Integrating KMD/TK Data with MoA and Other Information in a Weight of Evidence Approach

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Development of the KMD Concept

- HESI Working Group on Dose Dependent Transitions in Mode of Action (MoA)
  - Case studies on change in MoA at higher doses (Slikker et al. 2004a,b)
- HESI WG on Agricultural Chemical Safety Assessment
  - Use of kinetics early in a tiered testing approach (Carmichael et al. 2006)
- - Dow Chemical
  - Internal dosimetry in animal bioassays (Saghir et al. 2012)
  - Initial applications of KMD approach (Saghir et al. 2013, 2015)
- - Dow AgroSciences
  - Application of KMD in support of 3Rs: (Terry et al. 2014, 2015; Sewell et al. 2017)

Early Regulatory Support for Consideration of Toxicokinetics in Dose Selection

- OECD Guidance Document 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Supporting Test Guidelines 451, 452 and 453 – 2nd Edition (2012)

  - 90. If the main objective of the study is to identify a cancer hazard, there is broad acceptance that the top dose should ideally provide some signs of toxicity such as slight depression of body weight gain (not more than 10%), without causing e.g., tissue necrosis or metabolic saturation and without substantially altering normal life span due to effects other than tumours. Excessive toxicity at the top dose level (or any other dose level) may compromise the usefulness of the study and/or quality of data generated. Criteria that have evolved for the selection of an adequate top dose level include: (in particular) toxicokinetics; saturation of absorption; results of previous repeated dose toxicity studies; the MOA and the MTD.

  - 91. Toxicokinetic non-linearity should also be considered in the selection of the top dose to be used. Although top dose selection based on identification of inflection points in toxicokinetic nonlinearity may result in study designs that fail to identify traditional target organ or body weight effects, it must be appreciated that metabolic saturation in fact represents an equivalent indicator of biological stress. In this case, the stress is evidenced by appearance of non-linear toxicokinetics rather than appearance of histological damage, adverse changes in clinical chemistry, haematology parameters or decrease in body weight gain (Toxicokinetics is discussed in Section 3.4).

Recent Regulatory Support for Consideration of Toxicokinetics in Dose Selection

- OECD 443(2018) “Extended One-Generation Reproductive Toxicity Study”
- - “…all the relevant available information on the test chemical, i.e. physico-chemical, toxicokinetics (including species-specific metabolism), toxicodynamic properties, structure-activity relationships (SARs), in vitro metabolic processes, results of previous toxicity studies and relevant information on structural analogues should be taken into consideration in planning the Extended One-Generation Reproductive Toxicity Study.”
- - “Although not required, TK data from previously conducted dose range-finding or other studies are extremely useful in the planning of the study design, selection of dose levels and interpretation of results.”

