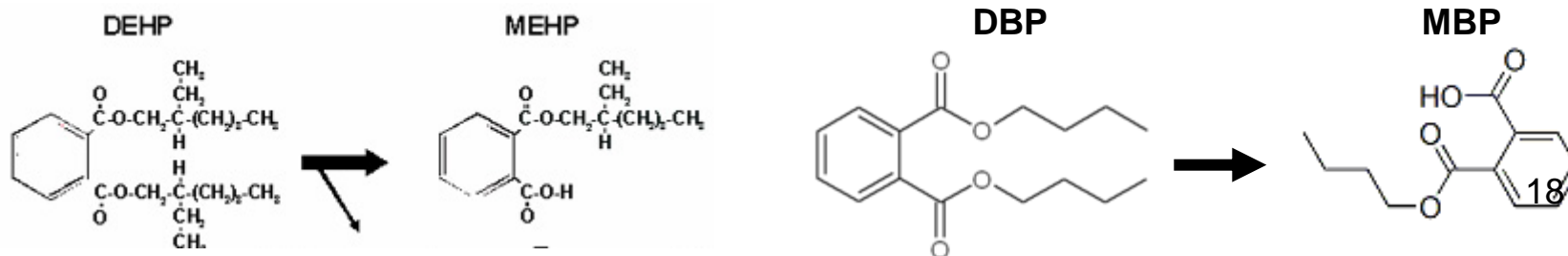
- Controversy about whether the increases are statistically significant
 - Low number of tumors
 - Ito et al. combined different types of liver tumors
- Increases in spontaneous liver tumors in PPAR α -null mice in an aging study
 - PPAR α *protects* against spontaneous liver tumor induction (Howroyd et al. (2004) Decreased longevity and enhancement of age-dependent lesions in mice lacking the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARalpha). Toxicol Pathol. 32(5):591-9.)

