Office of Pesticide Programs

Mode of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid) & Recommendations for Dose Response Extrapolation

Science Advisory Board
September 12-13, 2005

Introduction of EPA Staff

- Office of Pesticide Programs
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  - Dr. Vicki Dellarco
  - Dr. Anna Lowit
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- Special Acknowledgment
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Presentation

- Background
  - History & Objectives of Cacodylic Acid's Risk Assessment
- Highlights
  - Mode of Carcinogenic Action (V. Dellarco)
  - Point of Departure & Dose Response (A. Lowit)

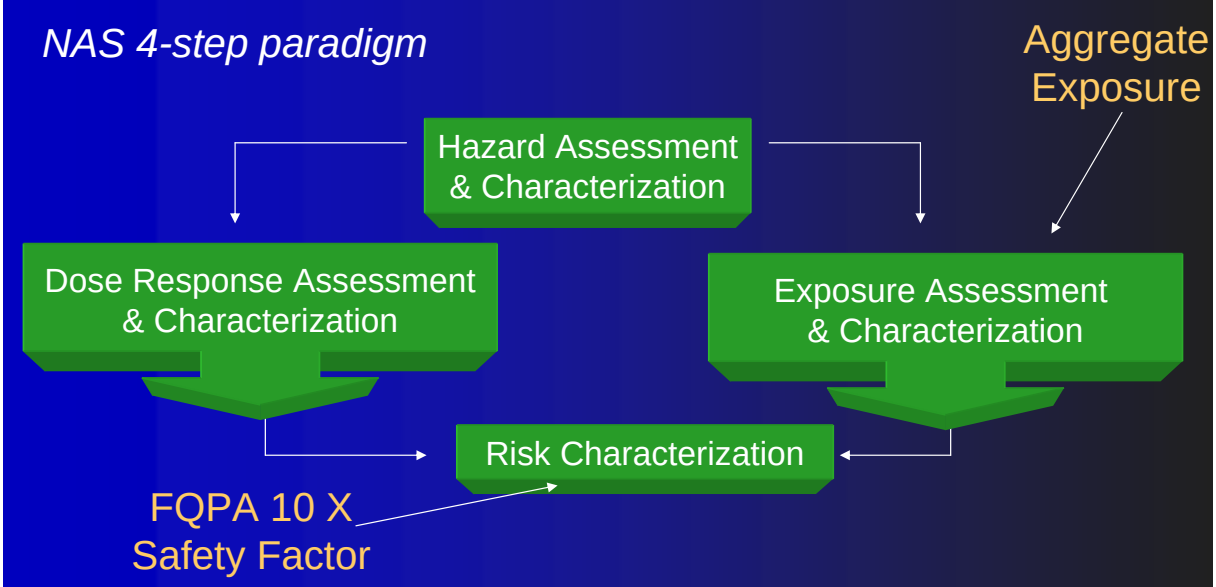
Background

Office of Pesticide Programs

- 1996 FQPA requires a safety review of all pesticide tolerances by August 2006
- Organic arsenicals
  - Monomethylarsonic acid (MMA^V)
    - Not carcinogenic in rodent studies
  - Cacodylic acid or Dimethylarsinic acid (DMA^V)
    - Carcinogenic in rodents
    - Special paper on mode of carcinogenic action

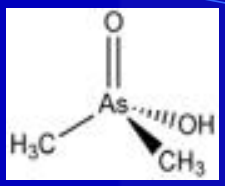
How Do We Assess Risk?