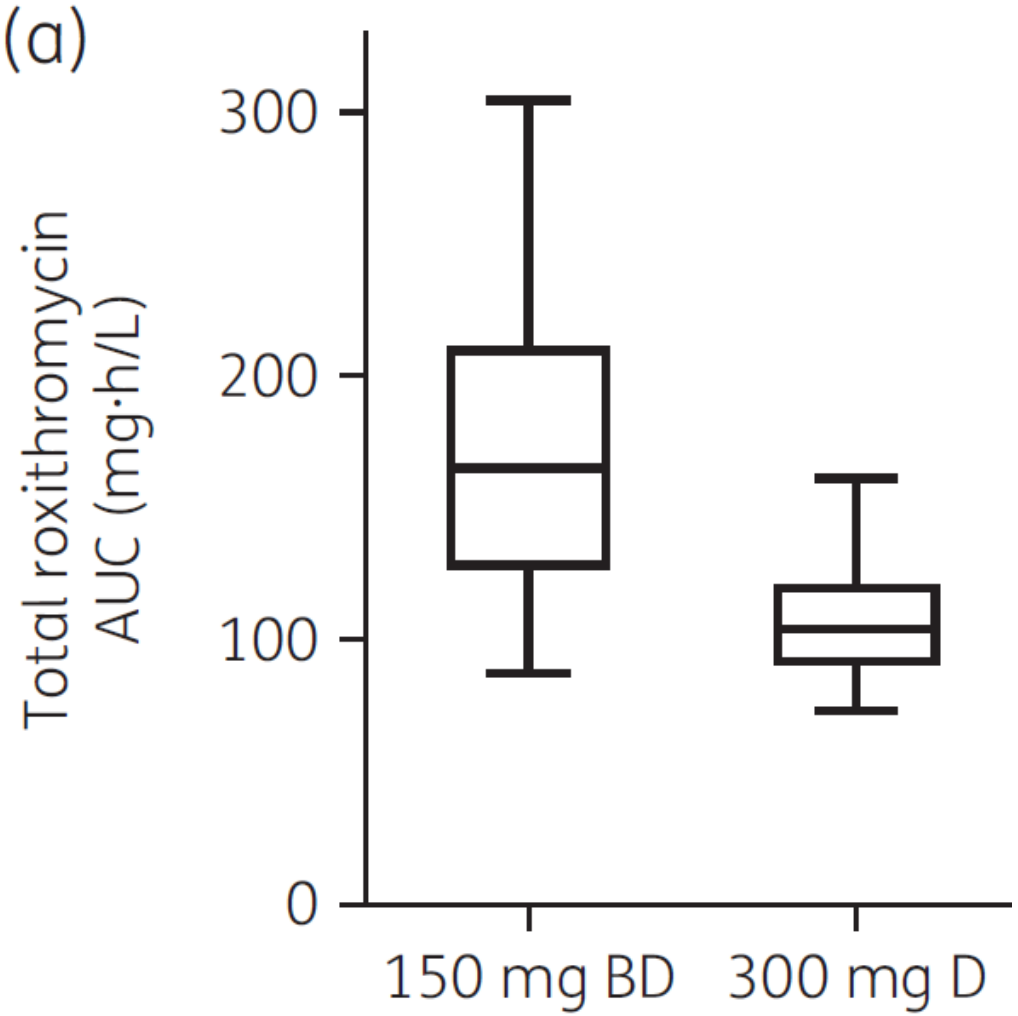
Problem Statement

A Kinetically-determined Maximum Dose:

- Relies on evidence of a nonlinearity (dose-dependent transition) in kinetics
- However, a nonlinearity in kinetics, by itself, does not support a conclusion that effects observed at doses above the KMD would be irrelevant to lower doses
- The presumption that exceedance of the KMD is associated with a transition to a more toxic mode of action needs to be supported by additional mechanistic evidence
  - To demonstrate a dose-dependent transition (DDT) in the Mode of Action (MoA) for toxicity (Slikker et al. 2004)

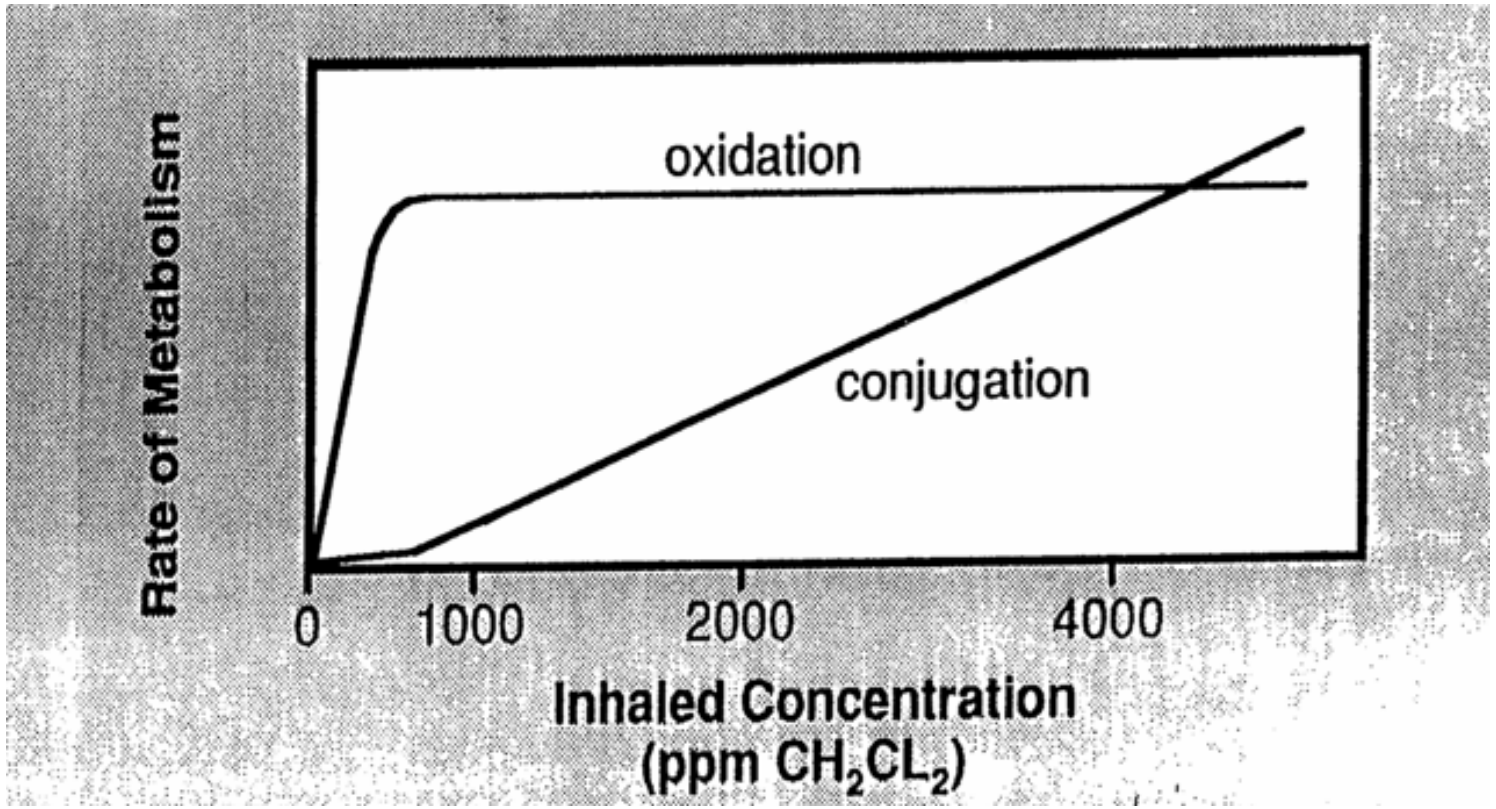
Examples of Potential Supporting Evidence for Application of a KMD to Limit Dosing

