Simulation Studies to Complement Observational Data: What can we learn? How should they be used?

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Outline

1. Introduction
2. The Example: Low dose effects in Ames Data
3. Re organizing the data to examine response at low doses.
4. Evaluating the impact of re-organizing- selection
5. Comparison of response at low doses
6. Conclusions

Introduction

- Basic Scientific method:
  - observe
  - record, enumerate, organize
  - summarize and compare
  - develop possible theories and hypotheses

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  - record, enumerate, organize
  - summarize and compare
  - develop possible theories and hypotheses
  - observe

Introduction

- Basic Scientific method
- Statistical Theory and Methods (20th century)
  - speed up the process
  - begin with theories, hypotheses
  - relies on hypothesis testing, estimation
  - firmer conclusions and theories
  - emphasize experimental data
    - Stepchild: observational data

Introduction

- Basic Scientific method
- Statistical Theory and Methods (20th century)
- Observational Data -suspect!
  - observations less valuable than from an experiment
  - theories more suspect
  - danger in lessons learned

Introduction

- Basic Scientific method
- Statistical Theory and Methods (20th century)
- Observational Data -suspect!
- Using Observational Data
  - observations are the basis of the Scientific Method
  - can re organize data to develop theories
  - use simulations to assess impact on organization
  - assess theories

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  - observations are the basis of the Scientific Method
  - can re organize data to develop theories
  - use simulations to assess impact on organization
  - assess theories
  - new observations

Introduction

- Basic Scientific method
- Statistical Theory and Methods (20th century)
- Observational Data--suspect!
- Using Observational Data
- Example
  - Mutagencity studies with Salmonella typhimurium
    - Mortelmans et al (1986), Zeiger et al., (1987, 1988)
  - 825 chemicals; 5 Ames tester Strains; +/- Hepatic S9
  - Examine response at low doses

Basic Ames Data

- 825 Chemicals
- Tester Strains: TA97, TA98, TA100, TA1535 TA1537
  - TA97- tested in only 5 chemicals by Mortelmans
  - TA1535 and TA1537- very low background colony counts (6-18 colonies/plate)

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Basic Ames Data Collection

- 825 Chemicals
- Tester Strains: TA97, TA98, TA100
- Tested with & without S9 Fractions
  - Standard 5-dose (log spacing)
  - Control (water, DMSO)
- 3-plates/dose
- Replicated

Basic Ames Observations

- 825 Chemicals
- Tester Strains: TA98, TA100 (+ or – S9 Fraction)
- Standard 5-dose (log spacing) & control
- Average response and SEM if Reps agreed
- Final replication if Reps didn't agree

- Notes:
  - Not all reps exactly the same
  - Focus is on increase in revertant counts (above control)
  - Basic original data is not available

Example Data

CHEMICAL	STRAIN	Control	SEM	DOSE 1	DOSE2 3	DOSE 3	DOSE 4	DOSE 5
4-Amino-2-Nitrophenol	TA100	91	2.6	86	96	95	100	108
1-Chloro-2-Propanol	TA100	127	7.5	135	131	127	130	149
Cresyl Diphenyl Phosphate, Mixed Isomers	TA100	122	3.2	127	123	120	127	136
Dichloroisocyanuric Acid, NA Salt	TA100	157	5.8	150	144	162	162	177
Dimethylol dihydroxyethylene Urea	TA100	123	3.1	131	128	120	123	182
Mercuric Chloride	TA100	148	2.0	144	141	151	150	190
Mercuric Chloride	TA100	150	4.1	130	149	148	145	171
N-Methyl Diethanolamine	TA100	177	4.2	173	183	159	165	200
Ninhydrin	TA100	143	10.7	159	161	150	150	160
Ninhydrin	TA100	151	6.8	154	169	163	145	190

Figure 1. Example of Responses for 4-Amino-2-Nitrophenol Assay

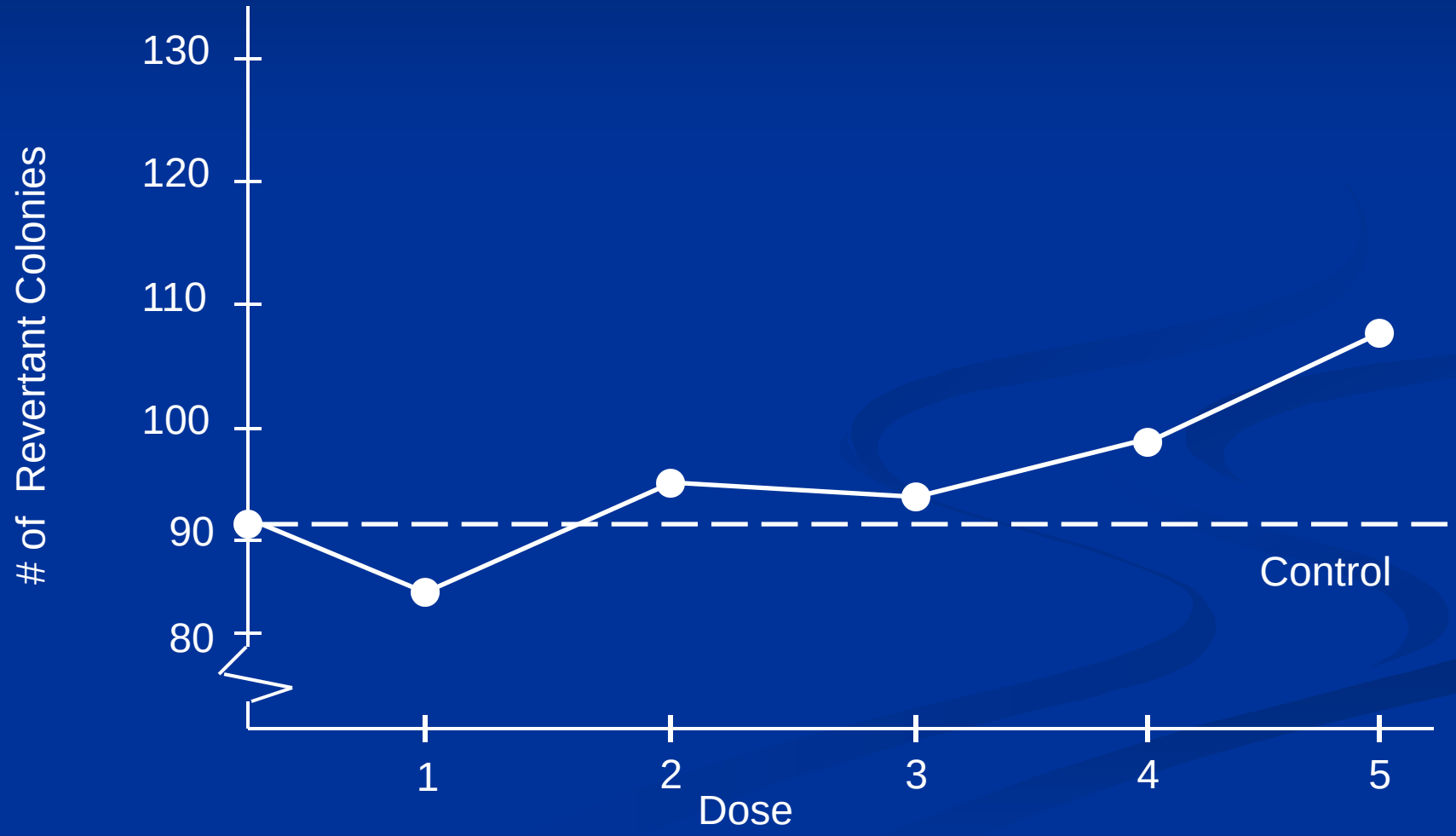
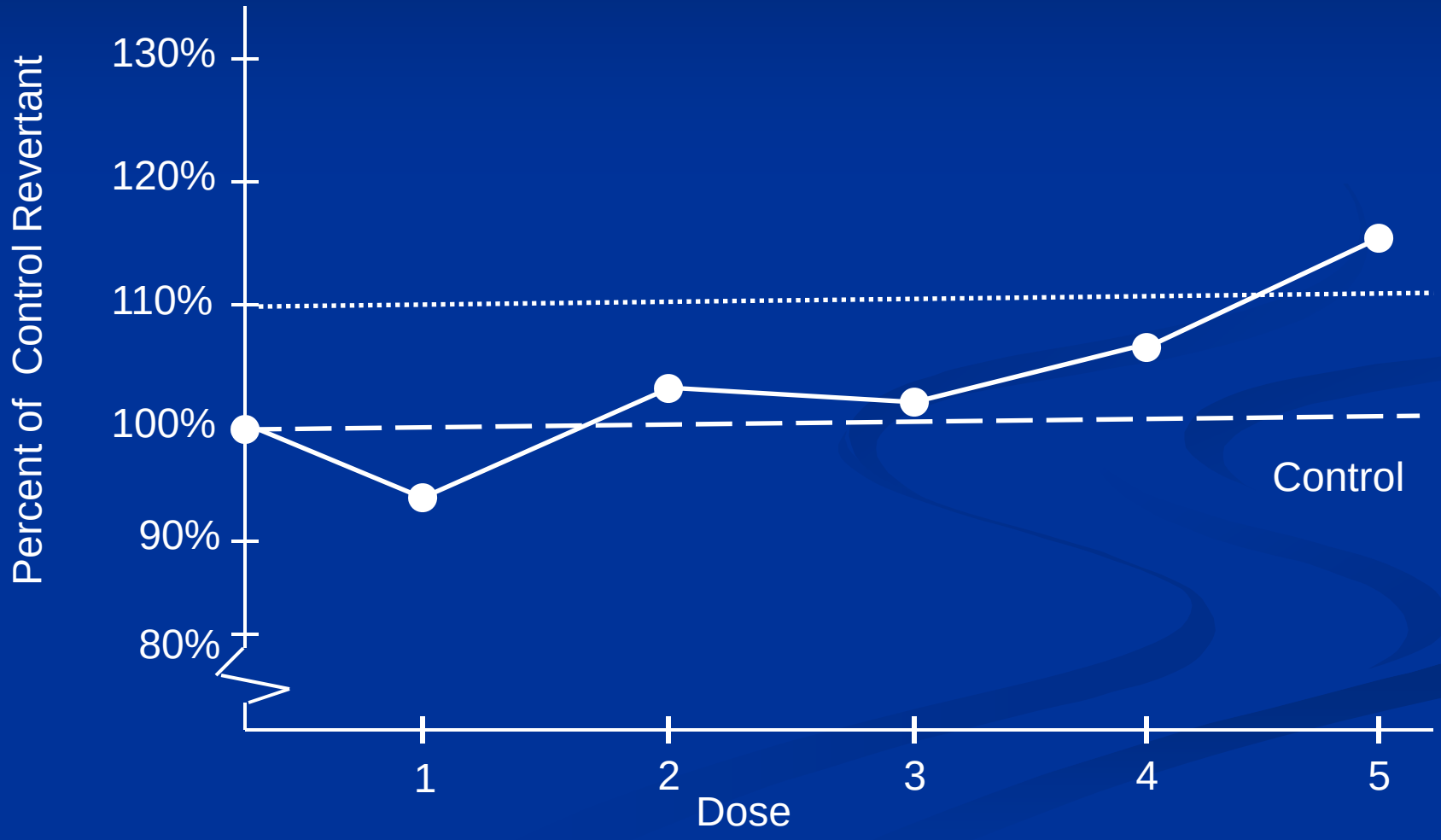