NAS 4-step paradigm



Risk Characterization

- Describes confidence in the assessment
  - <u>Transparency & Clarity</u>
    - Assumptions & uncertainties & their impact
  - <u>Reasonableness</u>
    - Sound conclusions based on best available science using generally-accepted scientific principles & knowledge



Cacodylic Acid (DMA^V) Cancer Risk Assessment

- **1994: OPP's 1st Cancer Peer Review**
  - Linear low dose default extrapolation based on rat bladder tumors
- **2005: Mode of Action Analysis**
  - Numerous studies pertinent to mode of action
  - Mode of action supports nonlinearity

Cacodylic Acid (DMA^V) 2005 Cancer Risk Assessment

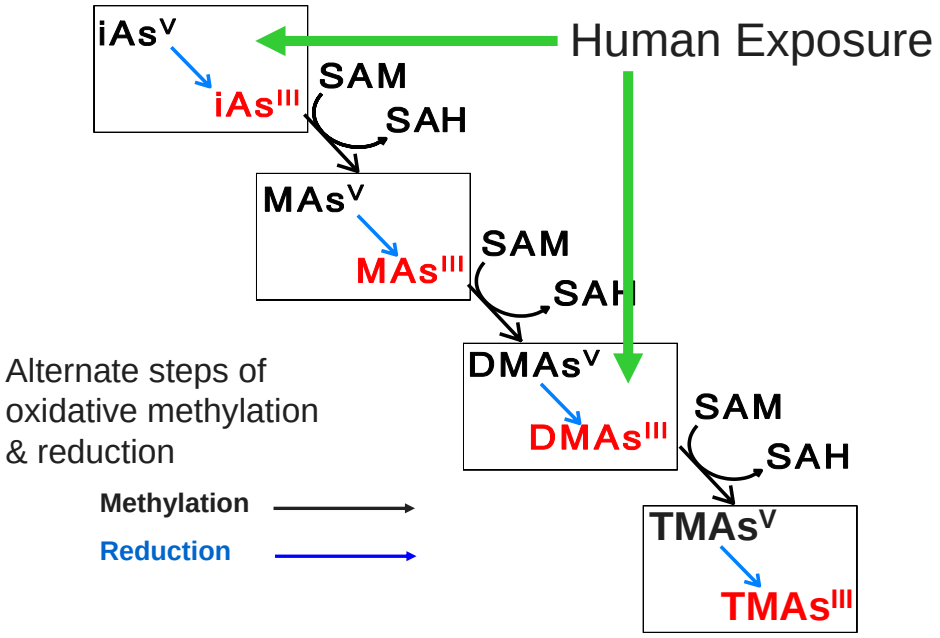
- Issue 1 Dataset for estimating cancer risk associated with exposure to cacodylic acid
- Issue 2 Mode of action underlying rodent tumor response & relevance to humans
- Issue 3 Dose response extrapolation approach

Issue 1: Dataset for Estimating Cancer Risk

- To what extent, if any, does the human epidemiology on inorganic As inform the cacodylic acid cancer risk assessment?

- Data considered
  - *In vitro & in vivo* metabolism studies
  - *In vitro & in vivo* toxicity studies

Metabolism of Arsenic



Issue 1: Dataset for Estimating Cancer Risk

- Recommendation/Conclusions for Cacodylic Acid
  - Use chemical specific data
    - Toxic & carcinogenic properties of exogenous inorganic arsenic, MMA^V, & DMA^V are distinct
      - For exogenous DMA^V--poor cellular uptake, not demethylated, fewer reactive metabolites
  - No epidemiology available on cacodylic acid
    - comprehensive data set that describes cancer development is available in the rodent (rat bladder tumors)

