Diisononyl Phthalate (DINP) Comments on the Transparency and Utility of Mechanistic Information

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Presentation of DINP Mechanistic Information

- In March 2014, EPA provided preliminary evidence tables for mechanistic information for the HBCD IRIS assessment.
- ACC and others noted that this was a positive step but not fully sufficient.
    - EPA did not provide study design information (doses, concentrations or exposure durations), or assay results.

- Now in August 2014, EPA has taken a step backwards, removed any evidence tables providing mechanistic information and is asking if what they have done is useful.
    - EPA also asks how mechanistic information can be used and makes no note of the discussions or comments from the March 2014 meeting.

Importance of Mechanistic Information

- Mechanistic/MOA information must not come second; it must be part of problem formulation.

Importance of Extracting the Relevant Information

- Detailed information, not just summary information is necessary.
  - See ACC comments to OEHHA 2014 (submitted to docket)

Table 1. Basic Study Information for Reproductive Toxicity (Inhalation Exposure)

Study Reference	Species/Strain	Age	Sex	Animals per Exposure Group	Exposure Concentration (ppb)	Exposure Length/Frequency
Author, Year	Rat, Wistar	6 weeks	Female	6	0, 0.1, 1, 10	4 h/d; 30 d
Author, Year	Rat, Sprague Dawley	4 weeks	Male	8	0, 5, 10, 20	4 h/d; 5 d/wk; 8 wk
Author, Year	Mouse, CD-1	8 weeks	Male	10	0, 0.025, 0.25, 2.5, 25	8 h/d; 5d/wk; 8 wk

Table 5 Study Outcomes for Reproductive Toxicity

Study Reference	Outcomes Assessed (Examples Below)										
	Sperm Count	Sperm Morphology	Sperm Motility	Testis Weight	Testis Histology	Estrous Cyclicity	Ovary Weight	Uterus Weight	Ovary Histology	Uterus Histology	Others…
Author, Year	X	X	X	X	X						
Author, Year						X					
Author, Year							X	X	X		

Table 7 Study Quality

Study Reference	Sample Size Calculation	Study Reliability (Klimisch Code)	Randomized Allocation to Experimental Groups	Blinded Outcome Assessment	Presence of Attrition Bias	Statistical Methods
Author, Year	Not performed	2 – Reliable with restriction (non-guideline study)	Not stated	Yes	Unknown	Appropriate
Author, Year	Sufficient study power	1 – Reliable without restriction (OECD guideline study)	Yes	Yes	Not likely	Appropriate

Importance of Extracting the Relevant Information (2)

- Detailed information, not just summary information is necessary.
  - See ACC comments to OEHHA 2014 (submitted to docket)

Table 8 Study Results by Outcome for Reproductive Toxicity (Sperm Count Example)

Study Reference	Species/Strain	Dose (mg/kg-d)	Sperm Count ($\times 10^7$ per g epididymal weight)	*P* Value
Author, Year	Rat, F344	0	2.2	-
		5	2.3	0.8
Author, Year	Mouse, CD-1	0	1.9	-
		0.1	1.8	0.1
		1	1.8	0.1
		10	**1.5**	**0.03**

- Approach can easily be adopted for mechanistic information

Importance of Extracting the Relevant Information (3)

- Detailed information, not just summary information is necessary.
  - See M.E. Kushman et al. / *Regulatory Toxicology and Pharmacology* 67 (2013) 266–277.

Table 3
Example entries into the evidence table for the "peroxisome proliferation" mechanism of action in rodents.

Study design and reference	Endpoint and assay	Results (% change from control)					
In Vivo Chronic Cancer Bioassays (David et al., 1999)	Palmitoyl-CoA Oxidase (nmol/min/mg prot), M		mg/kg/day DEHP [b]				
Rats (F344), M and F[a]			0	50	200	875	Recovery
Chronic (78 wks, and 78 wks followed by 26 wks of recovery)		1 week	0	nd	nd	255*	nd
		2 weeks	0	nd	nd	556*	nd
N = 6		13 weeks	0	nd	nd	390*	nd
		104 weeks	0	−29	71*	257*	−24
In Vivo Acute and Subchronic Studies (Hinton et al., 1986)	Palmitoyl-CoA Oxidase (nmol/min/mg prot), M	mg/kg DEHP					
Rats (Wistar), M and F		0	50	200	1000		
24 h		0	62*	57*	468*		
N = 3–4	Catalase (nmol/min/mg prot), M	mg/kg DEHP					
		0	50	200	1000		
		0	0	9	22*		
In Vitro Bioassays with Primary Hepatocytes (Goll et al., 1999)	Acyl-CoA oxidase activity (nmol/min/mg prot)	mM DEHP					
1° hepatocytes from male Sprague–Dawley Rats		0	0.1	0.25	0.5		
		0	10*	6	3		
72 h	Carnitine acetyltransferase activity (nmol/min/mg prot)	mM DEHP					
N = 3		0	0.1	0.25	0.5		
		0	10*	6	3		

