Using Empirical Data on Toxicity Pathways in the Prediction of Responses at Low Doses

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September 1, 2009

Nature of the Talk

- *Gedanken* experiment
- Goal is to explore statistical and toxicological concepts in low dose extrapolation
- Bring in concepts from NAS report Toxicity in 21 Century
- Suggest issues that toxicologists and statisticians should consider when talking to each other

The Great Divide

- Toxicologists have traditionally favored the concept of thresholds (the dose makes the poison)
  - Historical confidence that biology favors thresholds (after all we all are alive and are exposed to thousands of chemicals daily)
  - Thresholds should be the default assumption in risk assessment
- Animal data are a good marker for thresholds
  - Nothing is going on at doses 1-10 fold below the animal NOEL

The Great Divide

- Statisticians are suspicious of thresholds
  - Proving the existence of thresholds takes a lot of data (distinguishing between a low effect and “no” effect)
  - The value of data from animal studies is very dependent on the number of animals and noise-to-signal ratios
  - Animal tests have less statistical power then many toxicologists recognize
- Methodology used to deal with thresholds smacks more of policy then science (safety factors are statistically “icky”)

An Animal Study is Performed

- A toxicologist sees:
  - The apical effect at high doses
  - Precursors to the effect (but not the apical effect)
  - At the lowest dose sees no effect
- Conclusion: there is **no chance** of the apical effect occurring at low doses

An Animal Study is Performed

- The statistician is given the data on the occurrence of the apical effect
  - X animals affected @ high dose, 0 and mid dose, 0 at low dose, no background occurrence in controls
  - Fits data to multiple dose-response models, calculates lower limit to a robust benchmark dose
  - Asks toxicologist if they can rule out a non-threshold mechanism for the endpoint
  - If the answer is no, then use linear model
- Conclusion:
  - Low level risks will occur at low doses
  - Test enough animals and at any dose you will always find the apical effect

***You Did Visit the Same Country*, *Didn't You*?**

Ok, you probably caught what was going on

- Toxicologist and statisticians were not looking at the same data
- Toxicologists looks at precursor data and mentally did an assessment of contingent probability
  - No apical effect without the precursor effect
  - No precursor effect at lowest dose
  - Therefore at the lowest dose no chance of apical effect
- Statisticians only looked at data on occurrence of the apical effect (did not consider data on precursor effects)

Precursor Effects in Animal Studies

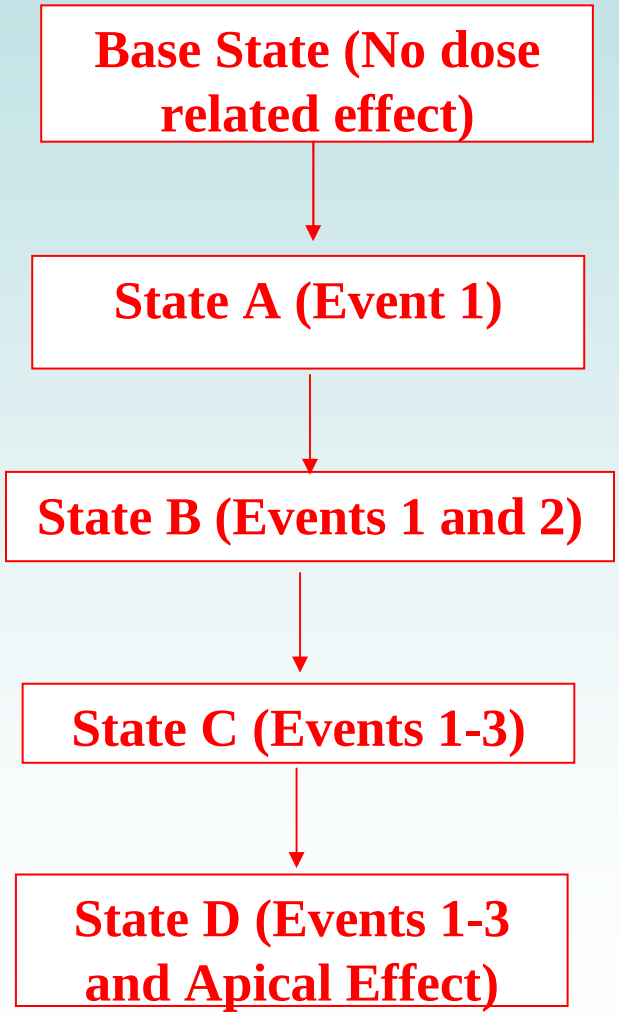
- Data on precursor effects are typically not discussed in regulatory toxicology and often not measured in animal studies
- Why?
  - They may not be measured (more endpoints in a study the more cost)
  - May not be required by GLP
  - If they are not used in regulation then why measure?
  - The regulated community is afraid to talk about precursor effects because they may be considered adverse (no upside)