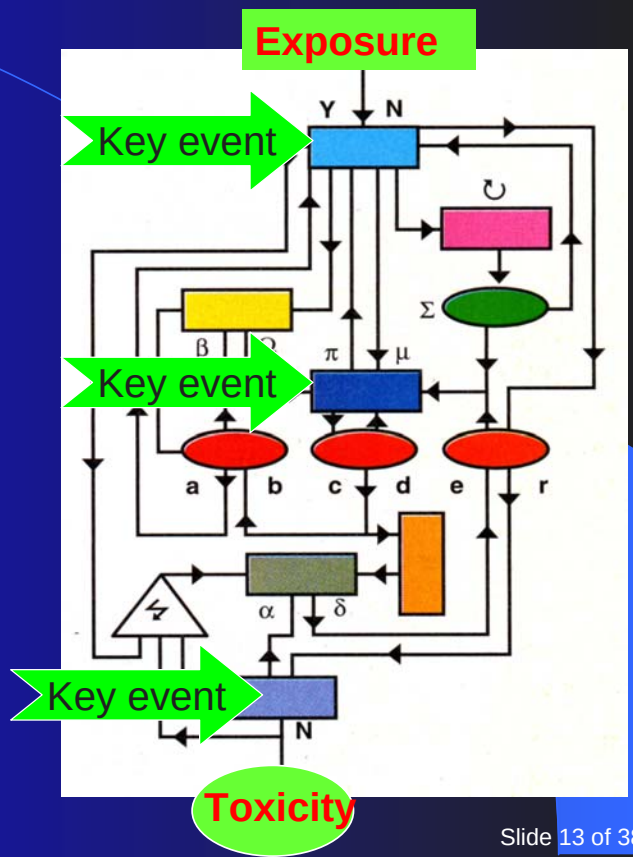
Issue 2: Mode of Action & Relevance to Humans

- Is there sufficient information to establish DMA^V's mode of action?
- Data considered
  - Laboratory animal data & *in vitro* studies on $DMA^{V/III}$
  - EPA's 2005 Cancer Guidelines--Mode of Action Framework Analysis

"<u>Mechanism</u> of action"
(more detailed understanding at biochemical & molecular level)

versus

"<u>Mode</u> of action"
(identification of **key & obligatory** steps)



Cacodylic Acid: Proposed Mode of Action

MultiStep Process of Carcinogenesis

Cacodylic Acid: Key Events Temporal Relationship

DMA^{III} ← DMA^{V}

6 hours	Urothelial Cytotoxicity
1 Week	Regenerative Proliferation
8-10 weeks	Hyperplasia
104 weeks	Tumors

Association of Key Precursor Events & Bladder Tumors in F344 Rats

Temporal →

Dose Response Concordance ↓

Dose (mg/kg bw/day)	Metabolism DMA^{V}→DMA^{III}	Urothelial Toxicity	Regenerative Proliferation	Urothelial Hyperplasia	Transitional Cell Carcinoma
0.2 (2 ppm)	+ (wk 3-0.03 ± 0.01 uM)	+ (wk 10-6/10, grade 3 or 4)	-	-	-
1 (10 ppm)	+ (wk 3-0.12 ± 0.02 uM)	+ (wk 3-2/7, grade 3) (wk- 10; 8/10, grade 3 or 4)	**slight** (wk 10-1.5X inc)	-	-
4 (40 ppm)	+ (wk 3-0.28 ± 0.09 uM)	+ (wk 3-7/7, grade 3) (wk 10-5/10, grade 3 or 4)	+ (wk 10-4.3X inc)	+ (wk 10- 4/10)	-
9.4 (100 ppm)	+ (wk 3-0.55 ± 0.15 uM)	+ (6 hrs-6/7, grade 3) (24 hrs-4/7, grade 3 or 4) (wk 2 6/10, grade 5)(wk 10-0/10, grade 4 or 5)	+ (wk 1- 2.2X inc) (wk 2-3.9X inc) (wk 10-4.2X inc)	+ (wk 8-7/10) (wk 10-9/10)	+ (papilloma first obs at wk 107; carcinoma first obs at wk 87)