- Saturation of absorption (roxithromycin)



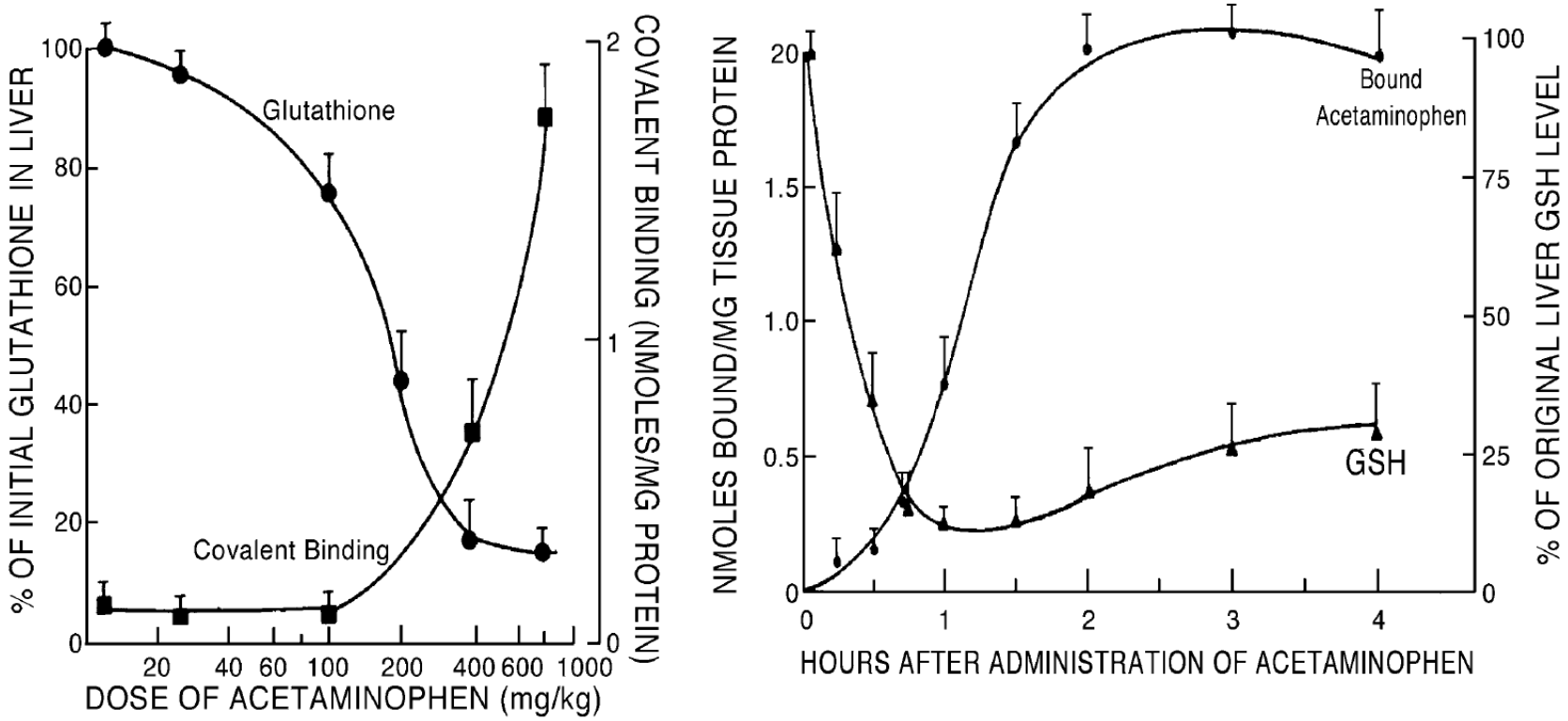
Examples of Potential Supporting Evidence for Application of a KMD to Limit Dosing

- Saturation of competitive metabolic detoxification (methylene chloride)