Figure 2. Example of Responses for 4-Amino-2-Nitrophenol Assay

Percent of Control



Objectives of Re-organization

- Evaluate response at low doses (as % control)
  - Pick low doses below 'response' range.
- See if data support "linearity" or "hormesis"

- Determine Eligible Assays for description
  - Evidence of 'high' revertant response
  - Evidence of lower dose response similar to control
  - Additional lower doses

Figure 3a. General Scheme Used to Select Assays

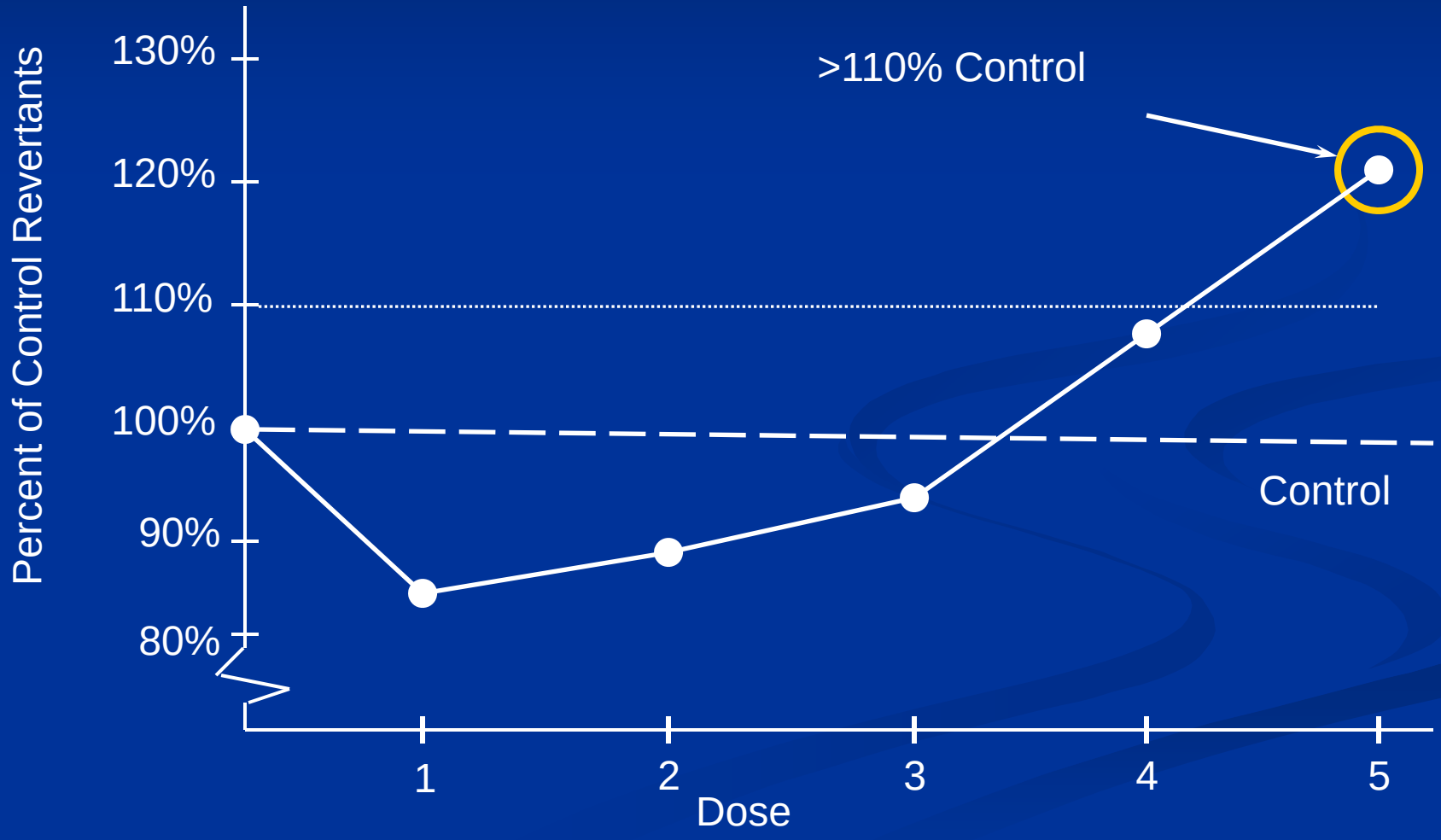


Figure 3b. General Scheme Used to Select Assays

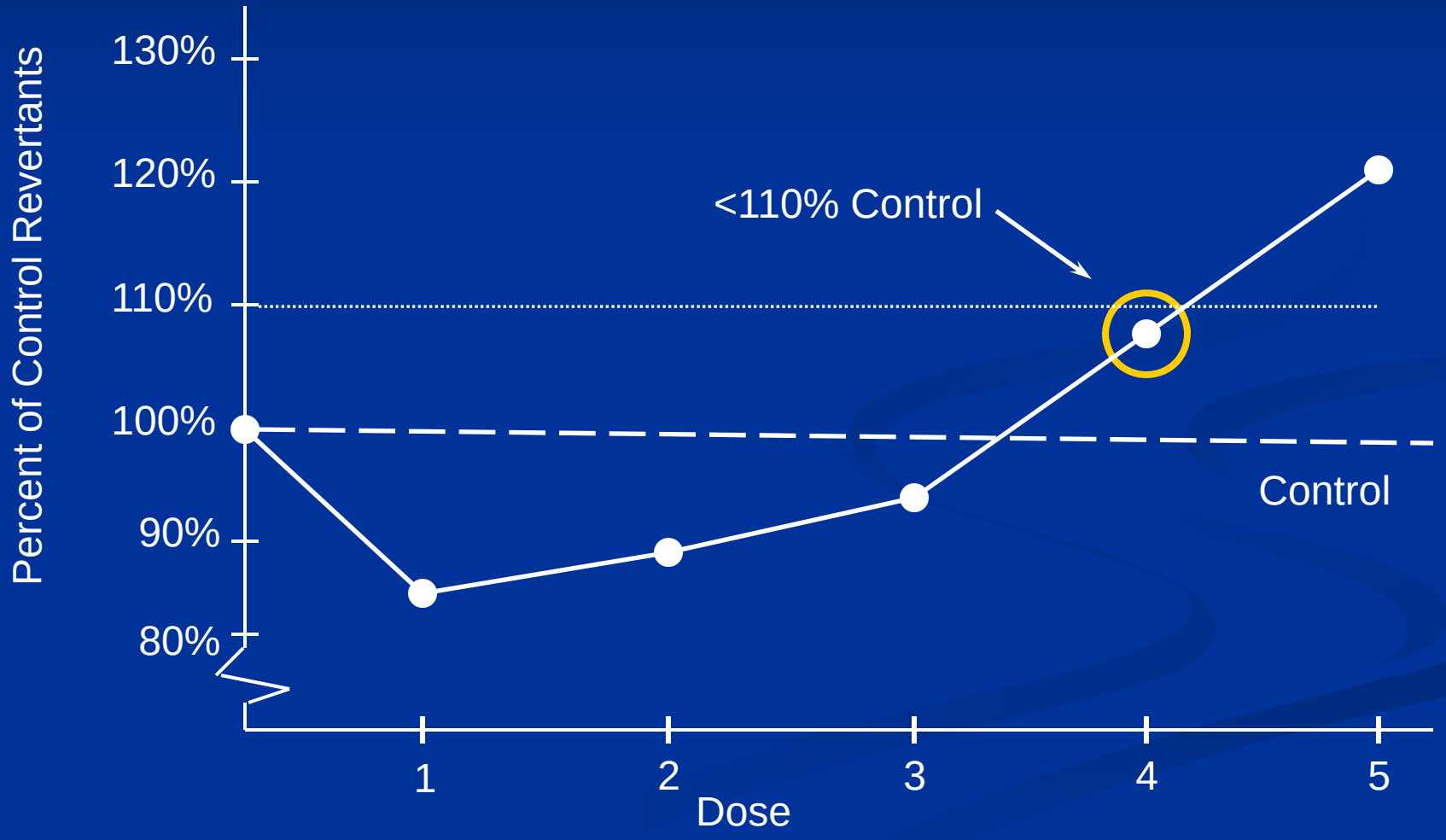


Figure 3c. General Scheme Used to Select Assays

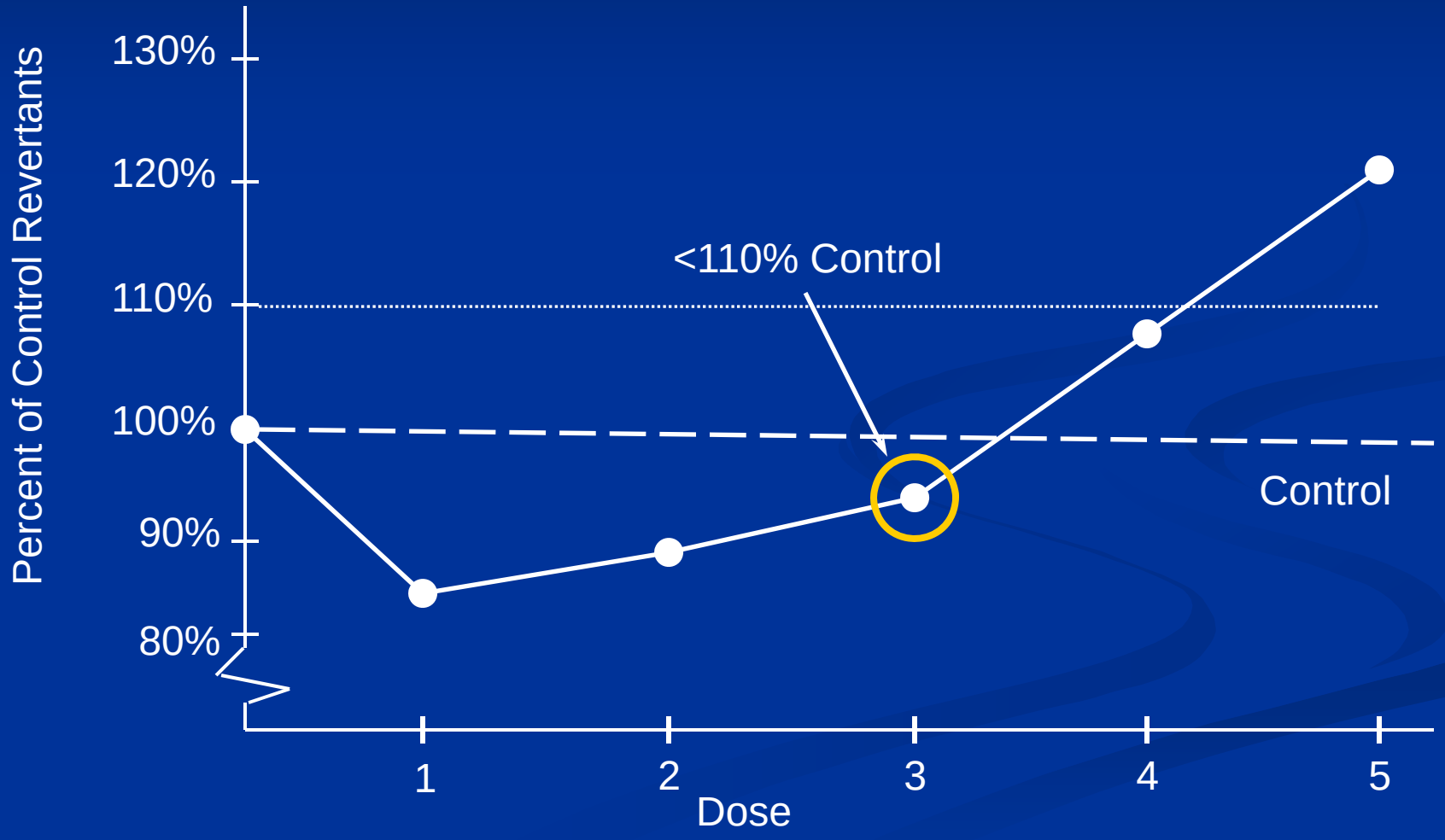


Figure 3d. General Scheme Used to Select Assays