Cacodylic Acid: Key Events Cytotoxicity/Regenerative Proliferation

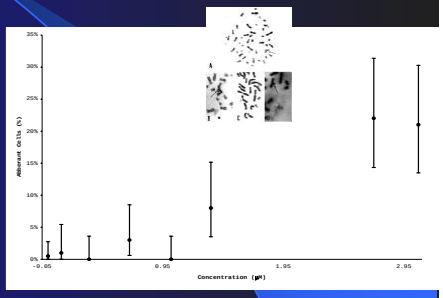
- Strength, Consistency & Specificity
  - Consistency of association found in repeated experiments within a lab & among different labs
  - Inhibition of DMA^{V} ➔DMA^{III} reduced cytotoxicity
  - Cessation of exposure to DMA^{V} results in recovery of tissue (i.e., hyperplasia)
- Biological Plausibility & Coherence
  - Regenerative proliferation associated with persistent toxicity appears to be a risk factor for bladder cancer in humans

Genotoxicity

In general: Consider

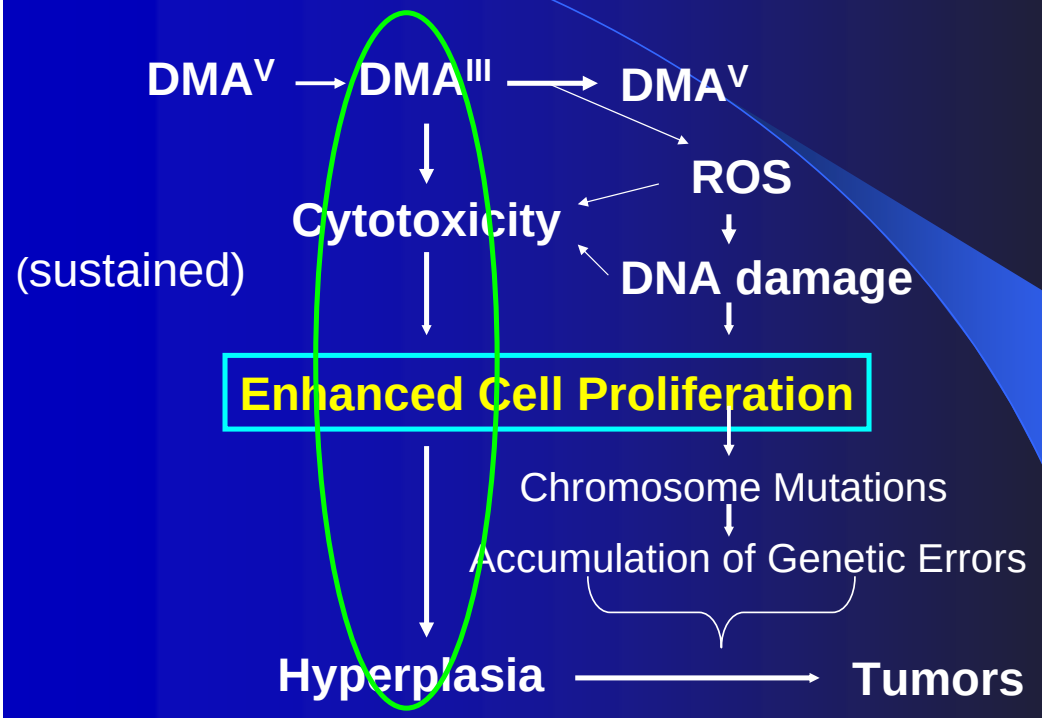
- Types of mutations
  - Point *versus* Chromosome
- Mechanism of genotoxicity
  - Direct interaction with DNA *versus* Indirect mechanisms

Characterization of Cacodylic Acid's Genotoxicity



- Neither DMA^{V} or DMA^{III} are direct acting point/gene mutagens
- Both are clastogenic but DMA^{III} is the more potent
  - *In vitro* data only
- DNA damage appears to result from an indirect mechanism (ROS/oxidative damage)
  - DMA^{III} → DMA^{V}

Cacodylic Acid: Key Events in Mode of Action



Mode of Action: Human Relevance

Are the Key events Common to Rats & Humans?