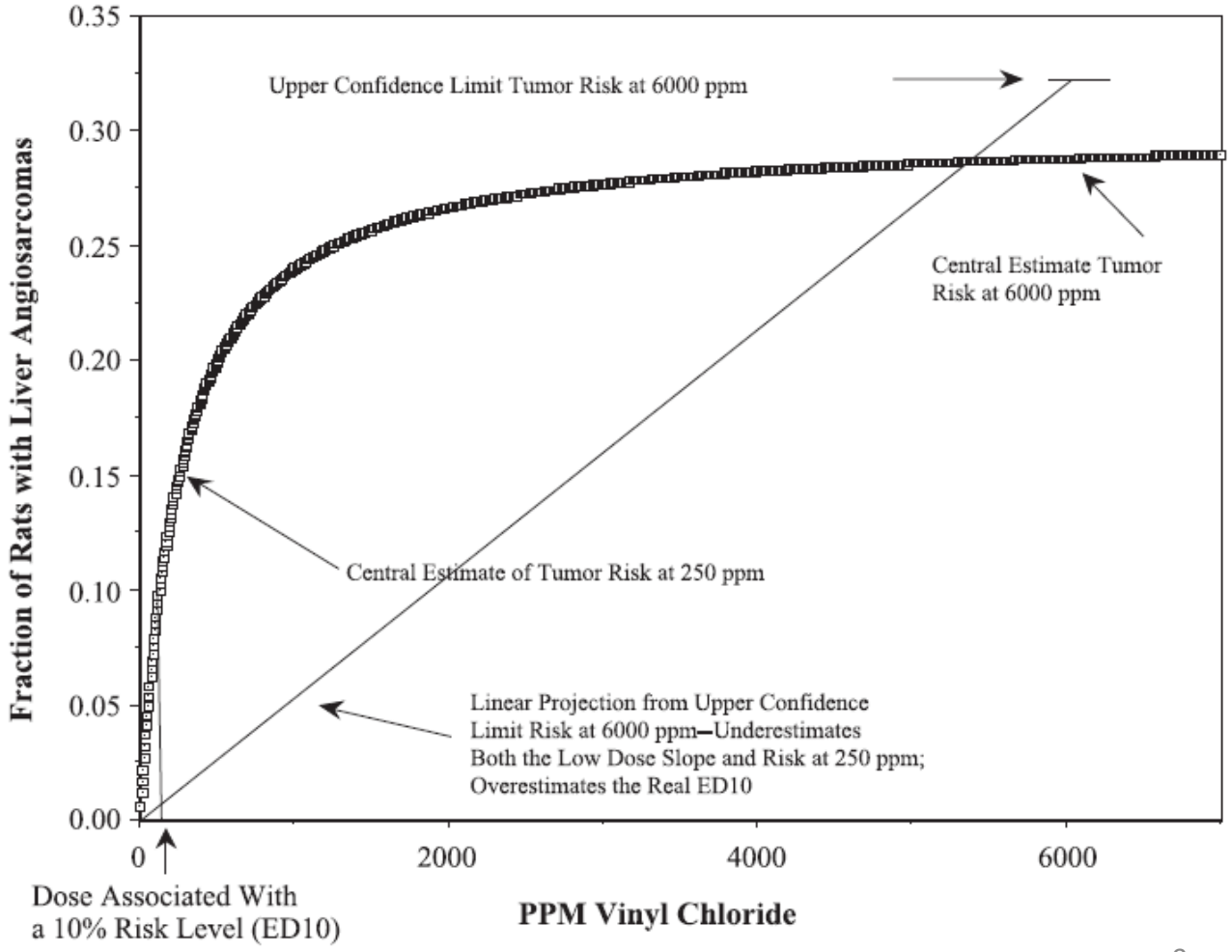
Examples of Potential Supporting Evidence for Application of a KMD to Limit Dosing

- Depletion of glutathione (Acetaminophen)



Examples of Potential Supporting Evidence for Application of a KMD to Limit Dosing

- Saturation of metabolic bioactivation (vinyl chloride)



Examples of Potential Supporting Evidence for Application of a KMD to Limit Dosing

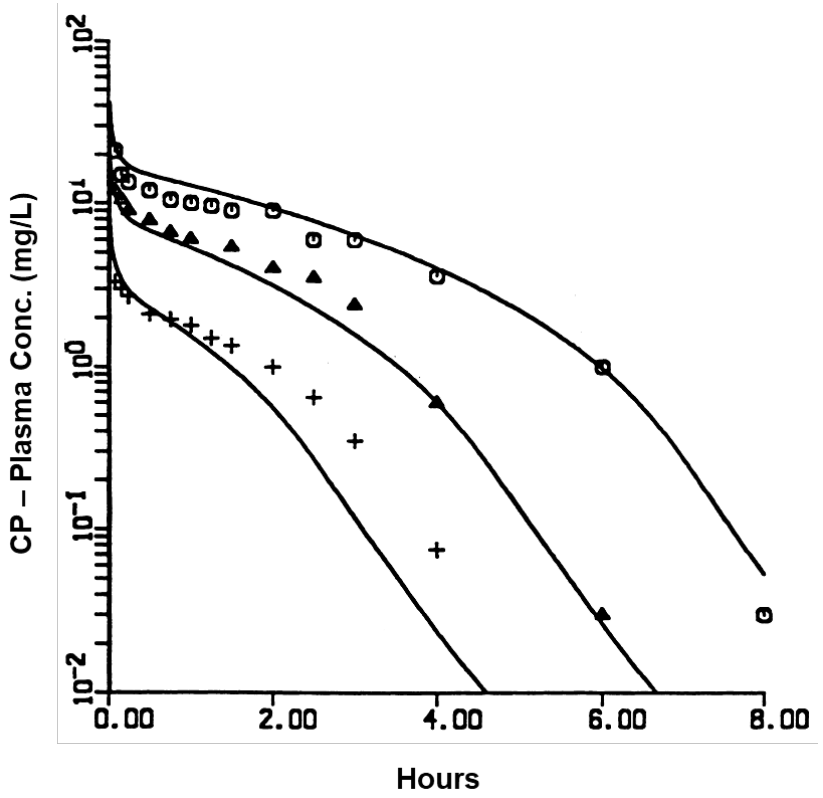
- Receptor activation associated with hepatomegaly (ETBE)