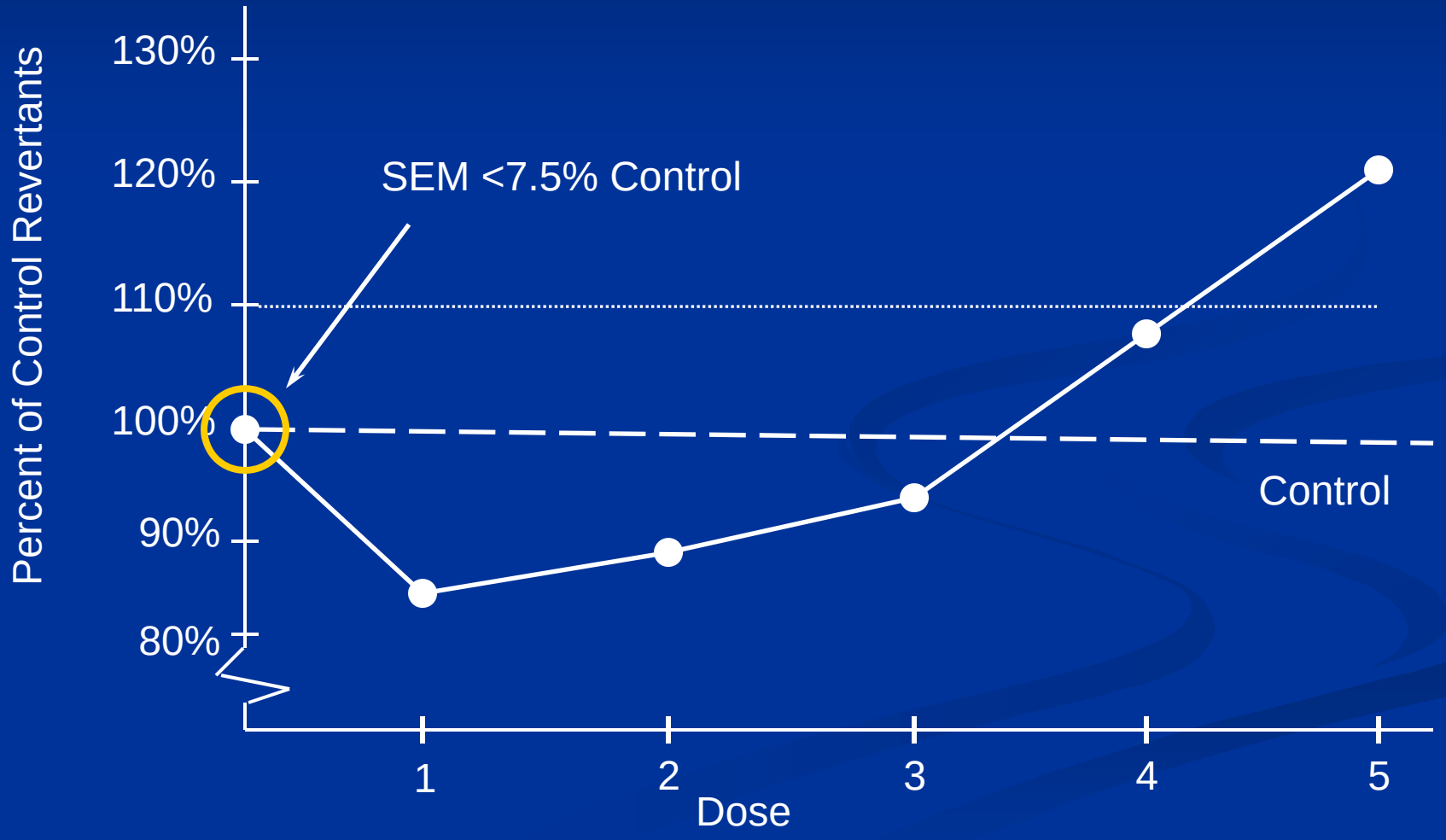
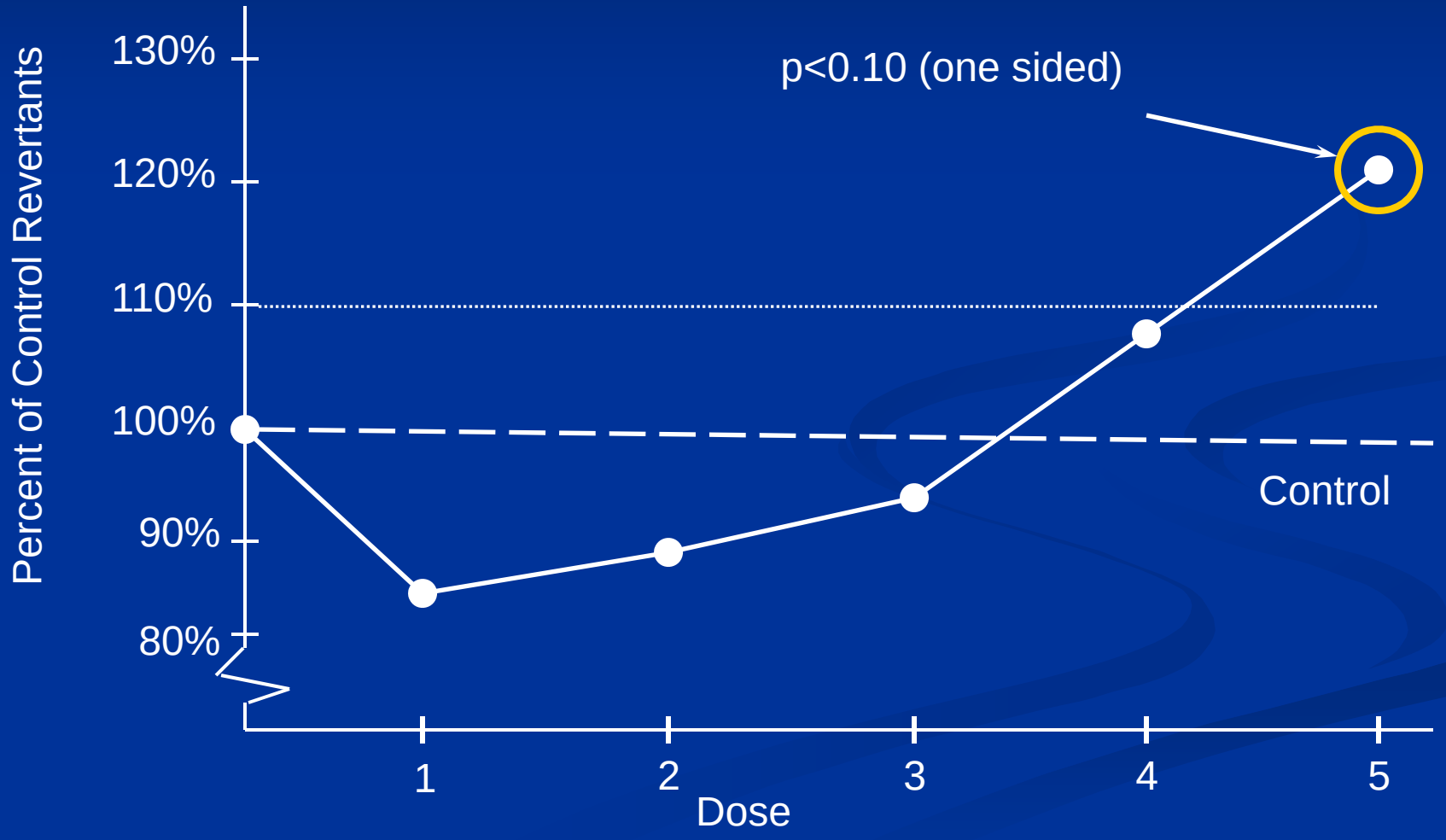


Figure 3e. General Scheme Used to Select Assays



START

Selection Process

~14,500 assays **included in the database:**

80% removed

~2900 **have a dose-5 response>110%**

66% removed

~942 **dose-4 response < 110%**

89% removed

107 **Assays that meet all criteria**

Evaluate response at low doses only in these assays

FINISH

on 95 Chemicals

START

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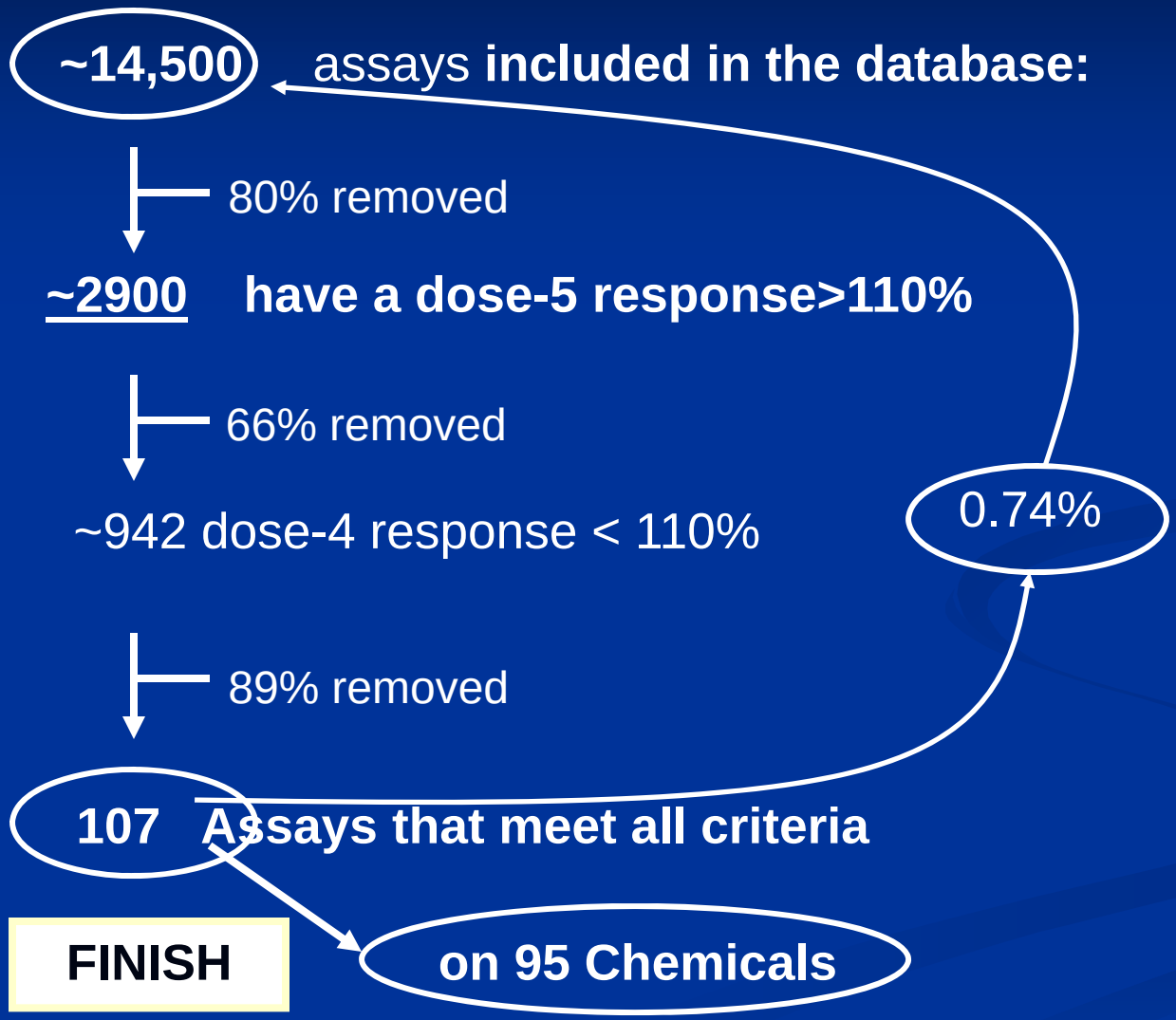

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107 **Assays that meet all criteria**

0.74%

Can this Process select assays that have lower response at low doses?