Dependent on a Sufficient Amount

DMA^{III} in Urine

Cell Injury & Death

Regenerative Proliferation

ROS/DNA Damage

Genetic Errors

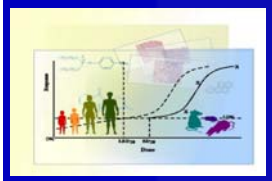
Issue 2 – Cacodylic Acid Mode of action underlying rat bladder tumor response & relevance to humans

Conclusions

- Sufficient evidence to establish a mode of action based on scientifically defensible key events
- Uncertainties remain, but they do not contradict the proposed MOA & conclusions
- No consistent scientific data do not support alternative pathways (i.e., other MOAs)
- Key events are plausible in humans

Issue 3- Dose response extrapolation approach

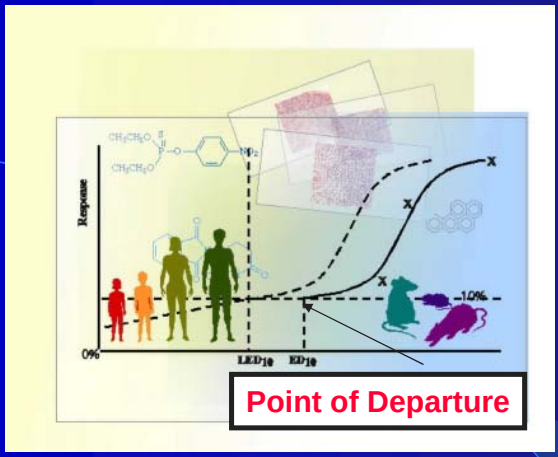
- How does the mode of action information inform the low dose response extrapolation?



Issue 3- Dose response extrapolation approach

Conclusions

- *Dose response extrapolation should be based on considerations of MOA which supports nonlinearity*
  - *Must be sufficient DMA^{III} to produce cell killing & sufficient cell killing to lead to regenerative proliferation*
  - *Cytotoxicity & enhanced proliferation need to be sustained*
  - *Frequency of chromosomal mutations dependent on enhanced proliferation & on generation of ROS (DMA^{III} ➔DMA^{V})*
- *Point of Departure based on cell proliferation should be protective of DMA's carcinogenic & promoting effects*



Cacodylic Acid: Dose Response Assessment

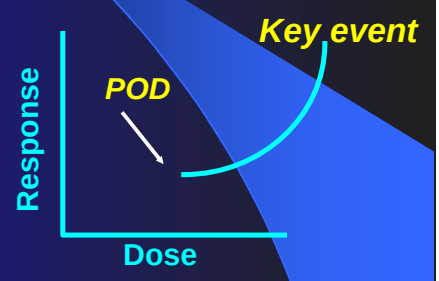
Dr. Anna Lowit
Office of Pesticide Programs