Toxicity in the 21st Century

- All toxic effects begin with perturbation of biological systems that have key precursor events that can be measured
- Data for key events in the pathways can be collected and should be considered
- Statisticians can and should model multi-step processes

Gedanken Experiment

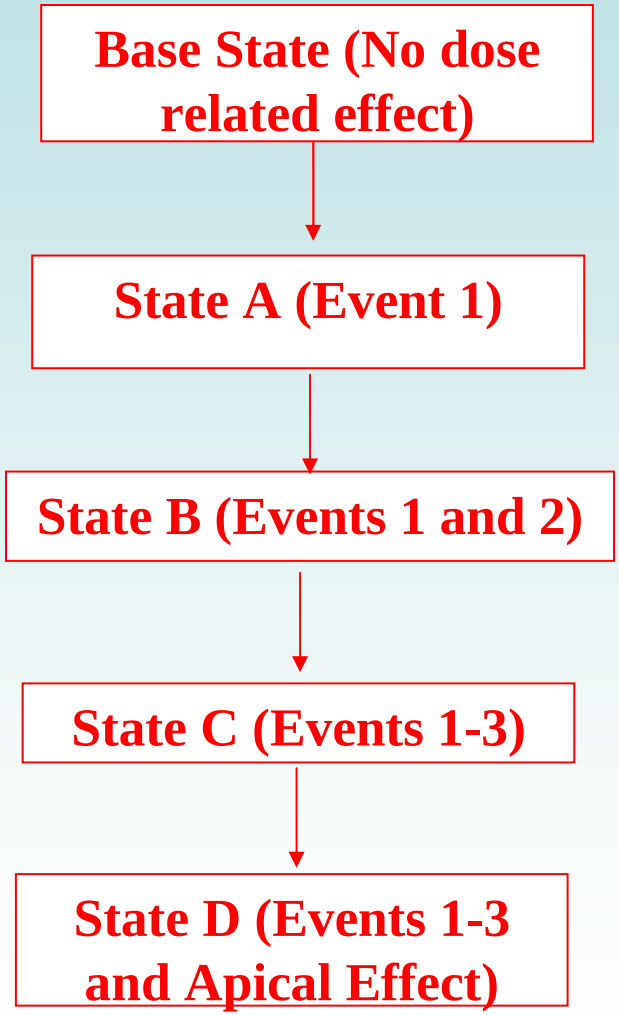
- A chemical has been tested
- Multiple dose groups
- Three key events measured on pathway to apical effect
- Animals are dosed
- All events and apical effect measured at the terminal sacrifice



Goal: Predict Low Dose Response in the Test Animals

Assumptions

- The occurrence of each event is contingent on the occurrence of prior effects.
- Each event is insufficient to cause subsequent effects.
- Each step in the pathway is dose dependent (these dose dependencies may or may not have thresholds).
- The events have background rates of zero.



Data

Number of Animals in Each State in Different Dose Groups						
Dose Group	Dose (d)	Base State	A	B	C	**D (Apical Effect)**
1	15	25	0	0	0	**0**
2	30	23	2	0	0	**0**
3	100	5	5	10	5	**0**
4	300	0	0	5	19	**1**
5	1000	0	0	0	10	**15**
6	3000	0	0	0	2	**23**

Three Options

Option 1: Only look at apical effect an assume threshold

- Detection limit on the apical effect based on sample size. For simplicity sake P_D is assumed to be ½ of the detection limit of 1 animal in 25 or:
  - $P_D = 0.5/25 = 0.02$
  - P_D is independent of dose.