FINISH

on 95 Chemicals

Figure 3e. General Scheme Used to Select Assays

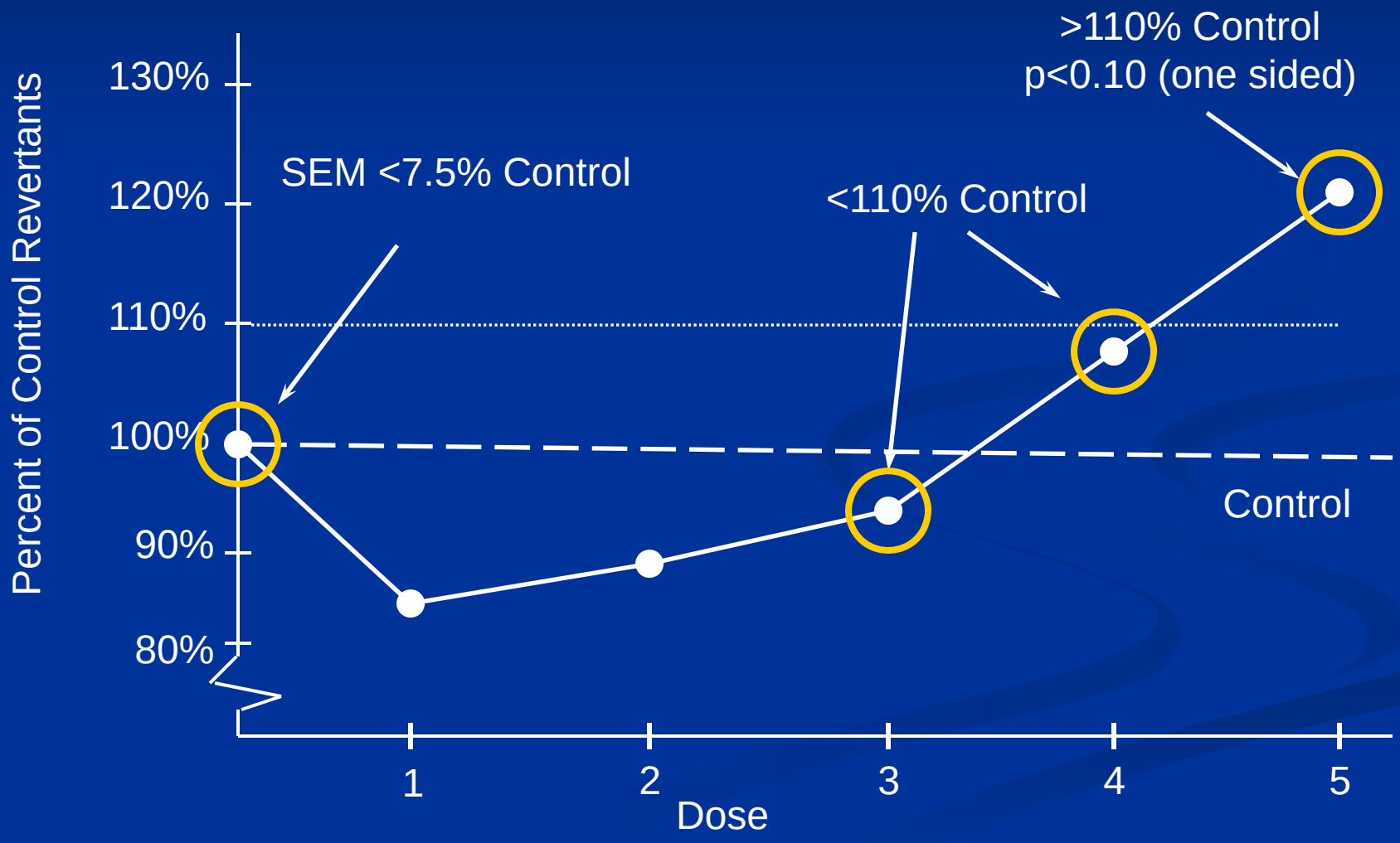


Figure 4. Focus of Analysis

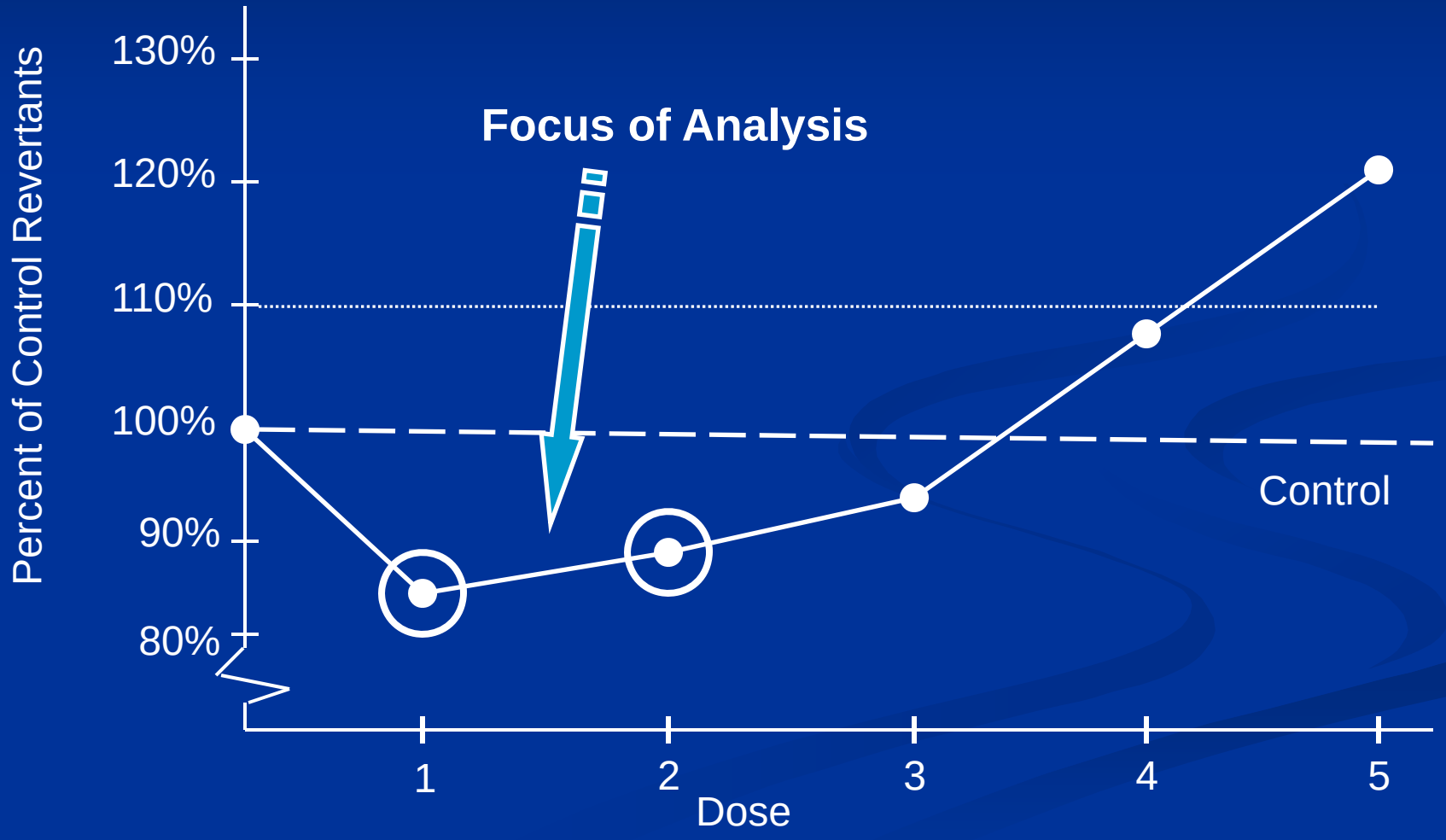


Figure 5a. Possible Selection? (Accurate Control Measure)