[a] Results for females are not shown.
[b] Converted to mg/kg/day from ppm (1 mg/kg = 20 ppm).
* Statistically significant results ($p < 0.05$).

Key Events (KE) and MOA

Table 4. Strawmen of PPARα mode of action key events.

Proposed mode of action of rodent liver tumors of PPARα activators			
	Strawman 1: taken from Corton (2010)	Strawman 2	Strawman 3: (taken from Klaunig et al., 2003)
KE #1	PPARα activation	PPARα activation	PPARα activation
KE #2	Increases in oxidative stress	Altered expression of genes involved in cell growth	a. Expression of peroxisomal genes b. PPARα mediated expression of cell cycle, growth and apoptosis c. Non-peroxisomal lipid gene expression
KE #3	NF-κB activation	Increased cell proliferation/decreased apoptosis	Increase in cell proliferation
KE #4	Increased cell proliferation/decreased apoptosis	Selective clonal expansion of preneoplastic foci cells	Clonal expansion of preneoplastic foci
KE #5	Increases in preneoplastic foci cells	Liver tumors	Liver tumors
KE #6	Liver tumors		

"Overall, the panel concluded that significant quantitative differences in PPARα activator-induced effects related to liver cancer formation exist between rodents and humans. On the basis of these quantitative differences, most of the workgroup felt that the rodent MOA is "not relevant to humans" with the remaining members concluding that the MOA is "unlikely to be relevant to humans."

From Corton et al. 2013. Crit Rev Toxicol DOI: 10.3109/10408444.2013.835784

Table 5. Occurrence of key events in the mode of action after exposure to PPARα agonists in rats.