DEHP-INDUCED LIVER TUMORS: EVIDENCE FOR DIFFERENT MOA IN WILD-TYPE AND PPAR α -NULL MICE

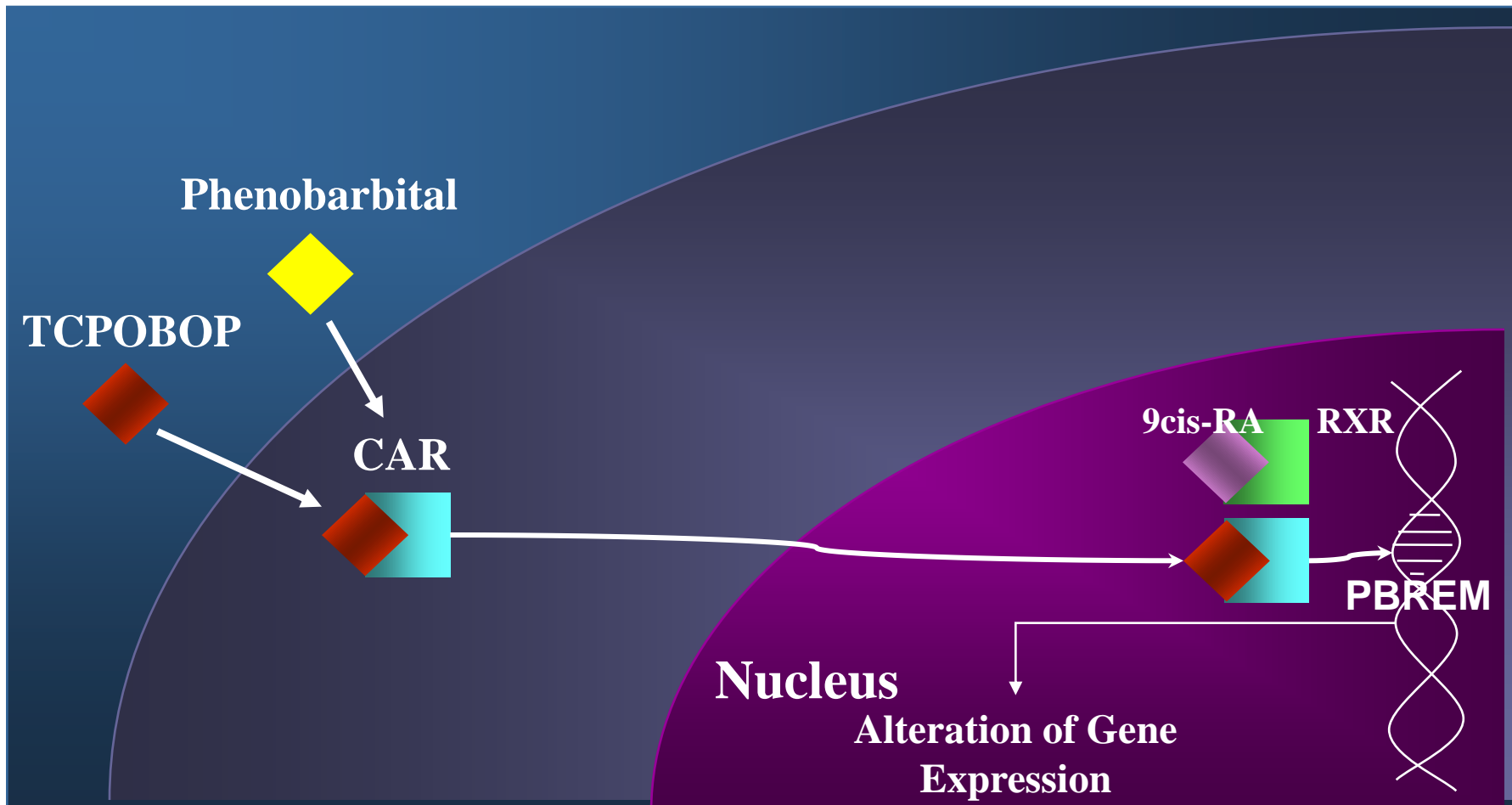
- Differences in tumor incidences in wild-type and PPAR α -null mice (Ito et al., 2007)
 - Increases in PPAR α -nulls (?) but not wild-type mice
 - PPAR α protective (?)
- Molecular differences in DEHP response in nontumor liver (Ito et al. 2007)
 - Increases in 8-OHdG, NF-kB and c-Jun in PPAR α -nulls but not wild-type mice
- Molecular characteristics of liver tumors from the two strains are different (Takashima et al. (2008). Different mechanisms of DEHP-induced hepatocellular adenoma tumorigenesis in wild-type and Ppar alpha-null mice. J Occup Health. 50(2):169-80.)
 - Expression profiles different

MOST CHEMICALS HAVE MULTIPLE MOLECULAR TARGETS

- Basis for “off-target” effects and toxicity of drugs and environmental chemicals in multiple tissues
- Phthalates activate other nuclear receptors in addition to PPAR α
 - DEHP and the major metabolite MEHP activate CAR and PXR (Hurst and Waxman, 2004; Baldwin and Roling, 2009; DeKeyser et al., 2009).
 - DBP and the major metabolite MBP activate CAR and PXR (Wyde et al., 2005)