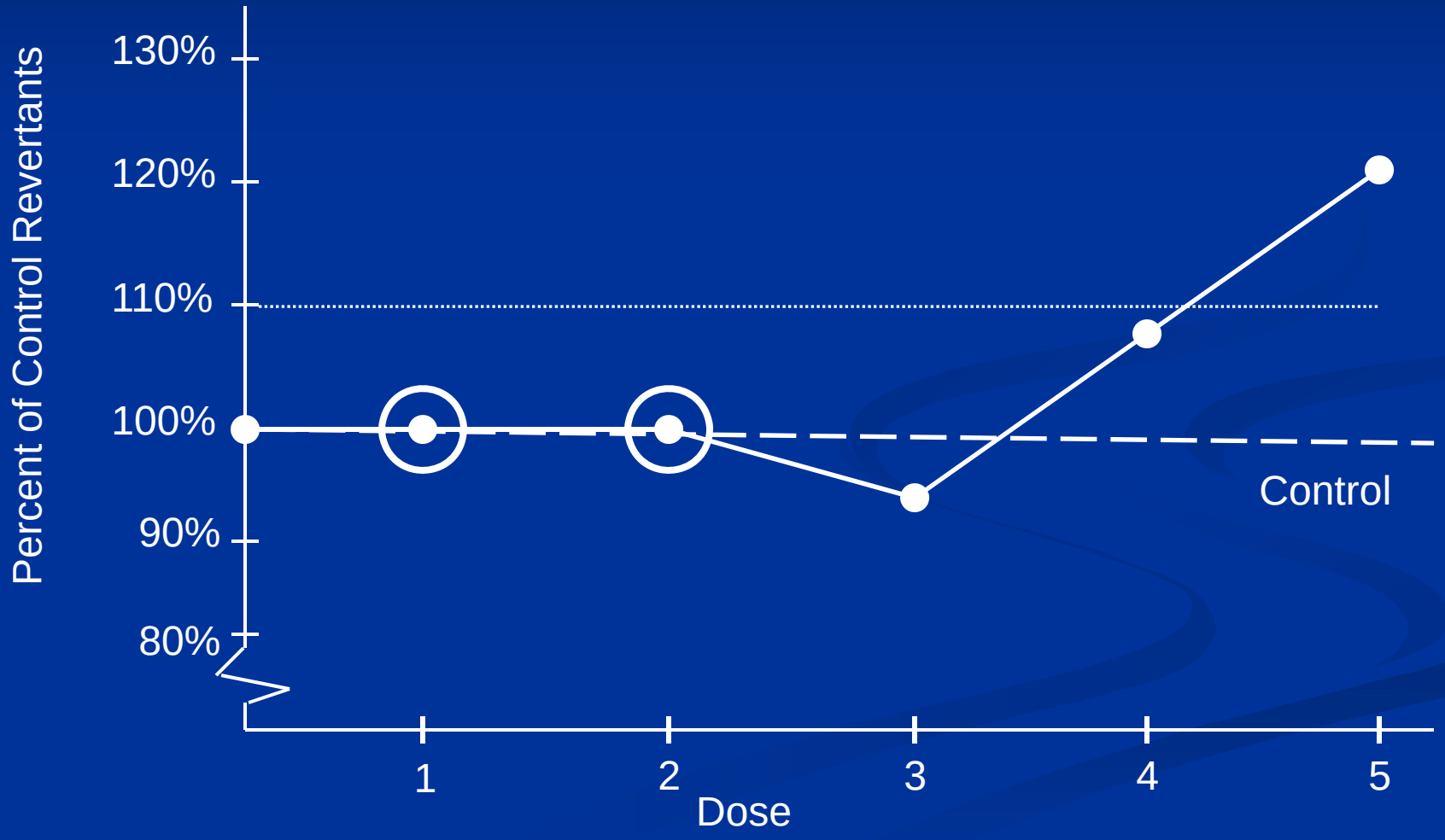


Figure 5a. Possible Selection? (Control too high)

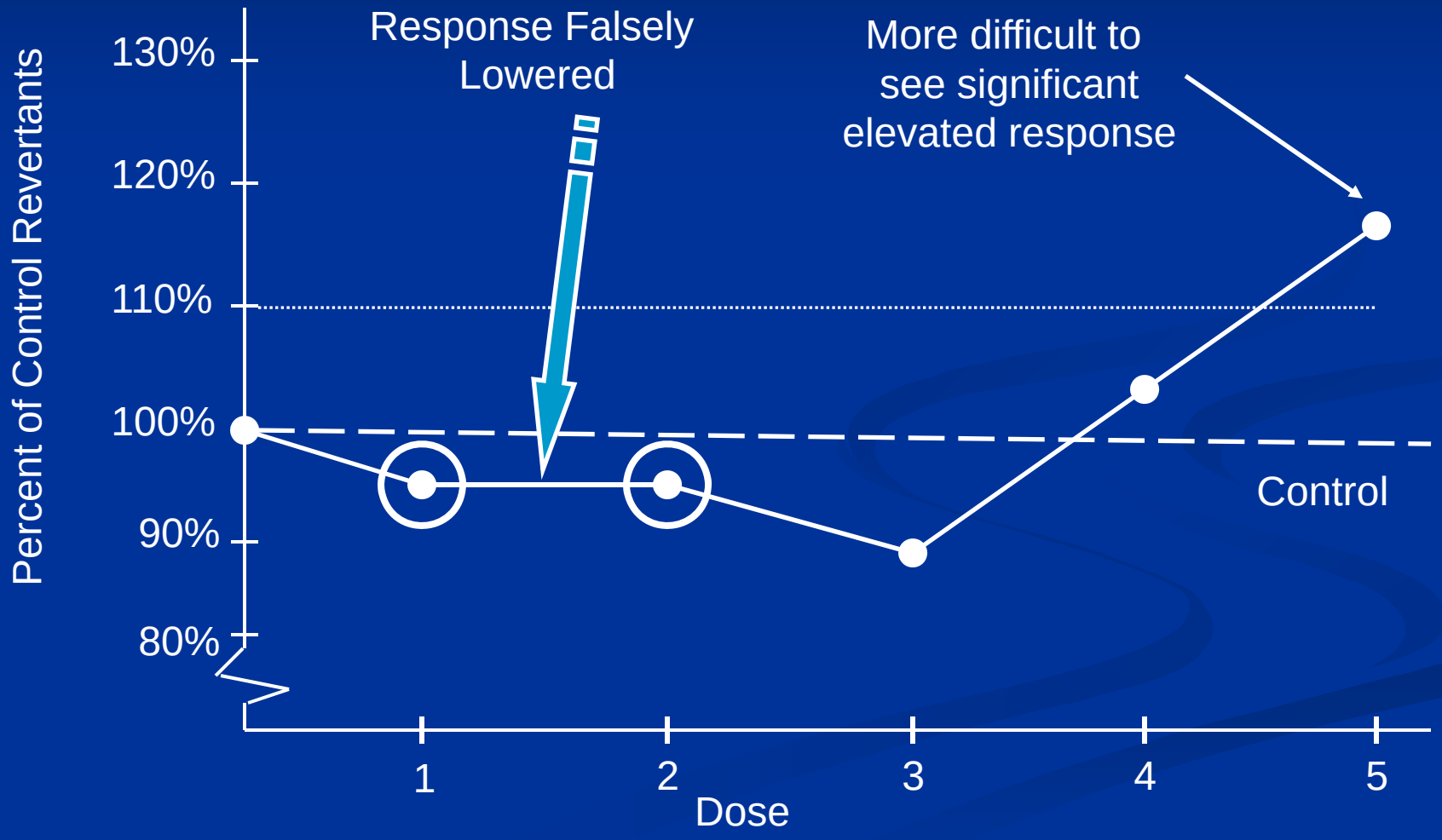
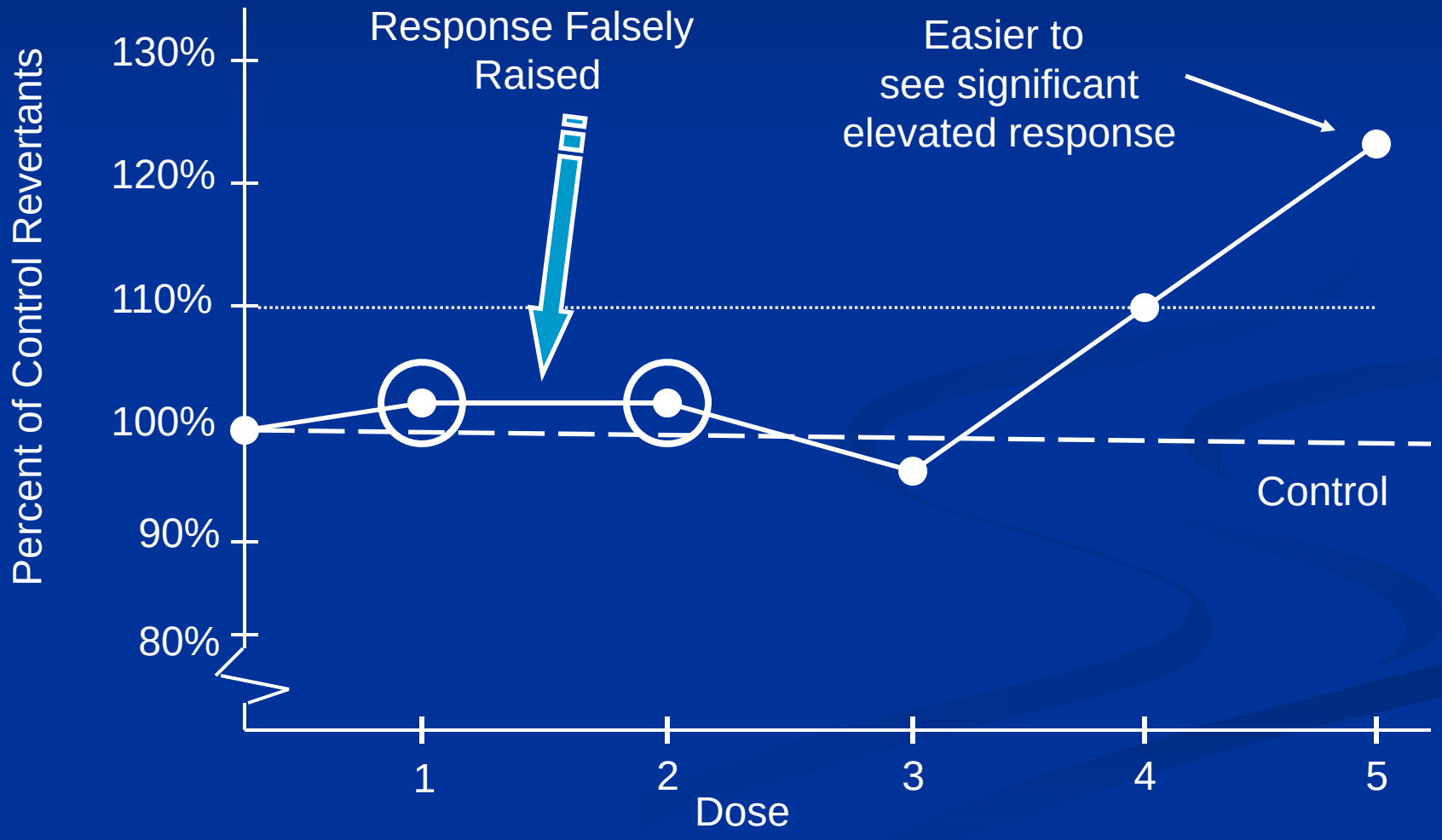


Figure 5a. Possible Selection? (Control too Low)



Simulation Study

- Assume no chemical effect
- Assume response error is normally distributed
  - Use estimate SEM from AMES assays for control
- Assume r=3 measures at control and each dose
- Follow the selection procedures for 50,000 assays
- Calculate the average response at each dose
- Determine bias

Figure 6a. Simulation (no selection criteria)