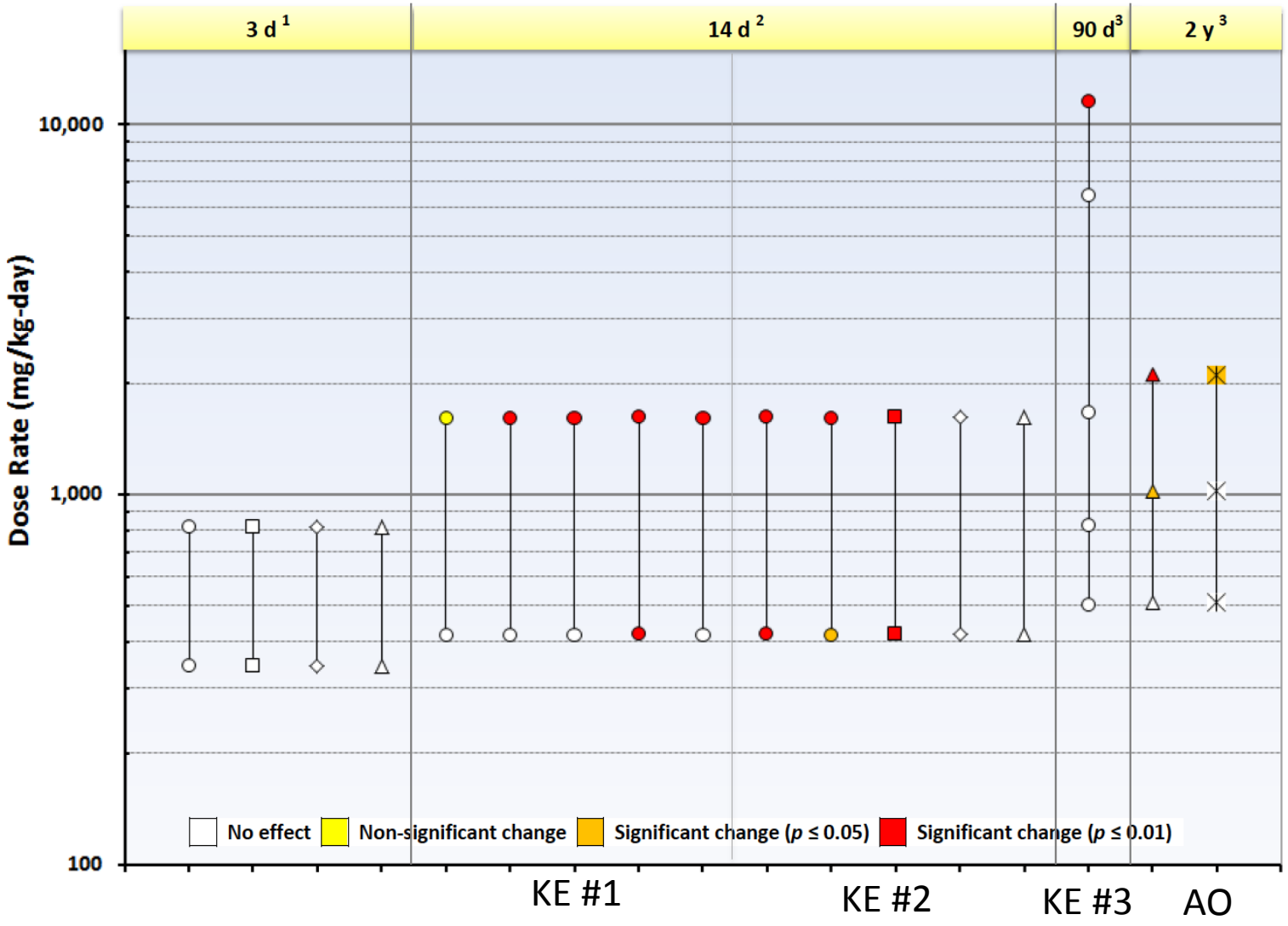
	Key events								
	KE#1	KE #3			KE#4	Modulating factors			Apical end point
	PPARα activation	Perturbation of cell growth and survival			Clonal expansion of preneoplastic foci				
Chemical		Increases in transient acute cell proliferation	Decreases in acute apoptosis	Increases in chronic cell proliferation		Oxidative stress	NF-κB activation	Alterations in gap junctions	Hepatic tumors
WY-14,643	+[1]	+[2]	+[3]	+[4]	+[5]	+[7] −[8]	+[9]	+[53]	+[6]
DEHP	+[10]	+[11]	+[12]	+/−[13]		+[14] −[15]		+[50]	+[14]
Clofibrate	+[16]	+[17]		+[18]		+[20] −[21]			+[19]
Nafenopin	+[22]	+[6]	+[23]	+[24] +/−[6]	+[25]	+[27] −[28]	−[29]	+[52]	+[26]
Ciprofibrate	+[22]	+[30]		+[31]	+[32]	+[34]	+[35]		+[33]
Methyl clofenapate		+[36]	+[37]	+[38]		+[39] −[40]			+[39]
Gemfibrozil (CI-718)	+[22]	+[57]			−[41]	+[42]	+[43]		+/−[41]
Di-n-butyl phthalate	−[10]					+[44]	+[43]		
Trichloroacetate	+/-[55]							+[54]	−[45]
Perfluorooctanoate	+[56]	+[46]				+[48] −[49]		+[51]	+[47]

Comments: In the table, (+) indicates that the chemical was found to lead to the event; (–) indicates that the chemical was found not to lead to the event; (+/–) indicates mixed results. PPARα activation was measured using transactivation assays. NF-κB activation refers to binding of NF-κB (p65:p50 heterodimer) to the NF-κB response element in electrophoretic mobility shift assays. Acute cell proliferation was measured in the livers of treated mice, usually with seven days or less of exposure. Apoptosis was mostly measured in primary hepatocytes, given the low background in intact livers. However, three studies have measured apoptosis in rodent livers after exposure to a PPARα agonist (Bursch et al., 1984; James et al., 1998a; Youssef et al., 2003). Chronic cell proliferation was measured in the livers of rats exposed to PPARα agonists, usually for more than three weeks.