"The SAB agrees that the Saito et al. (2013) study is well-conducted and well-reported, but the data for neoplastic liver lesions from inhalation exposure, by themselves, are not suitable for a quantitative analysis because tumors were only observed at the highest concentration. The SAB noted that the highest concentration is also where centrilobular hypertrophy, nuclear receptor activation, and induction of metabolism may have contributed to the outcome. With a statistically significant increase in tumors at the high dose only, the Saito et al. (2013) data are not sufficiently robust to provide a meaningful quantitative estimate of human cancer risk for ETBE.

EPA-SAB-19-001, February 27, 2019

Examples of Evidence That Does Not Support the Application of a KMD to Limit Dosing

- Saturation of metabolic clearance of a toxic parent compound (retinoic acid)

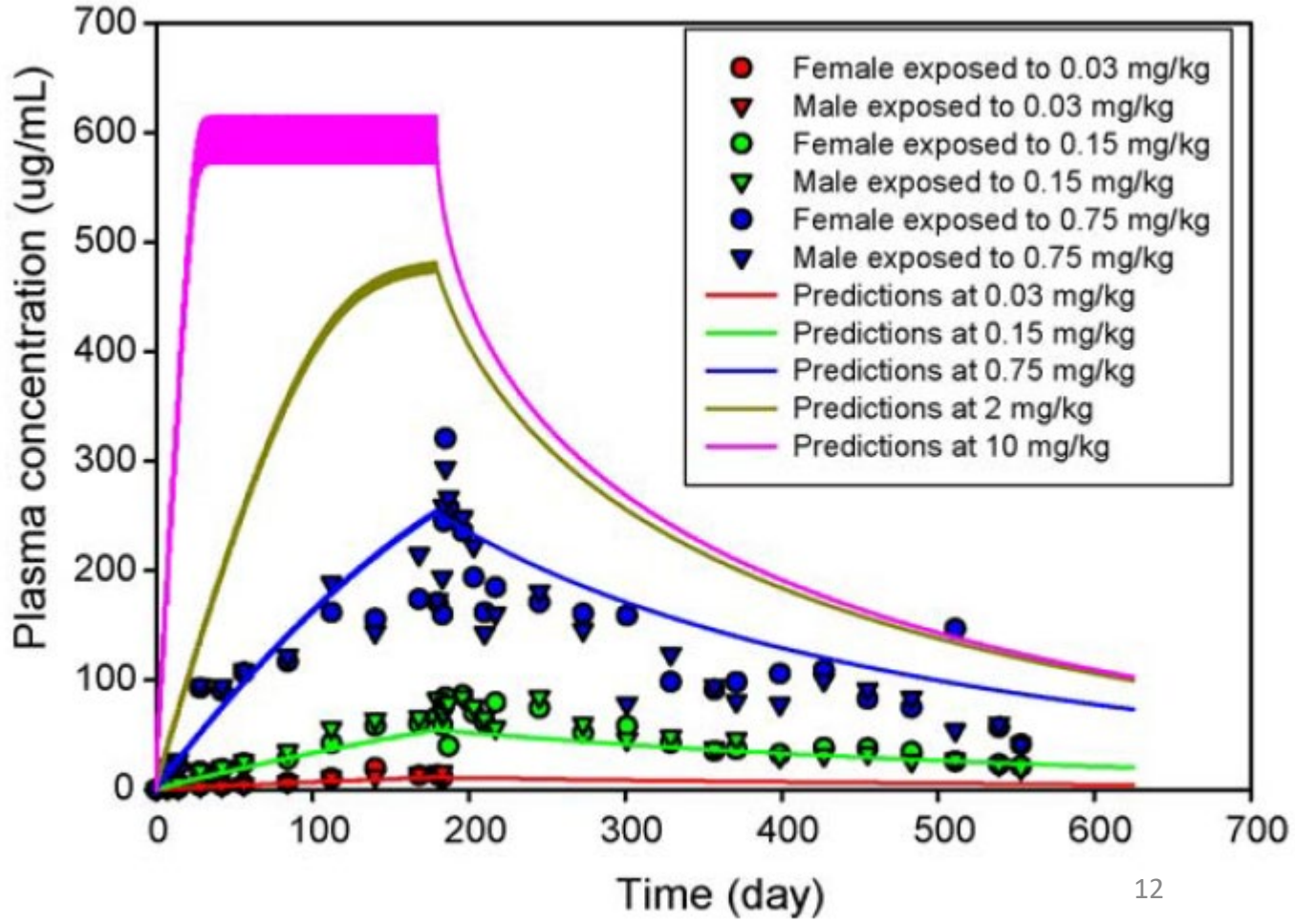


Rat, IV, ATRA
5.0, 0.25, and 0.015 mg/kg

Species	Route	Dose (mg/kg)	All-Trans-Retinoic Acid		Total Active Retinoids		Total Retinoids
			C_{Max} (ng/mL)	AUC (ng*hr/mL)	C_{Max} (ng/mL)	AUC (ng*hr/mL)	C_{Max} (ng-eq/mL)
Minimal Teratogenic Doses							
Mouse	Oral	4	1078	1516	2875	10188	3504
Rat	Oral	2.5	965	1763	1912	12204	2337
Monkey	Oral	5	2105	4768	2348	5715	5354
Clinical Doses							
Human	Oral	1.1	229	558	235	601	1305
Human	Topical	Therapy [a]	0.001	0.014	0.001	0.014	0.015
Human	Topical	Abuse [b]	0.035	0.44	0.035	0.44	0.51

Examples of Evidence That Does Not Support the Application of a KMD to Limit Dosing

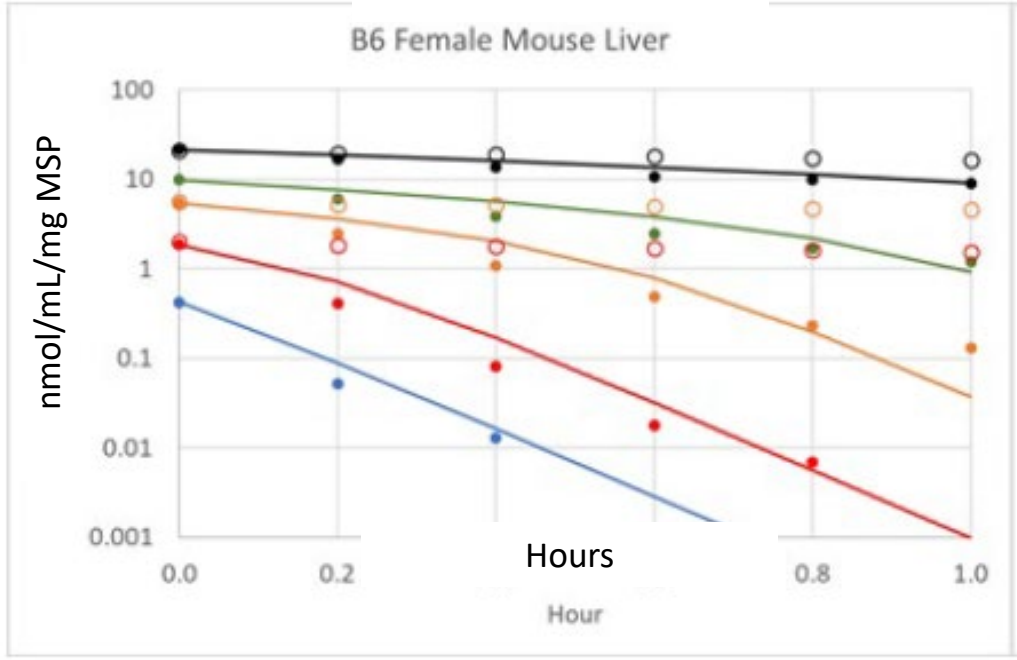
- Saturation of renal resorption of a toxic parent compound (PFOA)



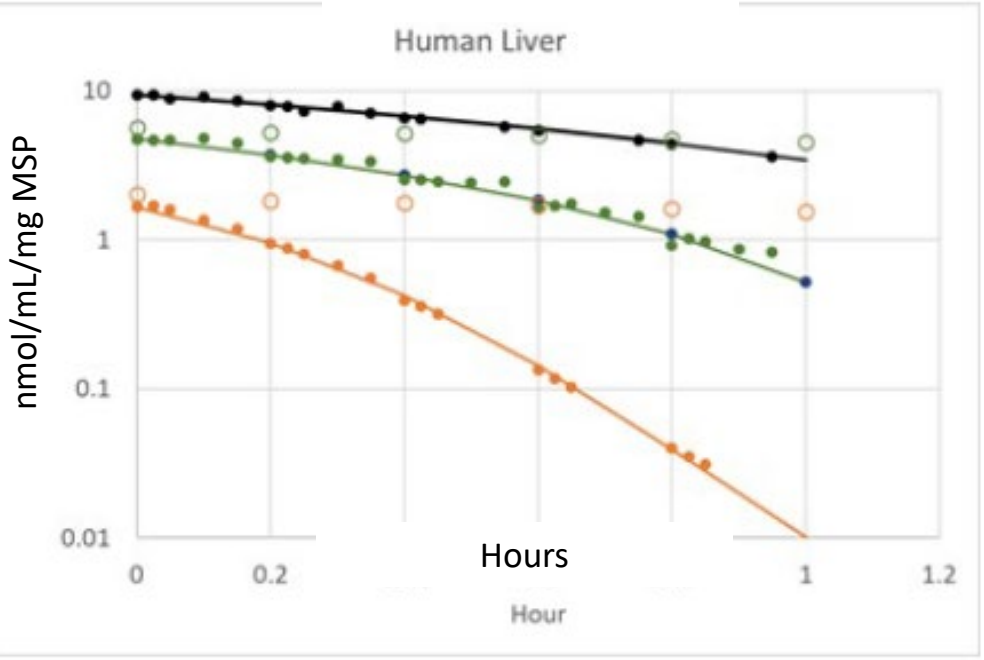
Non-animal approaches to Estimate and Support a KMD