Dose Response Considerations

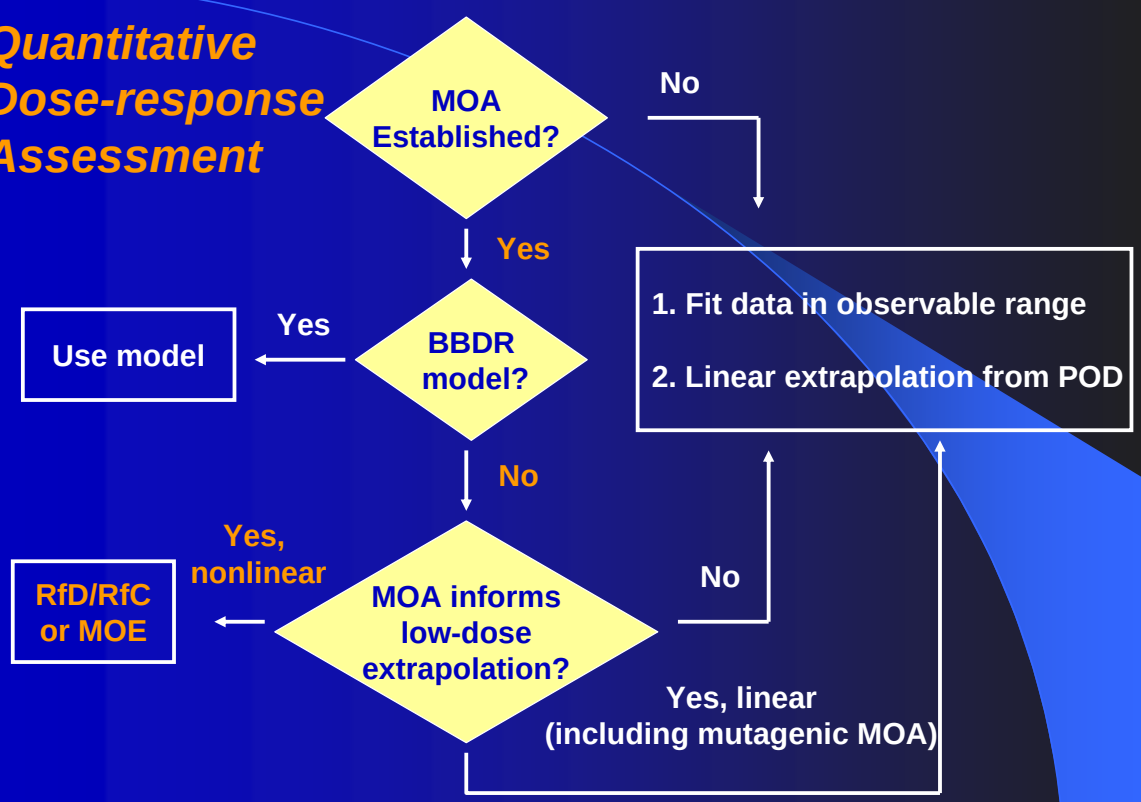
- Cancer Guidelines describe a two-step dose-response process which separates
  - Modeling the observable range of data
  - Extrapolation to lower doses
- *Risk = Hazard x Exposure*
  - Exposure assessment is on-going
  - Aggregate risk assessment
    - Multi-pathway

Dose Response Considerations

- Nonlinear extrapolation
  - Preferred approaches
    - PBPK Model--internal dosimetry at the target tissue
      - e.g. DMA^{III}
    - BBDR Model—predict biological effect
      - e.g., two stage clonal growth
  - Interim approach
    - Identify a point of departure (POD) based on benchmark dose modeling
    - Apply uncertainty and safety factors



Quantitative Dose-response Assessment



Point of Departure Considerations

Cytotoxicity/ cell killing	Dose-response data from multiple time points
Enhanced proliferation	Dose-response data
Chromosomal mutations	*In vivo* data at target site not available
Hyperplasia	Dose-response data from multiple time points and studies
Bladder tumors	Dose-response data from multiple studies