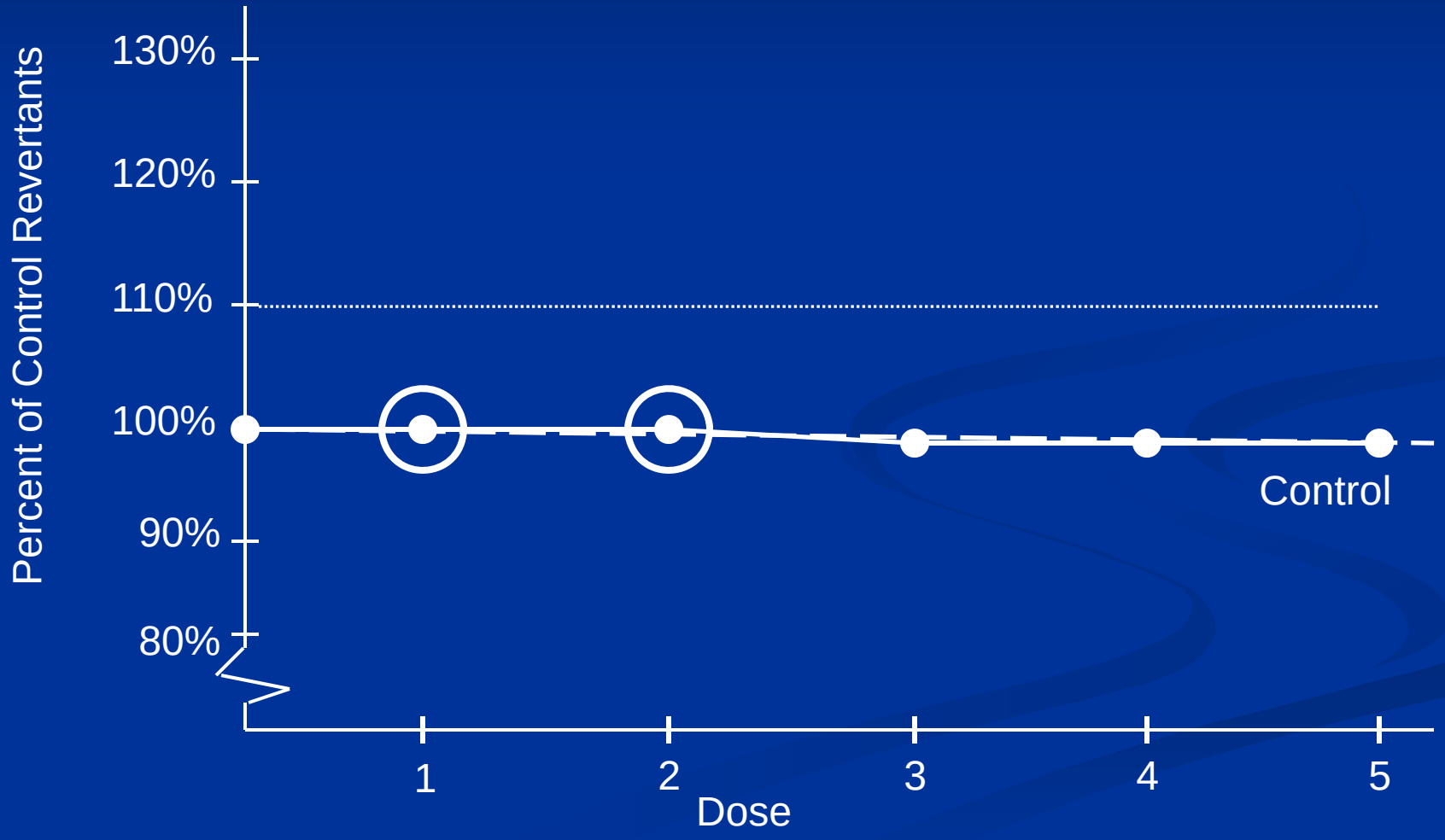


Figure 6b. Simulation Results(>110 Dose 5)

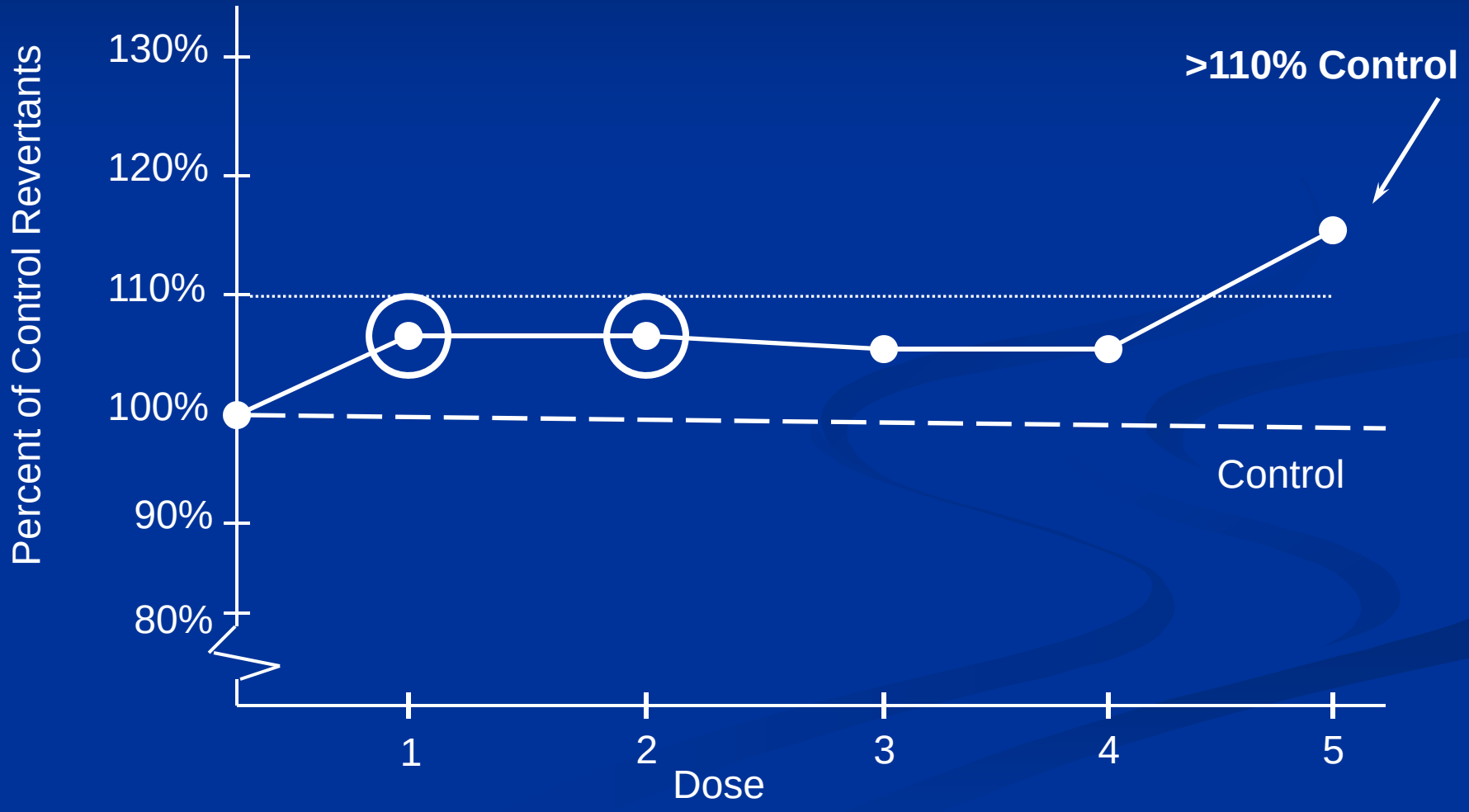


Figure 6c. Simulation Results (>110 Dose 5; Dose 4 < 110)

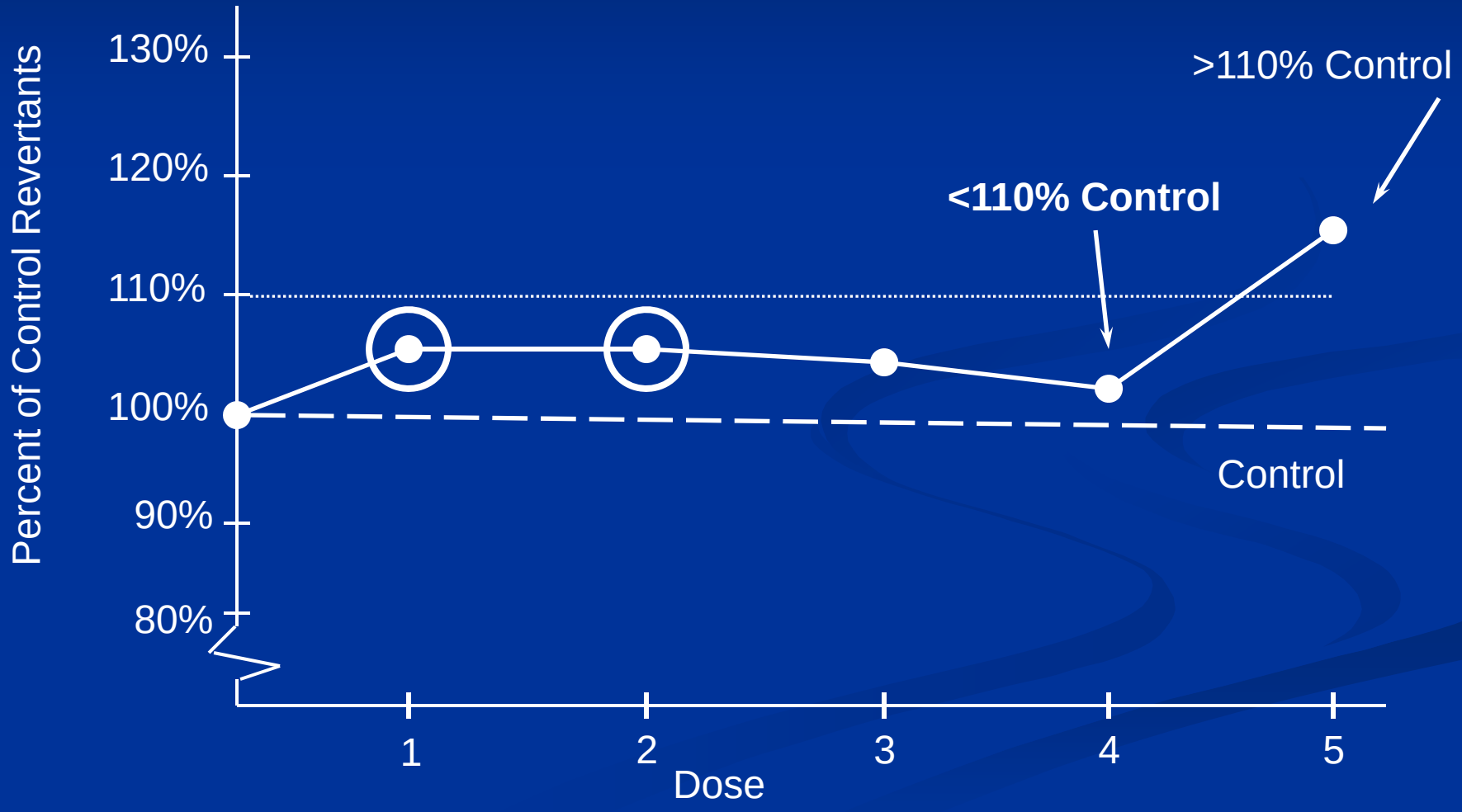


Figure 6d. Simulation Results (>110 Dose 5; Dose 3&4 < 110)