- *In vitro* metabolism studies can identify concentrations associated with a dose-dependent transition (DDT) in metabolism in both experimental animal and human hepatocytes
  - *In vitro* to *in vivo* extrapolation using the metabolism parameters identified in these studies can be used to determine both animal and human KMDs

Mouse Liver

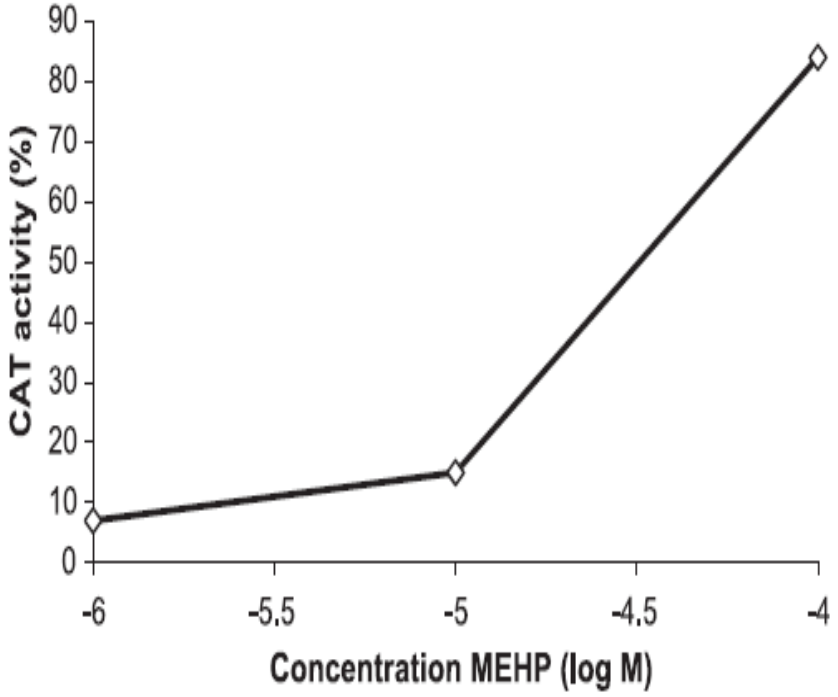


Human Liver



Non-animal approaches to Estimate and Support a KMD

- In some cases, dose non-proportionality may be secondary to toxicodynamic changes
  - e.g., glutathione depletion, nuclear receptor activation
- *In vitro* concentration-response studies can also identify concentrations associated with a dose-dependent transition (DDT) in Mode of Action
  - Examples: glutathione depletion, nuclear receptor activation
  - *In vitro* to *in vivo* extrapolation using the parameters identified by *in vitro* metabolism studies can be used to determine animal and human exposures equivalent to the *in vitro* DDT in MoA



The *In Vitro* KMD Approach

- Estimate a KMD using in vitro metabolism data and pharmacokinetic modeling to perform Quantitative In Vitro to In Vivo Extrapolation (QIVIVE)
- Provide support for the KMD using in vitro concentration-response studies
  - genomic responses of cells exposed in culture
  - BMD analysis to estimate onset of key changes in response