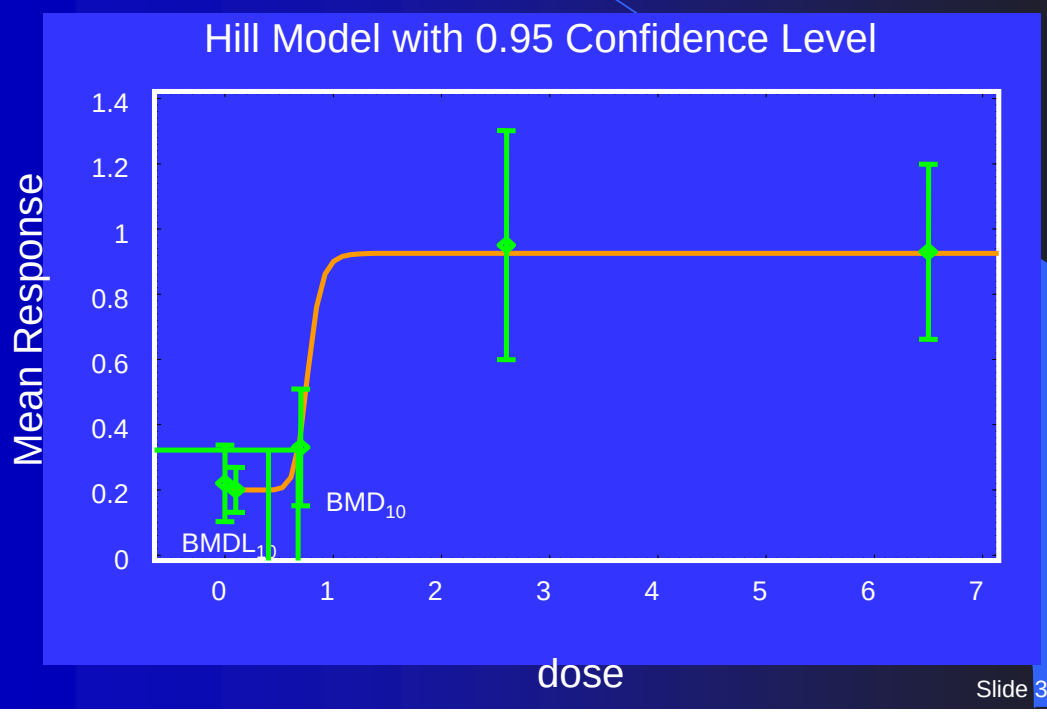
Point of Departure: Benchmark Dose Modeling

- Benchmark Responses Modeled
  - 1 & 10%
  - Other BMRs could be modeled (e.g. 5%)
- EPA's Benchmark Dose Modeling Software (BMDS)
  - Appendix D of DMA science issue paper

Benchmark Dose Modeling: Results from Feeding Studies

	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)
Urothelial cytotoxicity (3 weeks)	0.68	0.18
Regenerative proliferation (10 weeks)	0.65	0.29
Hyperplasia (10 weeks)	1.36	1.04
Tumors (104 weeks)	7.74	5.96

Benchmark Dose Modeling: Regenerative Proliferation



Point of Departure

- POD for cell proliferation adequately protective for carcinogenic & promoting activity
- POD based on BMDL
- Choice of benchmark response important

BMD_1	$BMDL_1$	BMD_{10}	$BMDL_{10}$
0.54 mg/kg/day	0.07 mg/kg/day	0.65 mg/kg/day	0.29 mg/kg/day

Uncertainty & Safety Factors

- When deriving RfDs from experimental animal data must consider uncertainty:
  - Interspecies – extrapolating animal data to humans
  - Intraspecies – variability among humans
  - OPP must consider FQPA safety factor for protection of children

Reference Dose (RfD)

$$\text{RfD} = \frac{\text{Point of Departure (e.g. BMDL)}}{\text{Uncertainty Factors}}$$

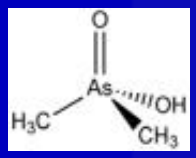
Risk = Hazard x Exposure

What will be included in OPP's risk assessment for cacodylic acid (DMA^V)?

- Aggregate risk assessment:
  - Multiple pathways of exposure to exogenous DMA^V and potential metabolites/degradates
    - e.g, food, water, lawn exposure
- Application of interspecies, intraspecies, and FQPA factors
- Statutory deadline of August, 2006 under the FQPA for tolerance reassessment

Dose Response Approaches Considered for Estimating Risk Associated with Direct Exposure to DMA^V

- Biologically &/or Physiologically Based Model
- Human Linear Cancer Slope Factor for inorganic As
- Linear Default Extrapolation for DMA^V rat data
- Nonlinear Default Extrapolation with appropriate uncertainty & safety factors for DMA^V rat data