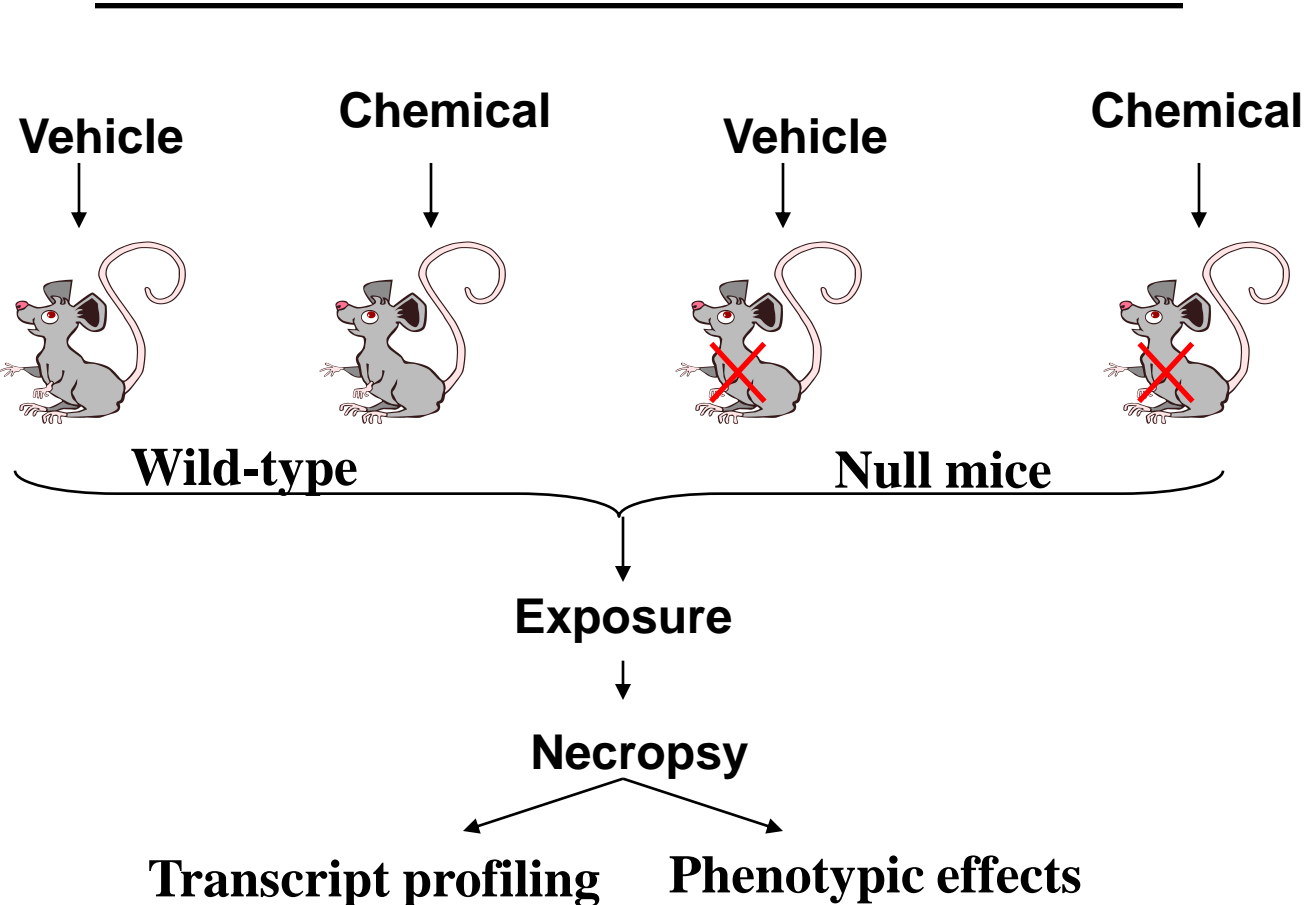
CAR IS ACTIVATED BY TWO MECHANISMS



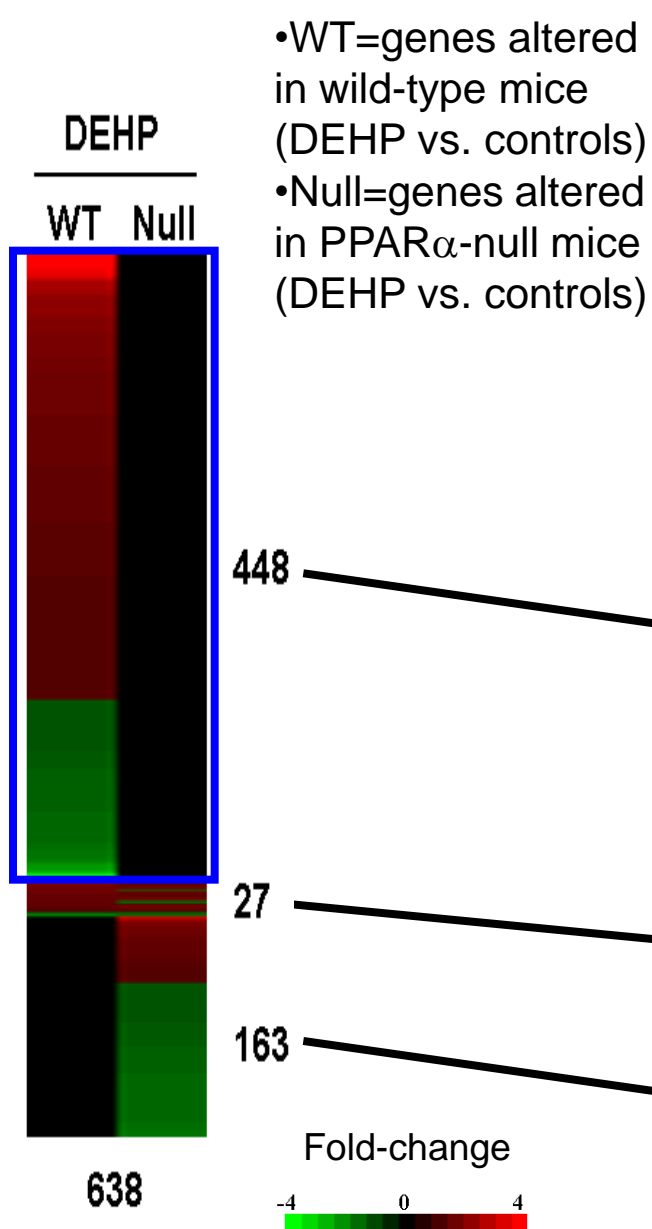
Phenotypic Responses

COMPARISON OF EFFECTS IN WILD-TYPE AND PPAR α -NULL MICE TO ASSESS PPAR α -INDEPENDENT EFFECTS

Study Design



ANALYSIS OF THE DEHP TRANSCRIPT PROFILE



- WT=genes altered in wild-type mice (DEHP vs. controls)