Option 2: Only look at apical effect assume no threshold

- Assume linear response for all doses below the LOAEL. The slope of the line (S) is:

  $S = R/d_l$

  where d_l is dose at the LOAEL and R is the response at the LOAEL
- D is 300 and Response is 0.04, therefore:
- $S = 0.04/300 = 0.00013\ d^{-1}$
- $P_D = d*S$

Option 3: Consider data on apical and precursor effects

Dose Response Modeling
(10th Grade Probability Theory)

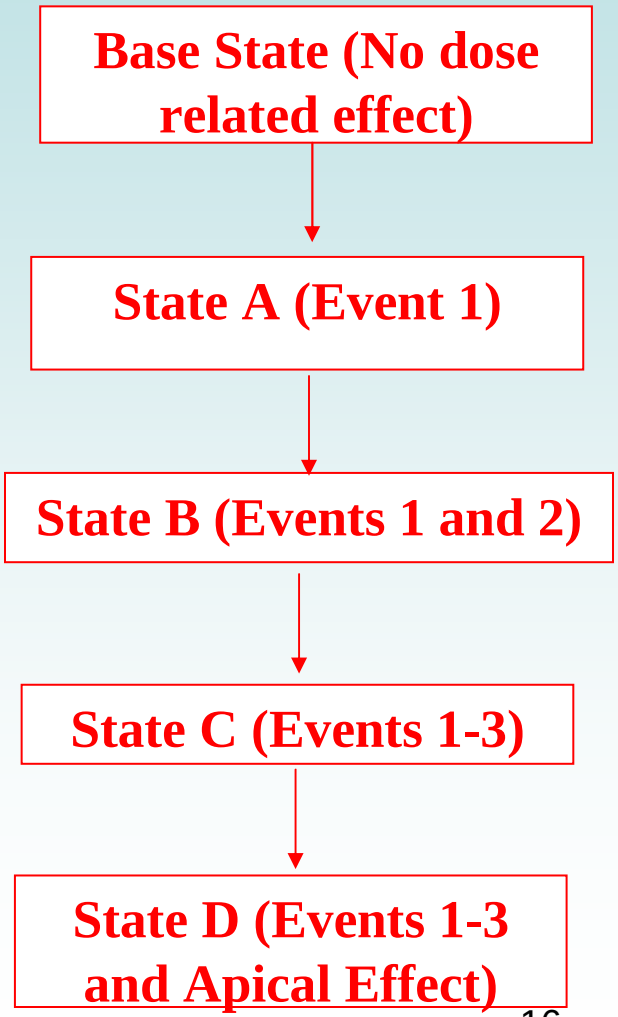
$$P_{Base\ State} = 1 - f_1(d)$$

$$P_A = f_1(d)*(1 - f_2(d))$$

$$P_B = f_1(d)* f_2(d)*(1 - f_3(d))$$

$$P_C = f_1(d)* f_2(d)* f_3(d)*(1 - f_4(d))$$

$$P_D = f_1(d)* f_2(d)* f_3(d)*f_4(d)$$



Number of Animals in each State (or Prior State) in Different Dose Groups						
Dose group	**Dose (d)**	**Base State**	**A**	**B**	**C**	**D (Apical Effect)**
1	15	25	0	0	0	**0**
2	30	25	2	0	0	**0**
3	100	25	20	15	5	**0**
4	300	25	25	25	20	**1**
5	1000	25	25	25	25	**15**
6	3000	25	25	25	25	**23**

Fraction of Tested Animals in each State (P_A, P_B, P_C and, P_D) in Different Dose Groups						
Dose group	**Dose**	**Base State**	**P_A**	**P_B**	**P_C**	**P_D**
1	15	1	0	0	0	**0**
2	30	1	0.08	0	0	**0**
3	100	1	0.8	0.6	0.2	**0**
4	300	1	1	1	0.8	**0.04**
5	1000	1	1	1	1	**0.6**
6	3000	1	1	1	1	**0.92**