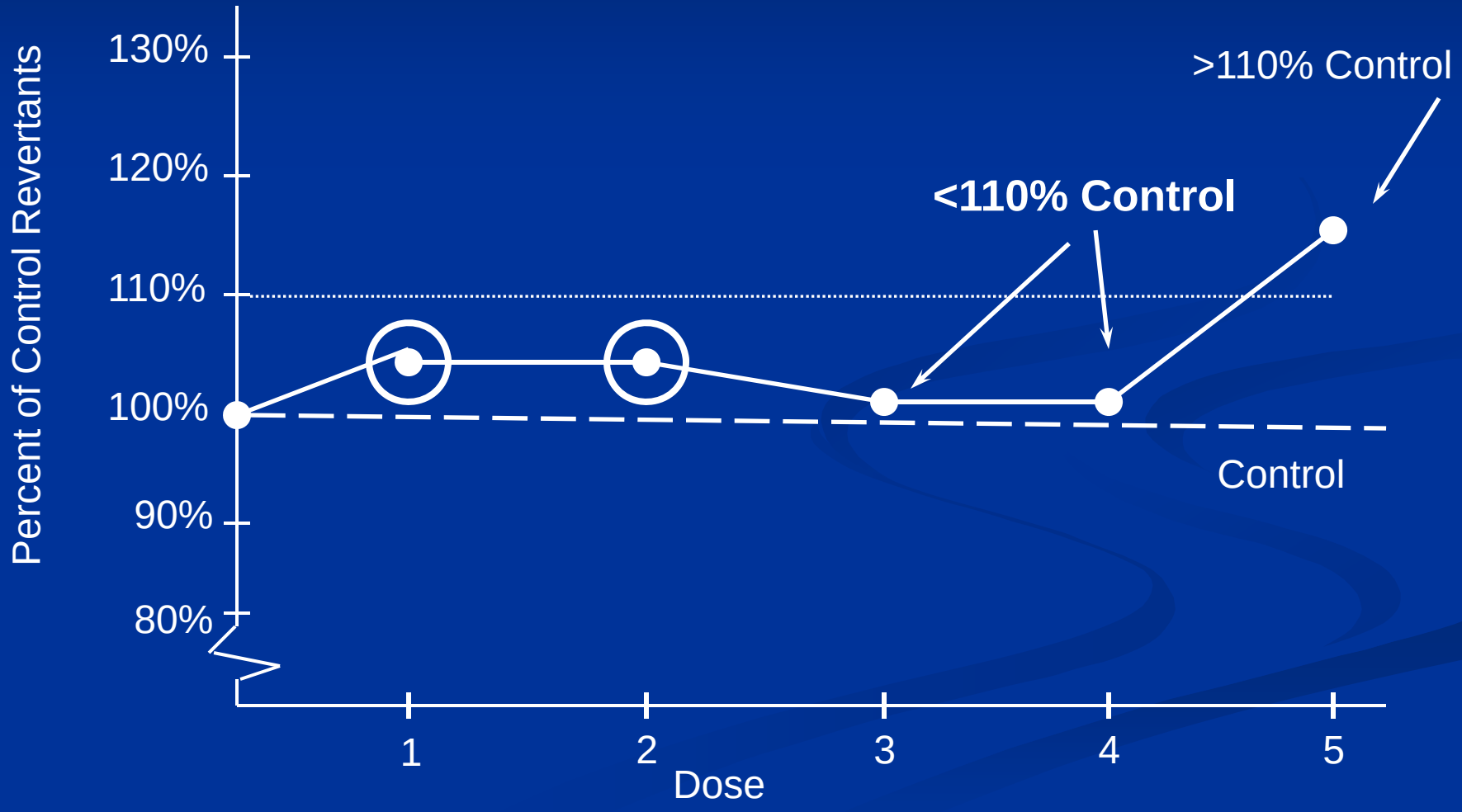


Figure 6e. Simulation Results (>110 Dose 5; Dose 3&4 < 110; SEM Cntl <7.5%)

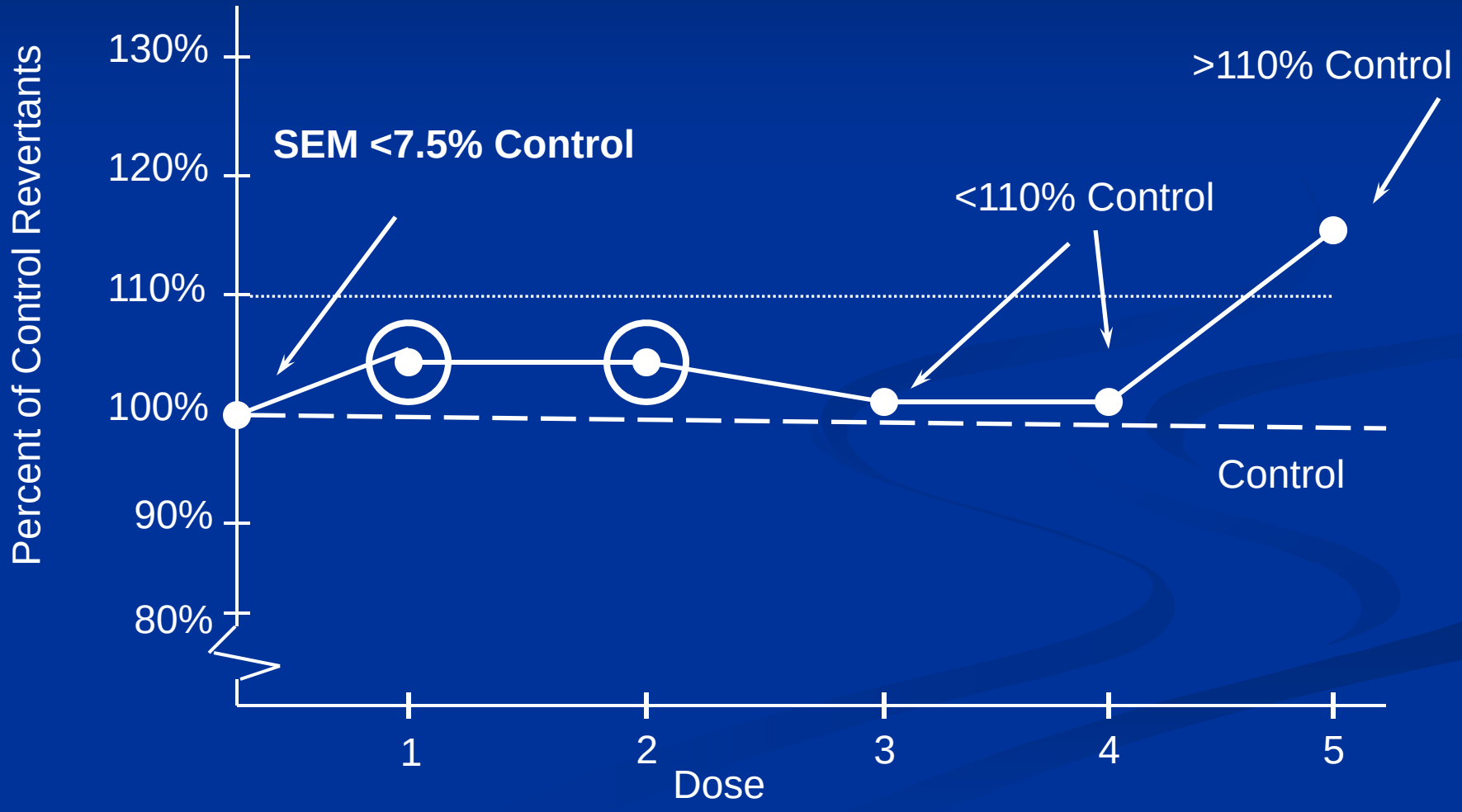
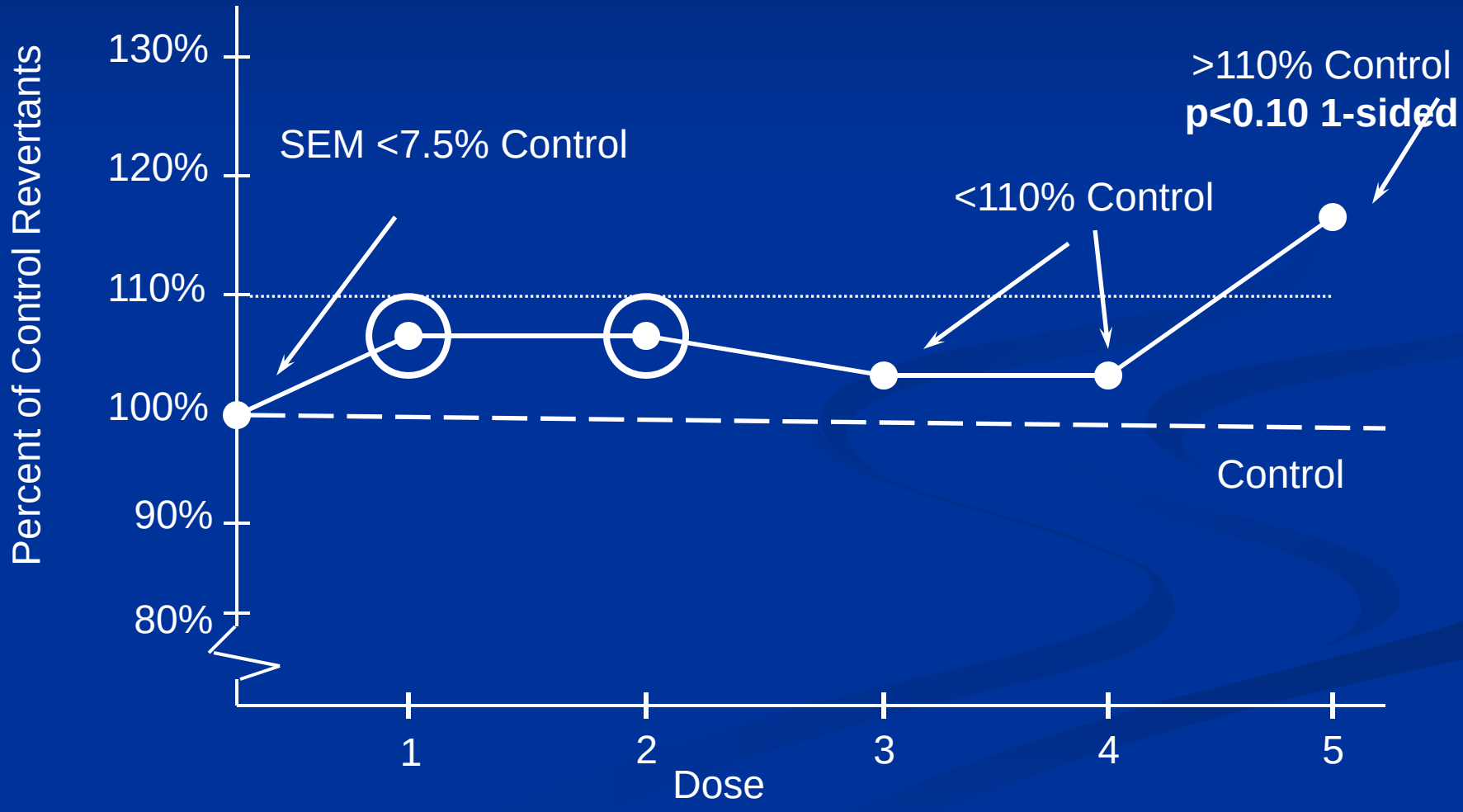


Figure 6f. Simulation Results (>110 Dose 5; Dose 3&4 < 110; SEM Cntl <7.5%)



Conclusions

- With no chemical effect, average response at low doses is expected to be above control (6%)
- We select assays where it appears that too many revertants occur at low doses
- The actual problem is that selection criteria favor control when response is too low
- True control revertants: 114
- Selected Study control revertants: 107

Figure 7a. Simulation Results (using all criteria) Dose 5 Response=100% Control Response