- Null=genes altered in PPAR α -null mice (DEHP vs. controls)

- Exposed wild-type and PPAR α -null mice to DEHP by gavage for 3 days (200 and 1150 mg/kg) (Ren et al., 2009)

- Examined gene expression using Affymetrix gene chips

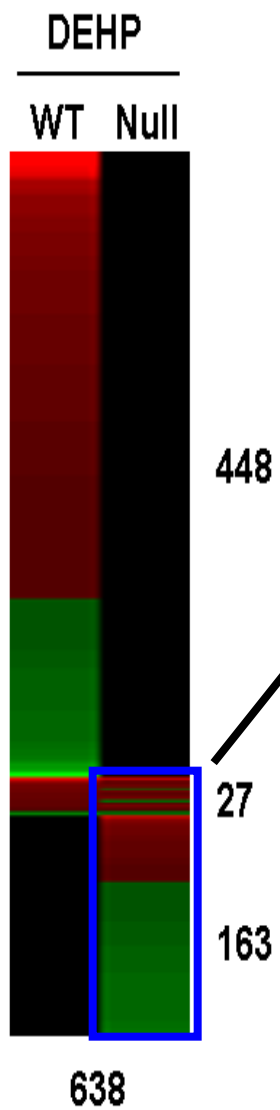
- Most of the genes (94%) regulated by DEHP in wild-type mice are PPAR α -dependent