Probability of Moving from One State to a Higher State						
Dose group	**Dose**	**$F_1(d)$**	**$F_2(d)$**	**$F_3(d)$**	**$F_4(d)$**	**Probability of Apical Effect *(=$F_1(d)$* $F_2(d)$* $F_3(d)$* $F_4(d)$)***
1	15	-	-	-	-	-
2	30	0.08	-	-	-	-
3	100	0.8	0.75	0.33	-	-
4	300	1	1	0.8	0.05	**0.04**
5	1000	1	1	1	0.6	**0.6**
6	3000	1	1	1	0.92	**0.92**

Low Dose Extrapolation for Key Event Dose Response

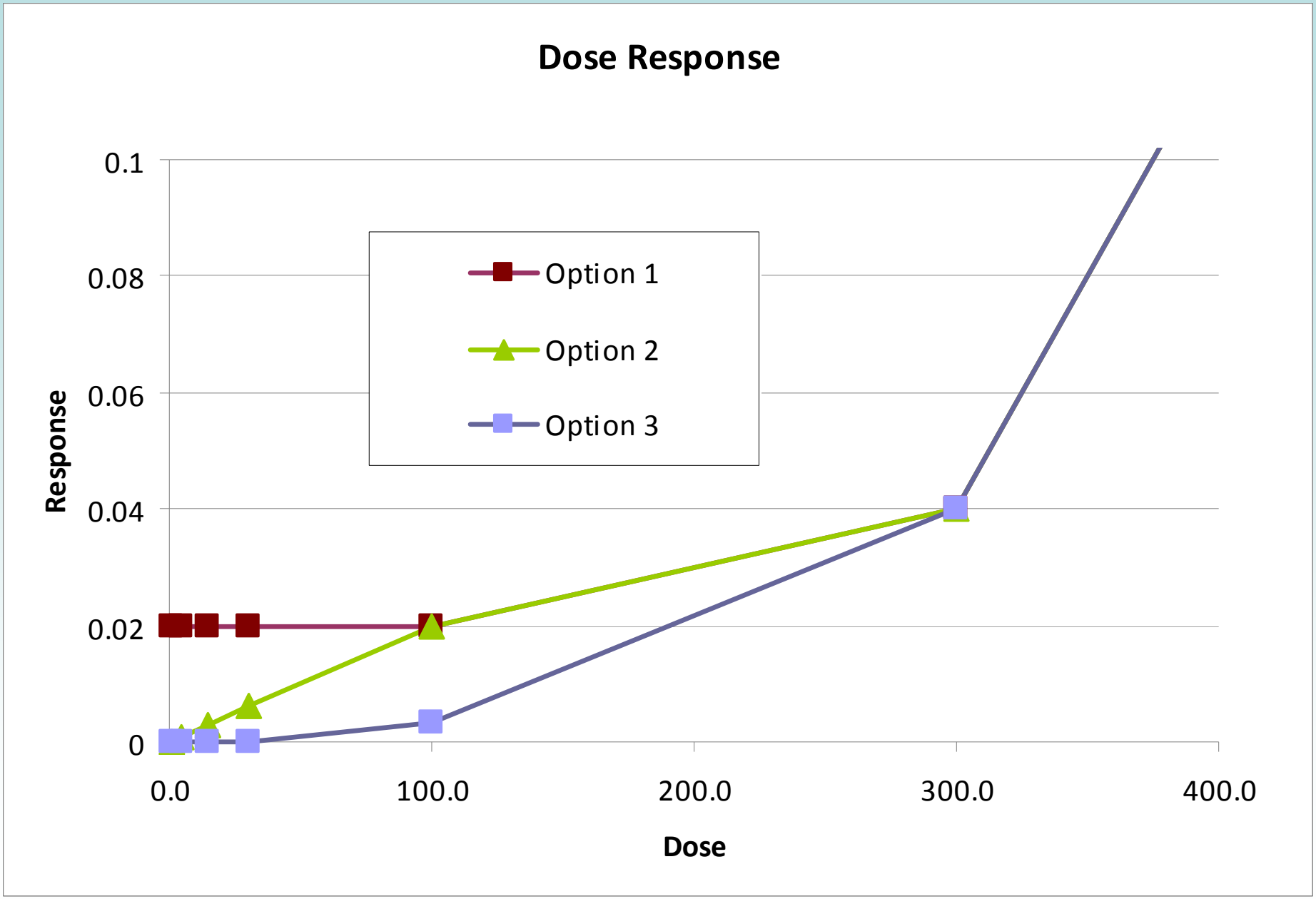
- Data are not available on the occurrence of apical and key events at doses below 300 and values of f_i can not be measured
- Lets assume no thresholds for the dose response for each step and estimate the response at lower doses by drawing a ling from the lowest dose producing the key event and zero