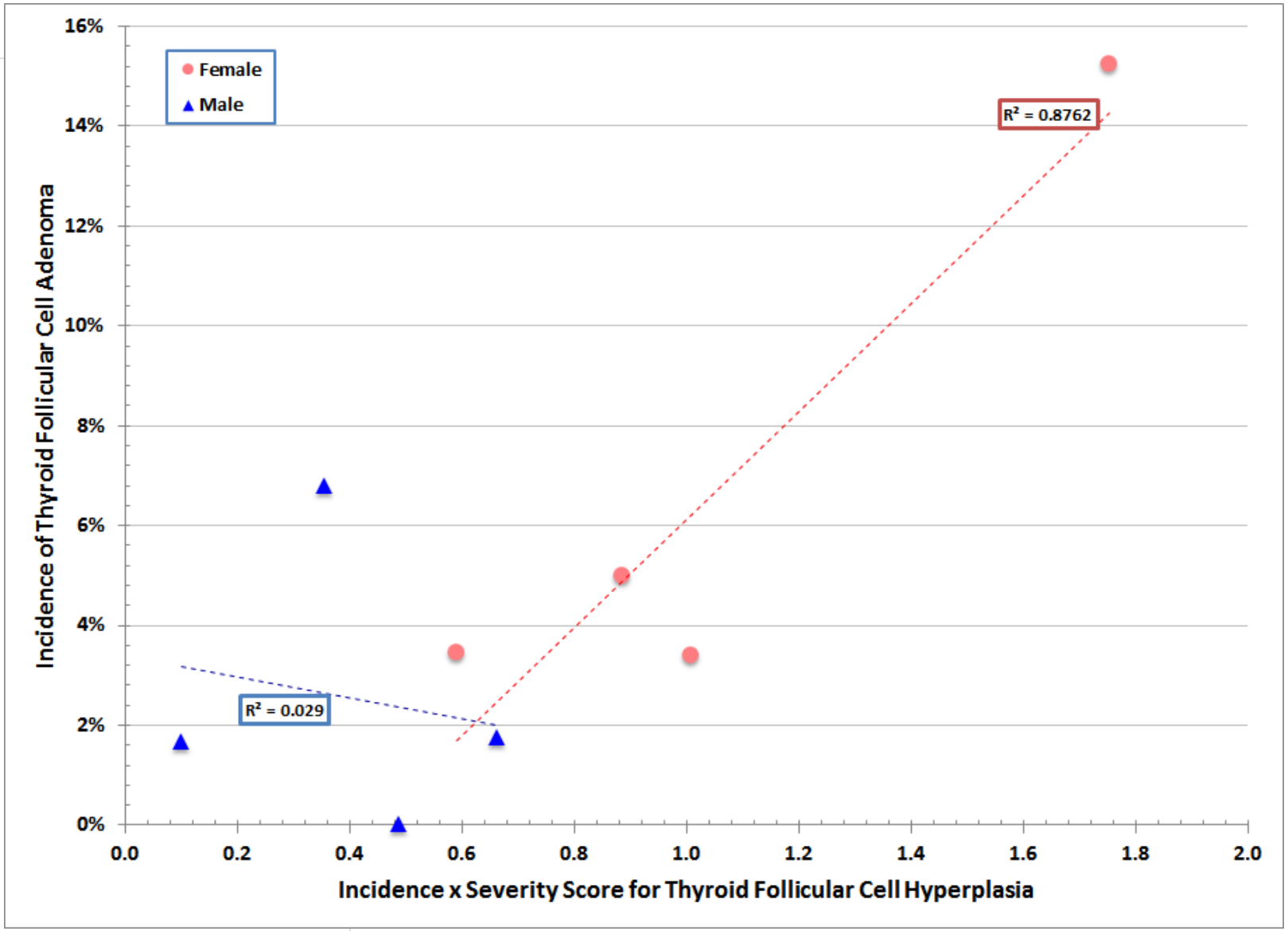
References:

1. Corton & Lapinskas, 2005; Gottlicher et al., 1992
2. Wada et al., 1992; Marsman et al., 1988, 1992; Lake et al., 1993
3. Youssef et al., 2003
4. Wada et al., 1992; Marsman et al., 1988, 1992; Lake et al., 1993
5. Marsman & Popp, 1994; Rose et al., 1999b
6. Lake et al., 1993