- 6% are PPAR α -independent

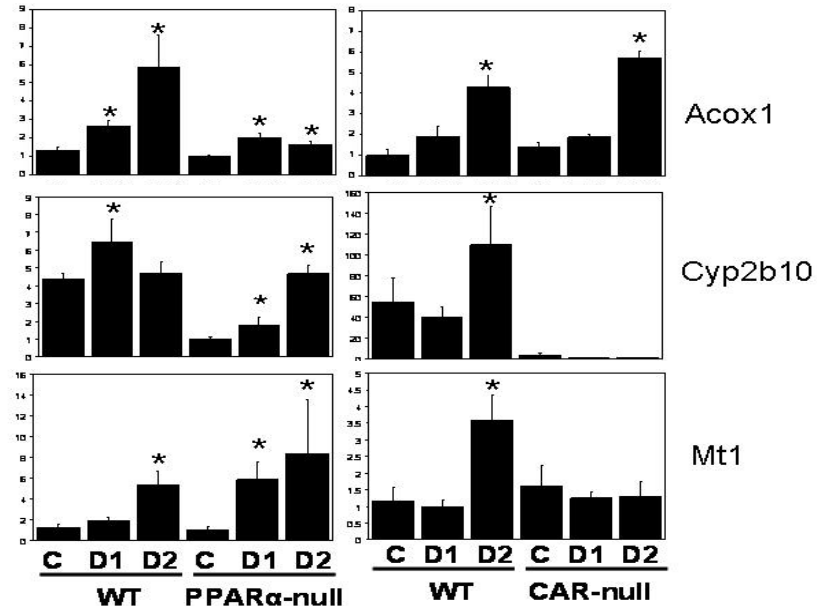
- Some genes are regulated only in the absence of PPAR α

ANALYSIS OF THE DEHP TRANSCRIPT PROFILE

- PPAR α -independent genes include those that are regulated by CAR
- CAR-dependence confirmed in CAR-null mice by RT-PCR



- *Acox1* is mostly PPAR α -dependent
- *Cyp2b10* and *Mt1* are PPAR α -independent but CAR-dependent