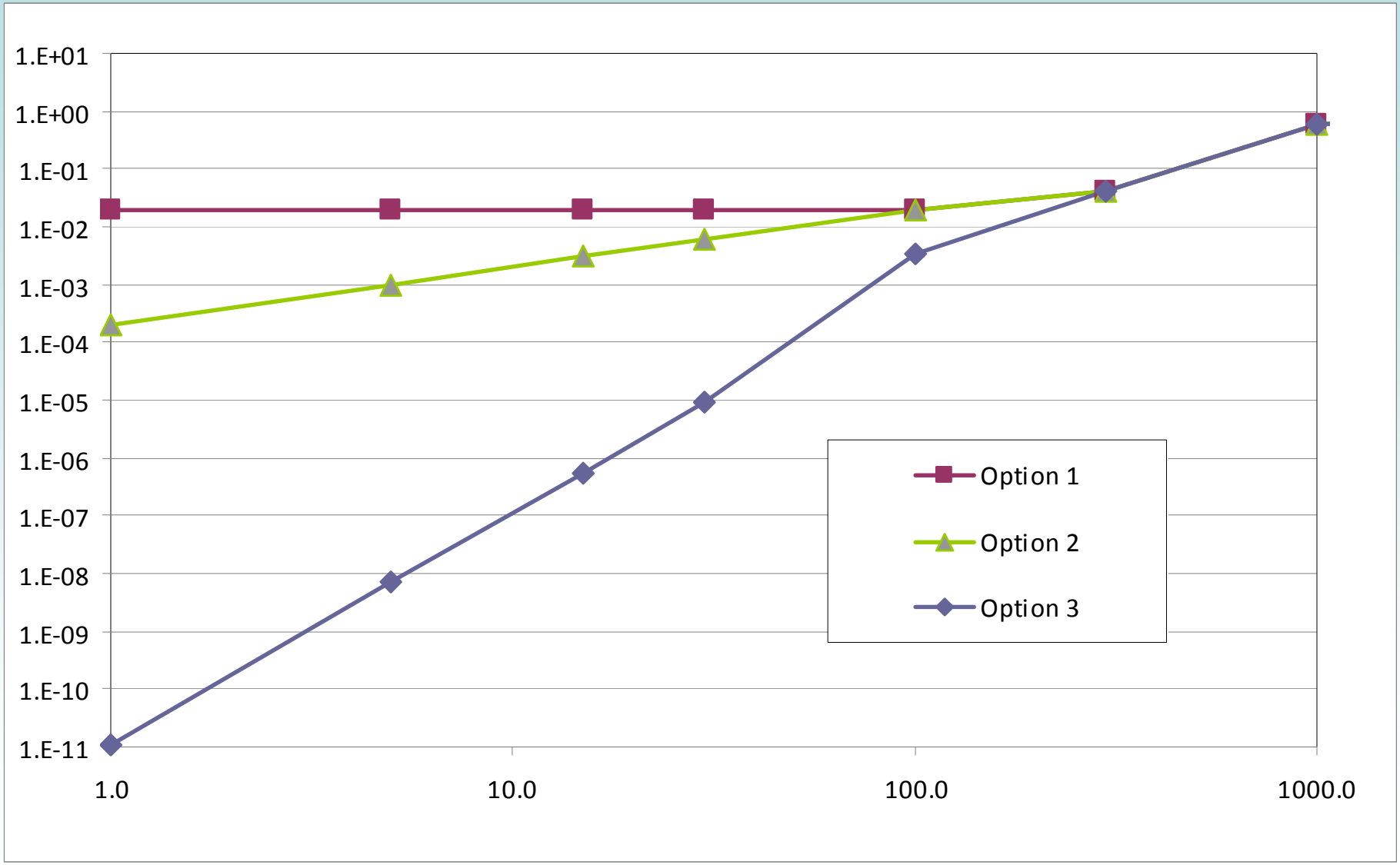
$$f_1(d) = d * S_1 = d * R_1 / d_1$$