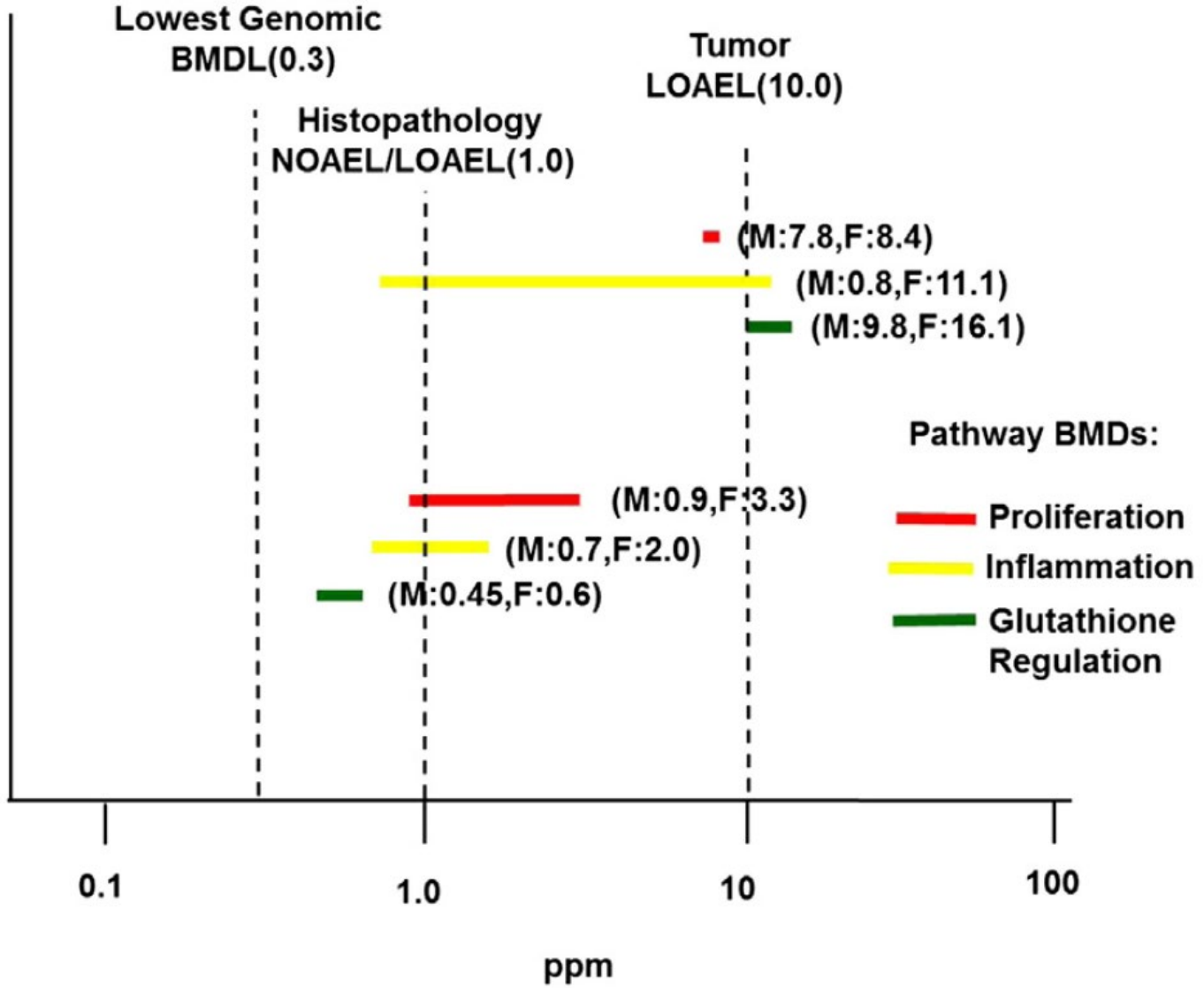
Table 2. Benchmark dose ranges for genes with a statistically significant dose-response trend in primary urothelial cells from most subjects after treatment with arsenite, MMA^{III}, and DMA^{III} (trivalent) mixtures.

Gene Name	Description	Number of subjects expressing the gene/total subjects	BMD range (μM)	BMDL range (μM)
HMOX1	Oxidative stress response	10/10	0.13–0.50	0.09–0.33
FKBP5	Protein folding	9/10	0.36–0.92	0.24–0.58
TXNRD1	Thioredoxin reductase	9/10	0.32–0.75	0.21–0.48
MT1E	Metallothionine regulation	8/10	0.24–0.77	0.16–0.49
DDB2	DNA damage sensing	8/10	0.30–0.88	0.20–0.56
TXN	Thioredoxin	8/10	0.26–0.76	0.17–0.48
LGALS8	Cell adhesion, growth regulation	8/10	0.16–0.92	0.11–0.58
THBD	Immune response	8/10	0.32–0.90	0.20–0.57

Beyond KMD

- In cases where there is a Dose-Dependent Transition in MoA that is not associated with a change in kinetics, in vitro concentration-response studies can be used to identify a toxicodynamic equivalent to the KMD to support a Maximum Relevant Dose (MRD)
  - genomic responses of cells exposed in culture
  - BMD analysis to estimate onset of key changes
    - glutathione depletion
    - receptor activation
    - oxidative stress / inflammation / proliferative signaling
    - DNA damage response

Using Genomic Concentration-Response Data to Identify a Dose-Dependent Transition in Mode of Action



Summary

- Identifying a kinetic basis for a KMD is only part of determining a Maximum Relevant Dose (MRD)
  - Additional evidence is also required to support the existence of a dose dependent transition to a more toxic MoA in the vicinity of the KMD
- In the future, both KMDs and MRDs can be determined using only *in vitro* studies
  - *In vitro* metabolism studies to identify a KMD
  - *In vitro* assays to provide MoA information to support use of the KMD
    - or to identify a MRD based solely on a dose-dependent transition in the MoA