From Ren et al., 2009

D1=200 mg/kg

D2=1150 mg/kg for 3 days

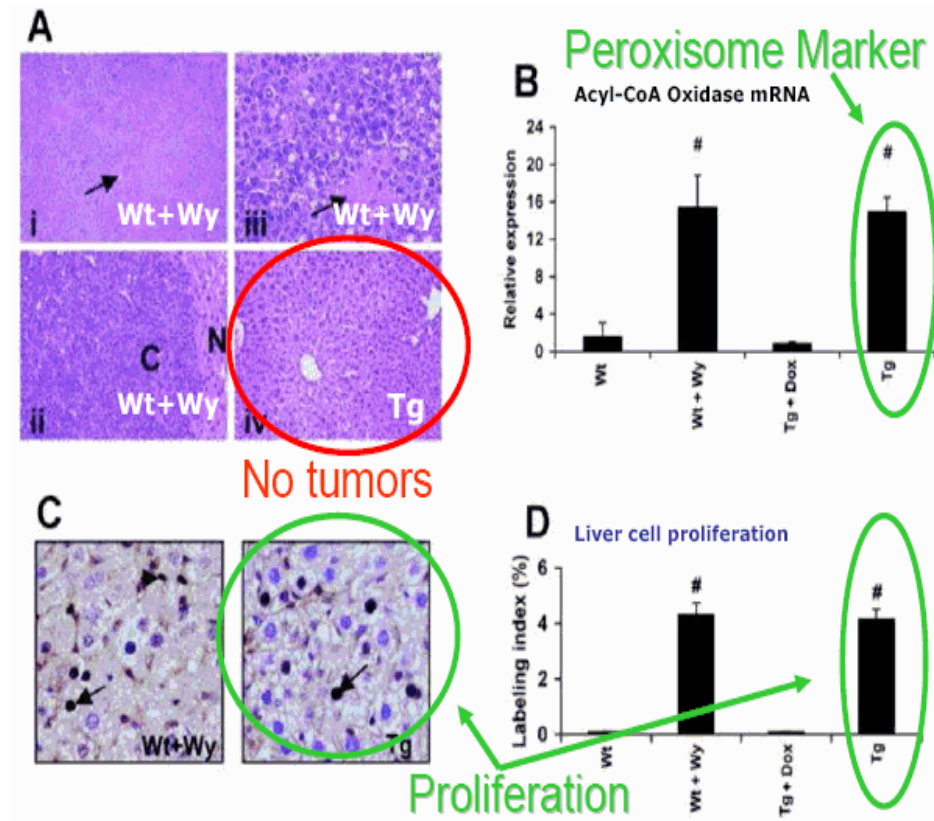
TRANSCRIPTIONAL RESPONSES ARE PRINCIPALLY THROUGH PPAR α

- PPAR α is the major determinant in mediating the transcriptional effects of PPC (WY, PFOA, PFOS, DEHP) in the liver
 - Dependence ranges from 85 – 99% of all altered genes
- Evidence that CAR and other PPAR subtypes play roles in the absence of PPAR α



- Hypothesis: DEHP causes liver tumors in PPAR α -null mice through CAR

PPAR α ACTIVATION IN HEPATOCYTES CAUSES CELL PROLIFERATION BUT NOT CANCER



•Yang et al. (2007) created a PPAR α -null mouse containing a PPAR α that is constitutively active (PPAR-VP16) in the absence of chemical exposure

•Compared responses to wild-type mice treated with WY

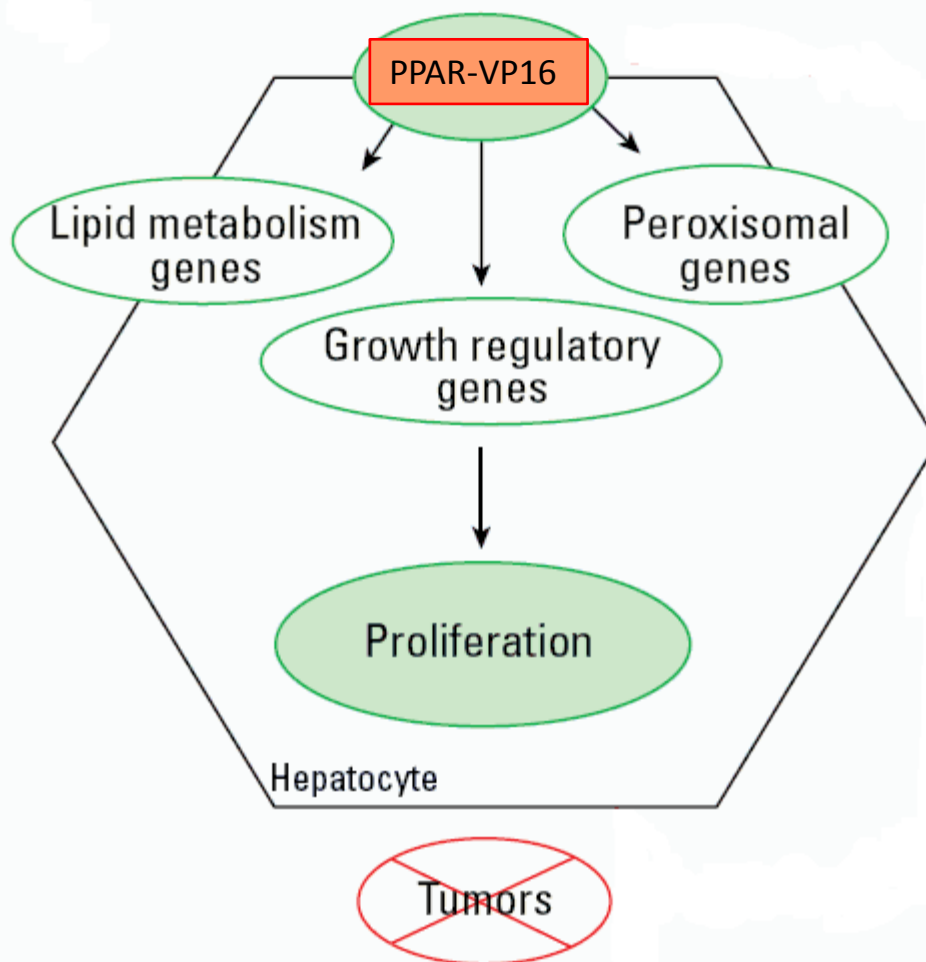
•Observed increases in markers of peroxisome proliferation and hepatocyte proliferation **but no proliferation of nonparenchymal cells**