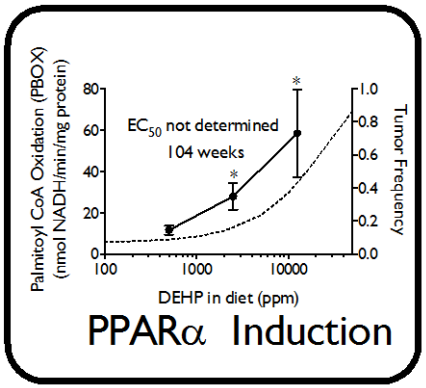
Dose Range Array

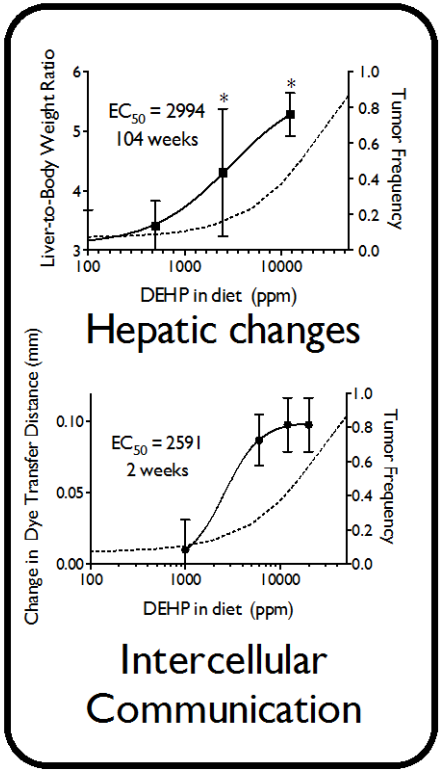


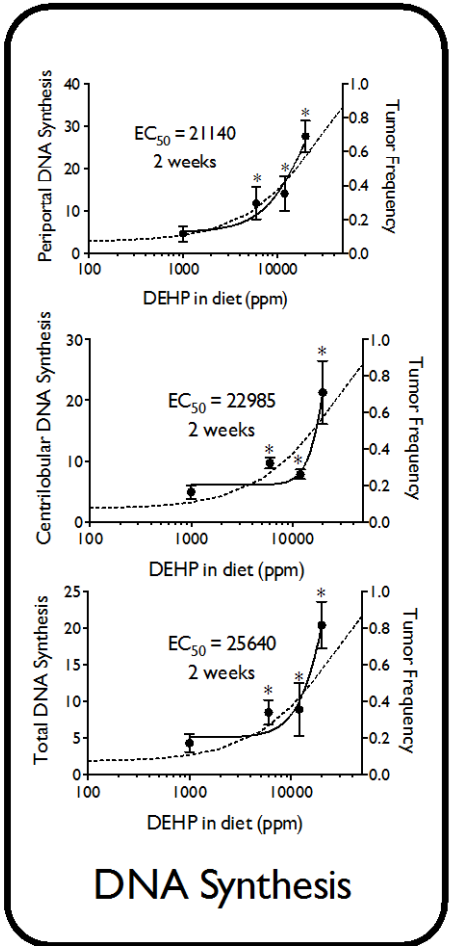
Correlation of Key Events (KE) with Adverse Outcomes

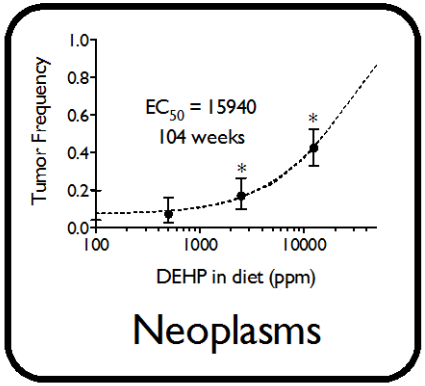


Comparison and Ordering of Dose-Response of Key Events





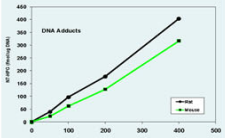
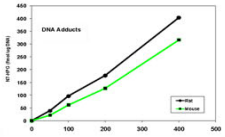
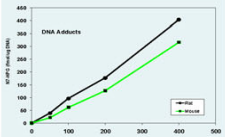