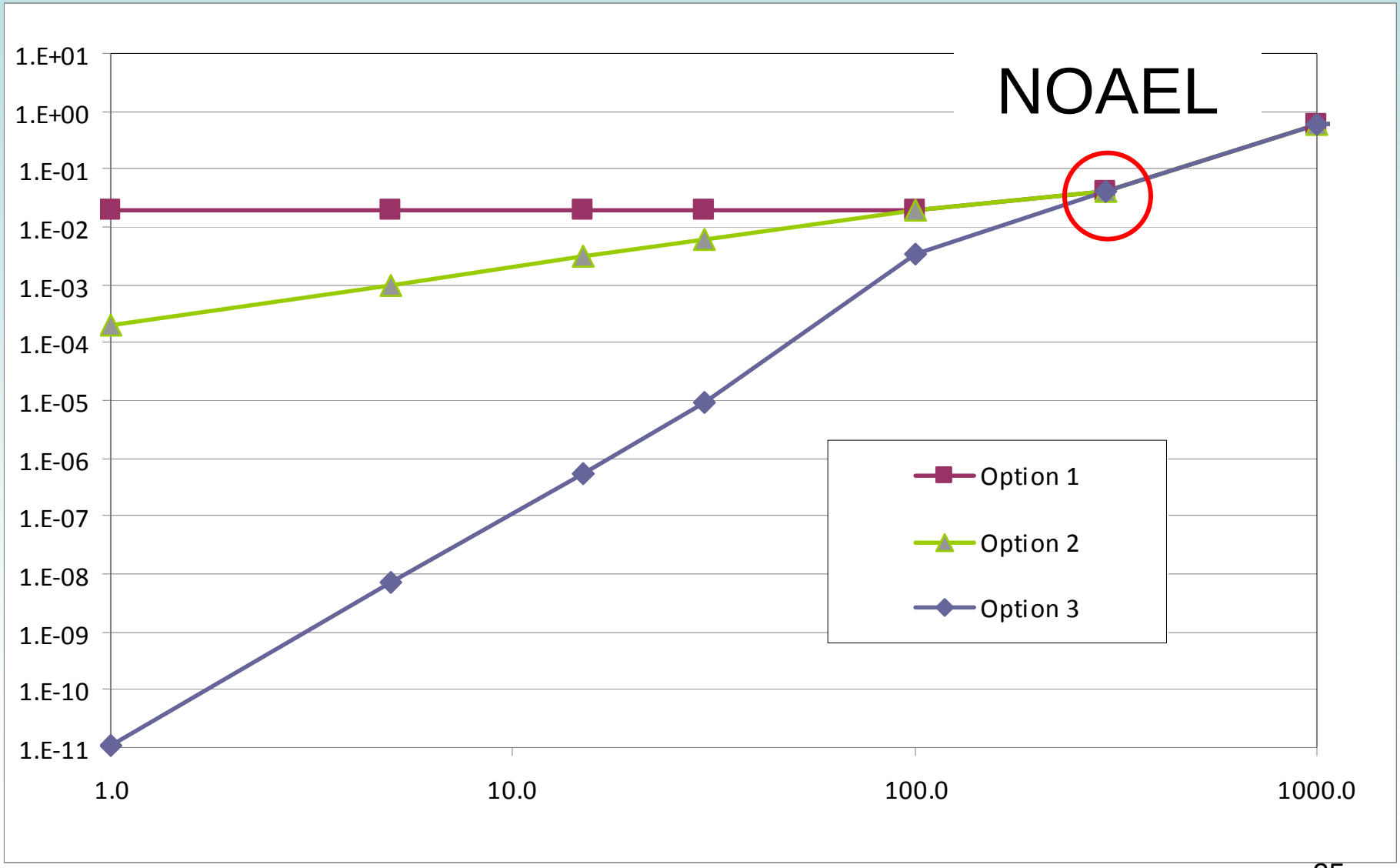
Probability of Moving from One State to a Higher State