•No tumors after 11 months

•Indicates that non-parenchymal cells are important for the tumor response

PPAR α ACTIVATION IN HEPATOCYTES CAUSES CELL PROLIFERATION BUT NOT CANCER

PPAR α -null mice



- Model of effects of PPAR-VP16 activity in the absence of a PPAR α activator
- Hepatocyte proliferation not sufficient to cause increases in tumors

IS THIS LOGICAL?

PPAR-VP16 expression increases hepatocyte proliferation but not liver tumors.

Therefore,

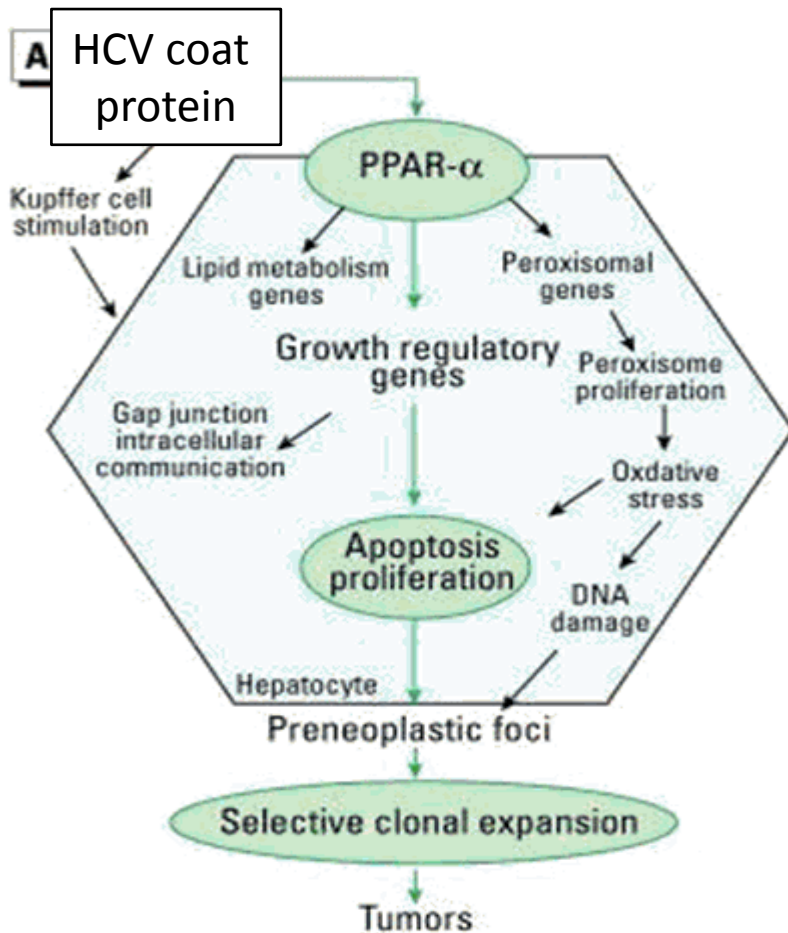
The PPAR α MOA is neither necessary or sufficient for peroxisome proliferators to cause liver cancer.

HEPATOCTE PROLIFERATION IS NECESSARY BUT NOT SUFFICIENT FOR LIVER CANCER

- Key steps in MOA are defined as necessary but not sufficient
- PPAR-VP16 effects are interesting but artificial
 - Cell proliferation only in hepatocytes
 - Proliferation of both hepatocytes and non-parenchymal cells in wild-type rodents upon exposure
- Many studies indicate that non-parenchymal cells are necessary for cell proliferation and tumor induction
 - Depleting Kupffer cells with glycine decreases cell proliferation and size of the tumors (Rose et al., 1997, 1999)
 - Non-parenchymal cells required for efficient proliferation of hepatocytes in culture (Hasmall et al., 2000a,b, 2001)
 - Non-parenchymal cell media required for proliferation of hepatocytes in culture (Parzefall et al., 2001)
- All in vivo data with PPAR α agonists demonstrate that hepatocyte proliferation is a necessary event in tumorigenesis, as tumors are not observed in the absence of induction of cell proliferation