From Corton et al. 2013. Crit Rev Toxicol DOI: 10.3109/10408444.2013.835784

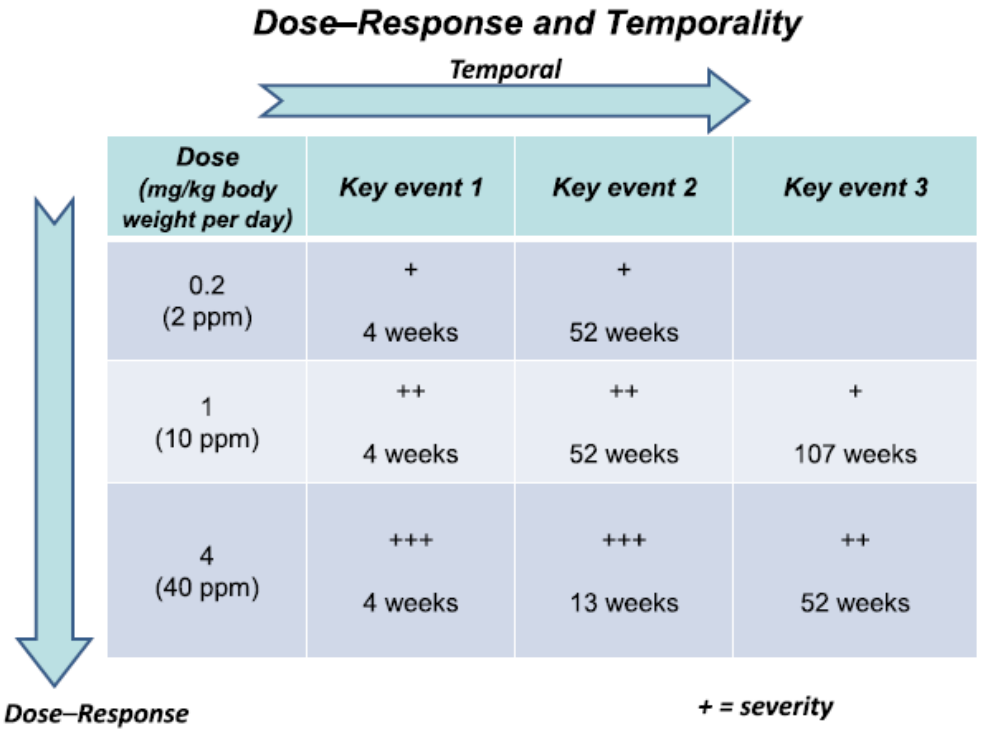
WHO/IPCS Framework, MOA and Bradford Hill

Concordance Table with Dose–Response

Key event / adverse outcome	Qualitative species concordance	Evidence base	Quantitative species concordance	Quantitative dose–response
Metabolism by cytochrome P450 2E1	Relevant enzyme in kidney and liver of humans	Considerable in animals; limited but relevant to humans	Physiologically based pharmacokinetic model incorporating metabolic rates, enzyme affinities and distribution based on *in vitro* human data supported by *in vivo* data	
Sustained cell damage and repair (cytotoxicity, proliferation)	Liver and kidney target organs in humans	Considerable in animals; possible in humans, but limited data	No data	
Liver and kidney tumors	Possible in humans	Considerable in animals; highly plausible in humans	No data	

- Meek, Boobis, Cote, Dellarco, Fotakis, Munn, Seed, Vickers. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis *J. Appl. Toxicol*. 2014; 34: 1–18

WHO/IPCS Framework, MOA and Bradford Hill



Dose (mg/kg body weight per day)	Key event 1	Key event 2	Key event 3
0.2 (2 ppm)	+ 4 weeks	+ 52 weeks	
1 (10 ppm)	++ 4 weeks	++ 52 weeks	+ 107 weeks
4 (40 ppm)	+++ 4 weeks	+++ 13 weeks	++ 52 weeks

- Meek, Boobis, Cote, Dellarco, Fotakis, Munn, Seed, Vickers. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis *J. Appl. Toxicol.* 2014; 34: 1–18.

- Meek, Palermo, Bachman, North, Lewis. Mode of action human relevance (species concordance) framework: Evolution of Bradford Hill considerations and comparative analysis of weight of the evidence. *J. Appl. Toxicol.* 2014; 34: 1–18. online Feb 2014, DOI: 10.1002/jat.2984.

Improving Presentation and Use of Mechanistic Information

- EPA must extract sufficient mechanistic information from studies.
  - Simply counting the number of studies that provide data on each mechanistic category is not helpful.
- Multiple approaches for presenting this information already exist (see citations provided in slides).
- Early consideration of hypothesis based key events in the MOA/AOP during problem formulation will facilitate incorporation of data from different sources and provide a framework for organization which can be linked at different levels of biological organization.

❖ Mechanistic/MOA information must not come second; it must be part of problem formulation.

Questions and Discussion