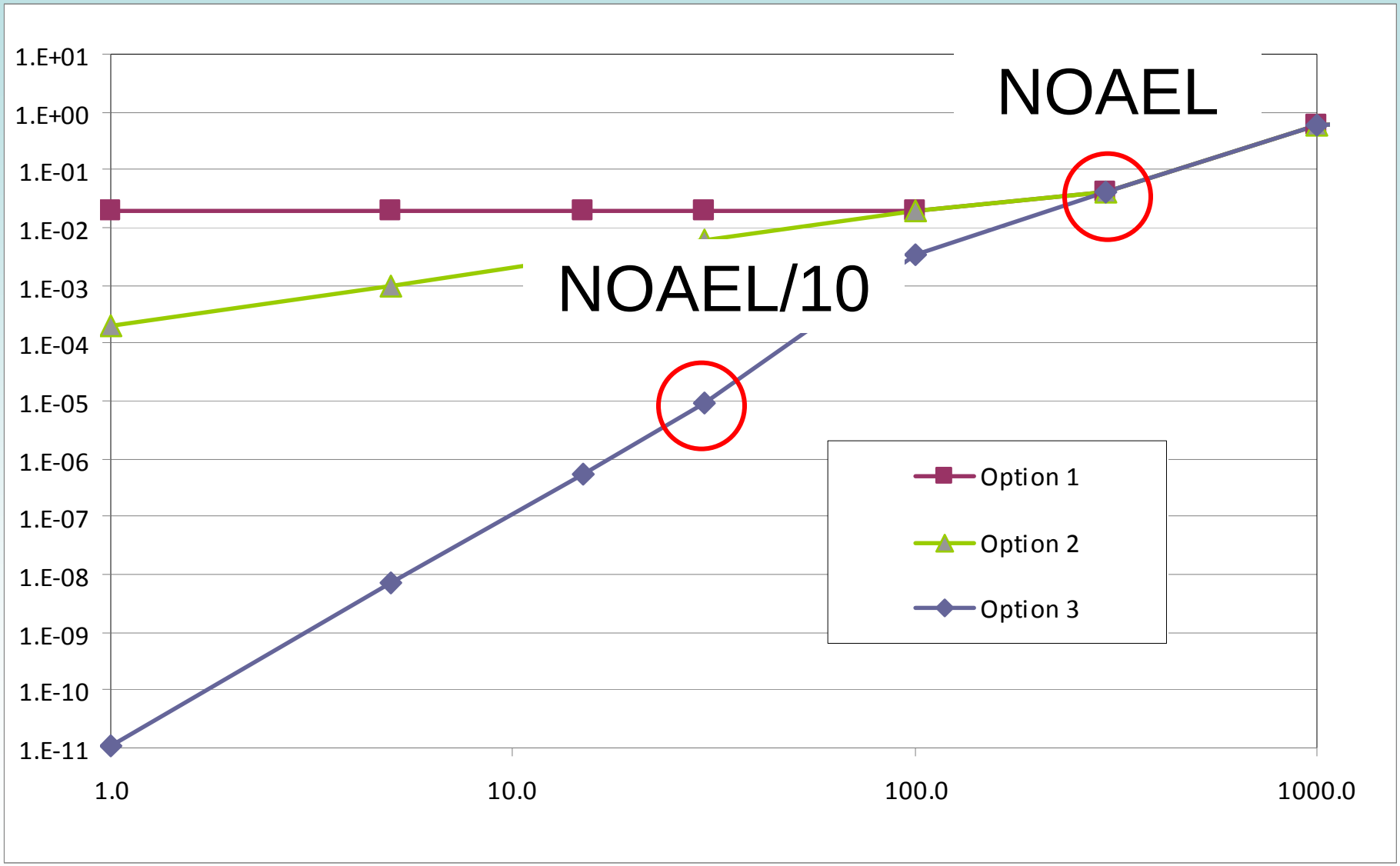
Dose group	Dose	$F_1(d)$	$F_2(d)$	$F_3(d)$	$F_4(d)$	**Probability of Apical Effect** ***(=$F_1(d)$* $F_2(d)$* $F_3(d)$* $F_4(d)$)***
1	15	-	-	-	-	-
2	30	0.08	-	-	-	-
3	100	0.8	0.75	0.33	=100/300* 0.05	-
4	300	1	1	0.8	0.05	**0.04**
5	1000	1	1	1	0.6	**0.6**
6	3000	1	1	1	0.92	**0.92**