THE PPAR α MOA IS CHEMICAL-INDEPENDENT

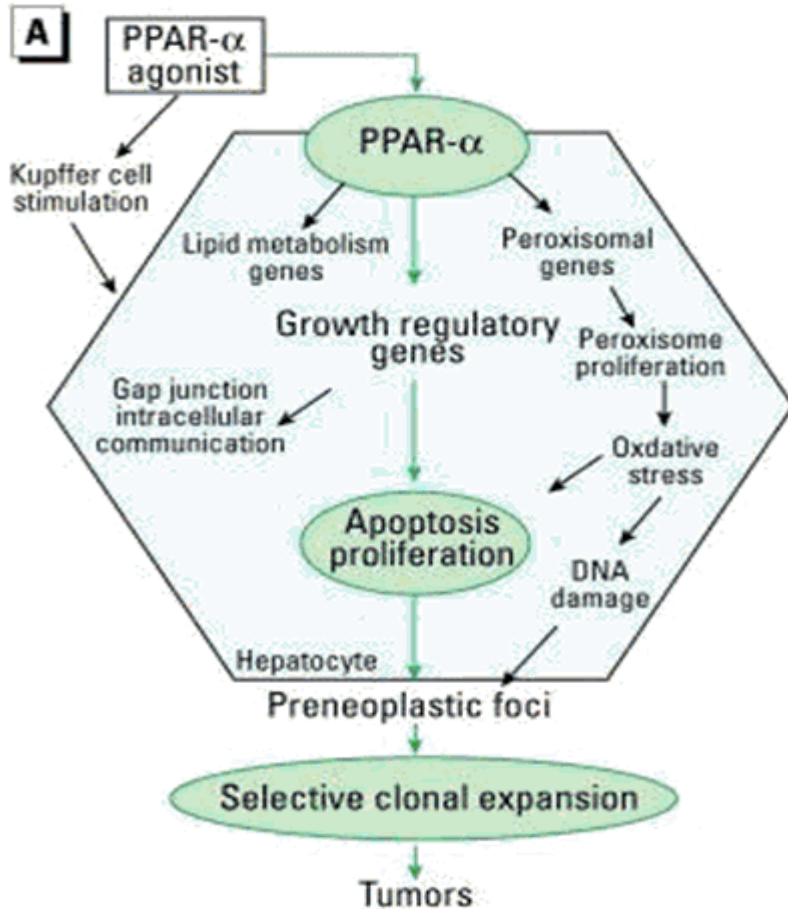
Wild-type mice



- Liver tumors induced by over-expression of the HCV core protein (Tanaka et al., 2008)
- Increases in
 - Oxidative stress
 - Hepatocyte proliferation
- PPAR α -dependent
- Activation of PPAR α may be due to increases in endogenous activators

CONCLUSIONS

Wild-type mice



- The PPAR α MOA is still relevant
- “Challenges” to the MOA have logical explanations
 - DEHP-induced liver tumors in PPAR α -null mice – a chemical-specific effect
 - Hepatocyte proliferation in the absence of tumors
- Data gaps remain but pertain to molecular mechanisms and chemical-specific effects not MOA

Meeting: Dose-response approaches for nuclear receptor-mediated modes of action

- Sept. 27-29, 2010
- NIEHS, Research Triangle Park, NC
- Broad participation from industry, academia, government (FDA, EPA)
- Goal: characterize/update MOA for nuclear receptors in liver cancer induction
 - PPAR α
 - CAR/PXR
 - Dioxin Receptor
- <http://www.tera.org/peer/nuclearreceptor/>

Thank you for
your interest!

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