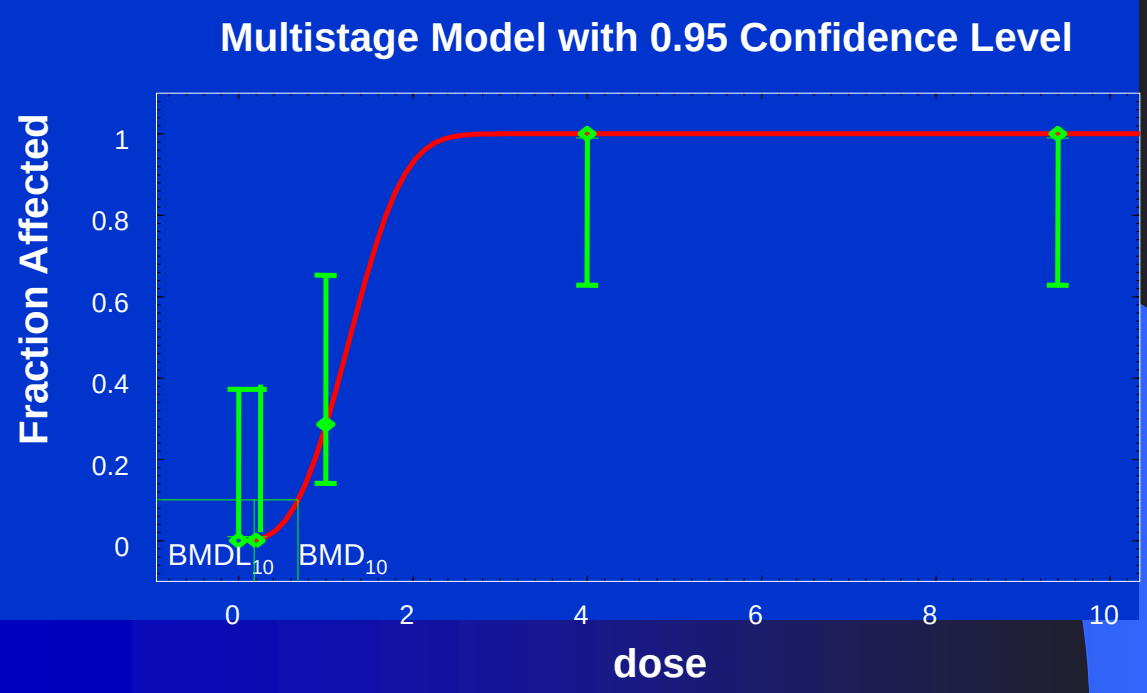


Summary and Conclusions: Cacodylic Acid (DMA^V)

- Rat is a relevant model for DMA^V and provides the most complete dataset for cancer
- Convincing MOA based on scientifically defensible key events, which support nonlinearity
  - RfD approach will include
    - POD based on enhanced cell proliferation
    - Uncertainty and safety factors
- Aggregate exposure assessment will address multiple pathways of exposure to exogenous DMA^V residues

Back pocket slides

Cacodylic Acid: Summary of benchmark dose estimates and lower 95% confidence limits for cytotoxicity, BrdU labeling index, hyperplasia and tumor data. (Doses in mg/kg/day)

Biological Event	Duration	Feeding				Duration	Drinking water			
		10%		1%			10%		1%	
		BMD (mg/kg/day)	BMDL (mg/kg/day)	BMD (mg/kg/day)	BMDL (mg/kg/day)		BMD (mg/kg/day)	BMDL (mg/kg/day)	BMD (mg/kg/day)	BMDL (mg/kg/day)
Tumor	104 weeks	7.74	5.96	6.80	2.22	104 weeks	1.92	1.21	0.88	0.14
Hyperplasia	10 weeks	1.36	1.04	0.42	0.32	104 weeks	1.63	1.04	0.74	0.14
	104 weeks	1.97	1.61	0.93	0.66					
BrdU labeling (proliferation)	10 weeks	0.65	0.29	0.54	0.07	Not determined. Available data not suitable for modeling.				
Cytotoxicity	3 weeks	0.68	0.18	0.31	0.02	No reliable dose-response data available				
	10 weeks	0.02	0.008	0.002	0.0007					

cytotoxicity