Probability of Moving from One State to a Higher State						
Dose group	**Dose**	**$F_1(d)$**	**$F_2(d)$**	**$F_3(d)$**	**$F_4(d)$**	**Probability of Apical Effect *(=$F_1(d)$* $F_2(d)$* $F_3(d)$* $F_4(d)$)***
1	15	**0.04**	**0.1**	**0.05**	**.03**	**6 E-7**
2	30	0.08	**0.2**	**0.1**	**.005**	**9 E-06**
3	100	0.8	0.75	0.33	**0.17**	**0.003**
4	300	1	1	0.8	0.05	**0.04**
5	1000	1	1	1	0.6	**0.6**
6	3000	1	1	1	0.92	**0.92**


Dose Response
0.1
0.08
0.06
0.04
0.02
0
Response
Option 1
Option 2
Option 3
0.0
100.0
200.0
300.0
400.0
Dose


1.E+01
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
1.E-06
1.E-07
1.E-08
1.E-09
1.E-10
1.E-11
1.0
10.0
100.0
1000.0
Option 1
Option 2
Option 3


NOAEL
1.E+01
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
1.E-06
1.E-07
1.E-08
1.E-09
1.E-10
1.E-11
1.0
10.0
100.0
1000.0
Option 1
Option 2
Option 3


1.E+01
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
1.E-06
1.E-07
1.E-08
1.E-09
1.E-10
1.E-11
1.0
10.0
100.0
1000.0
NOAEL
NOAEL/10
Option 1
Option 2
Option 3

NOAEL/10 gives a 10^{-5} response Looks like a threshold!

Discussion

- Option 2 reflects the current policy of linear modeling
- Option 3 is a power model that results from the assumption that dose affects multiple steps in the pathway
- If:

  $P_D = f_1(d)* f_2(d)* f_3(d)*f_4(d)$

  and $f_1(d) = D * S_1$

- Then:

  $P_D = D*S_1* D*S_2* D*S_3* D*S_4$ or

  $P_D = D^4*S_1*S_2*S_3*S_4$

$R= D^4*K$ or $R= D^{(N+1)}*K$ where N is the number of key events

Hasn't this Been Done Before?

- Cancer multistage models always defaulted to linear models. Why is this different?
- The key issue is background
  - Cancer rates in animals are not zero this implies that there are some animals at each stage of the pathway
  - Any background level of a key event (B) would make the dose response of earlier events irrelevant
  - At low doses the risk of the apical effect reduces to:

$$P_D = B*D*S_4$$

R = KD

Conclusion

- Consideration of multiple steps in the dose response process leads to power functions not linear models at low doses
- Such power functions result in threshold like effects.
  - Dose 10X below the NOAEL can result in very low response rates $<10^{-5}$

Messages to Toxicologists

- Animal studies need to include:
  - Measurement of pre-adverse effects
  - Measures of internal dose and kinetics
- Arguments about mechanism of action need to address:
  - Background rates of key events
  - Dose response relationships need to be determined for both apical and key events

Message to Statistician

- Demand data on precursor effects
- Use the data to inform the estimates of low dose effects when the apical effect dose not occur at detectable levels
- A finding of a linear response in one step of a mechanism of action does not imply that the final low dose response will be linear

Thank you!