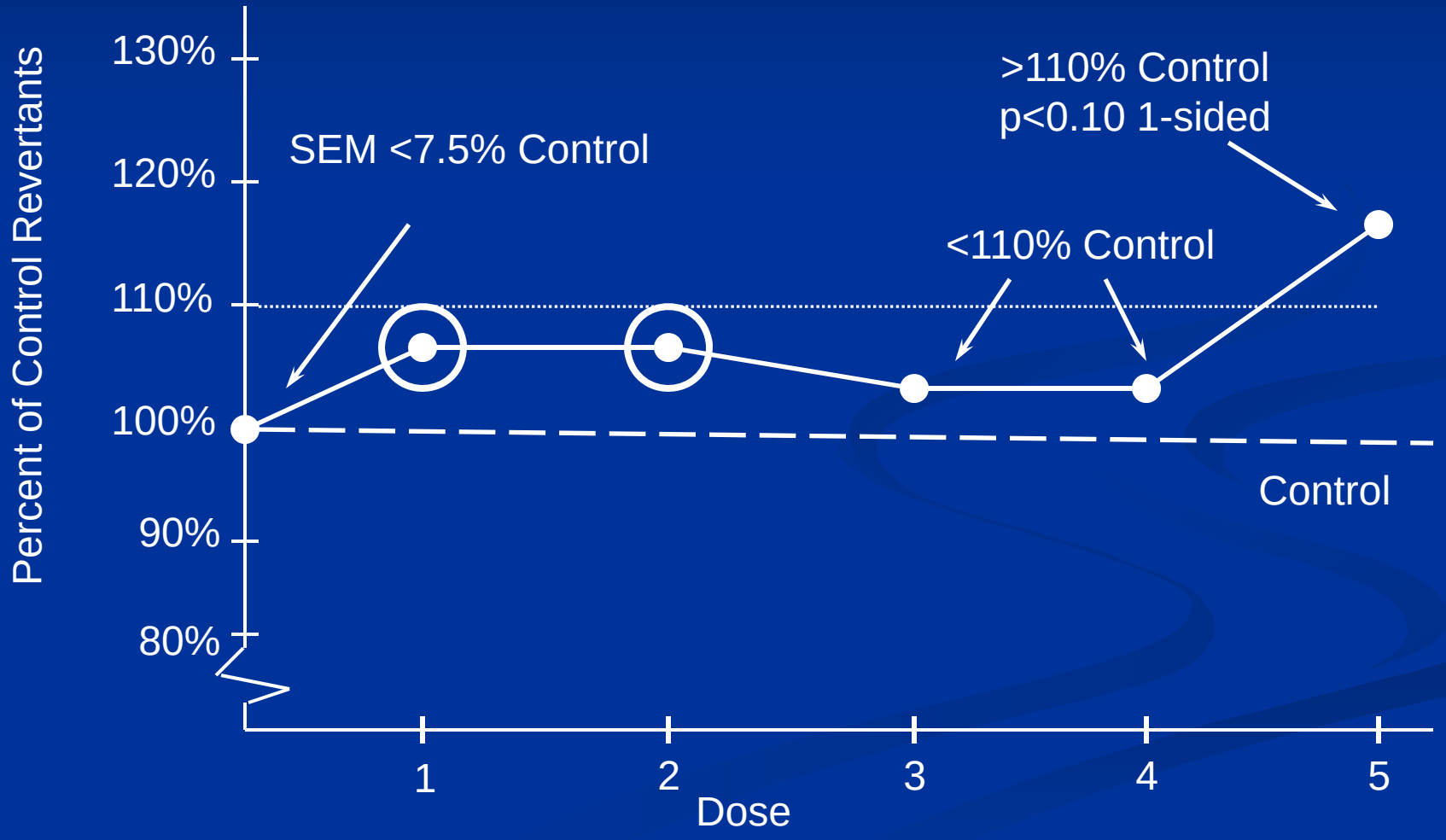


Figure 7b. Simulation Results (using all criteria) Dose 5 Response=105% Control Response

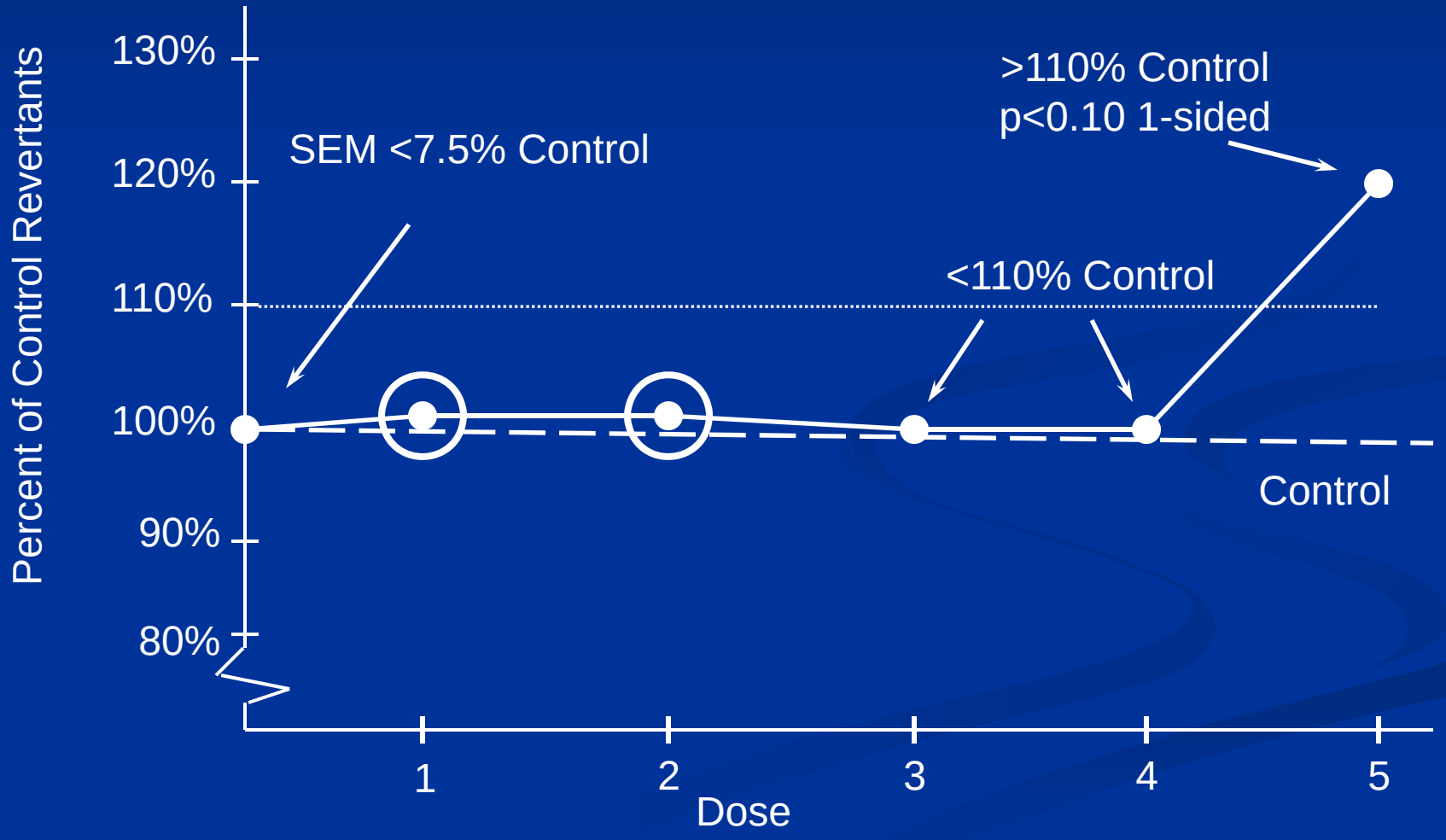


Figure 7b. Simulation Results (using all criteria) Dose 5 Response=107.5% Control Response

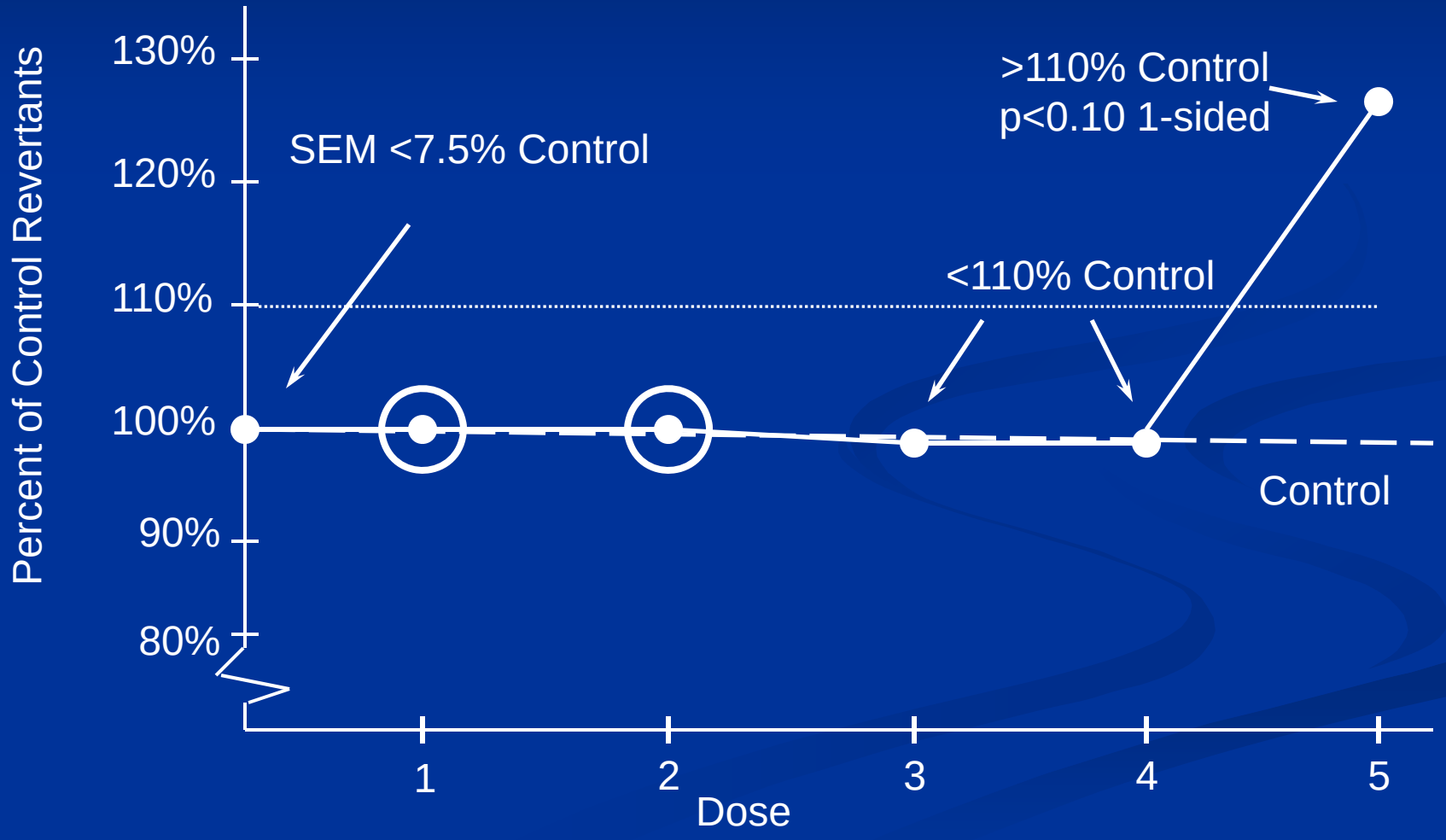
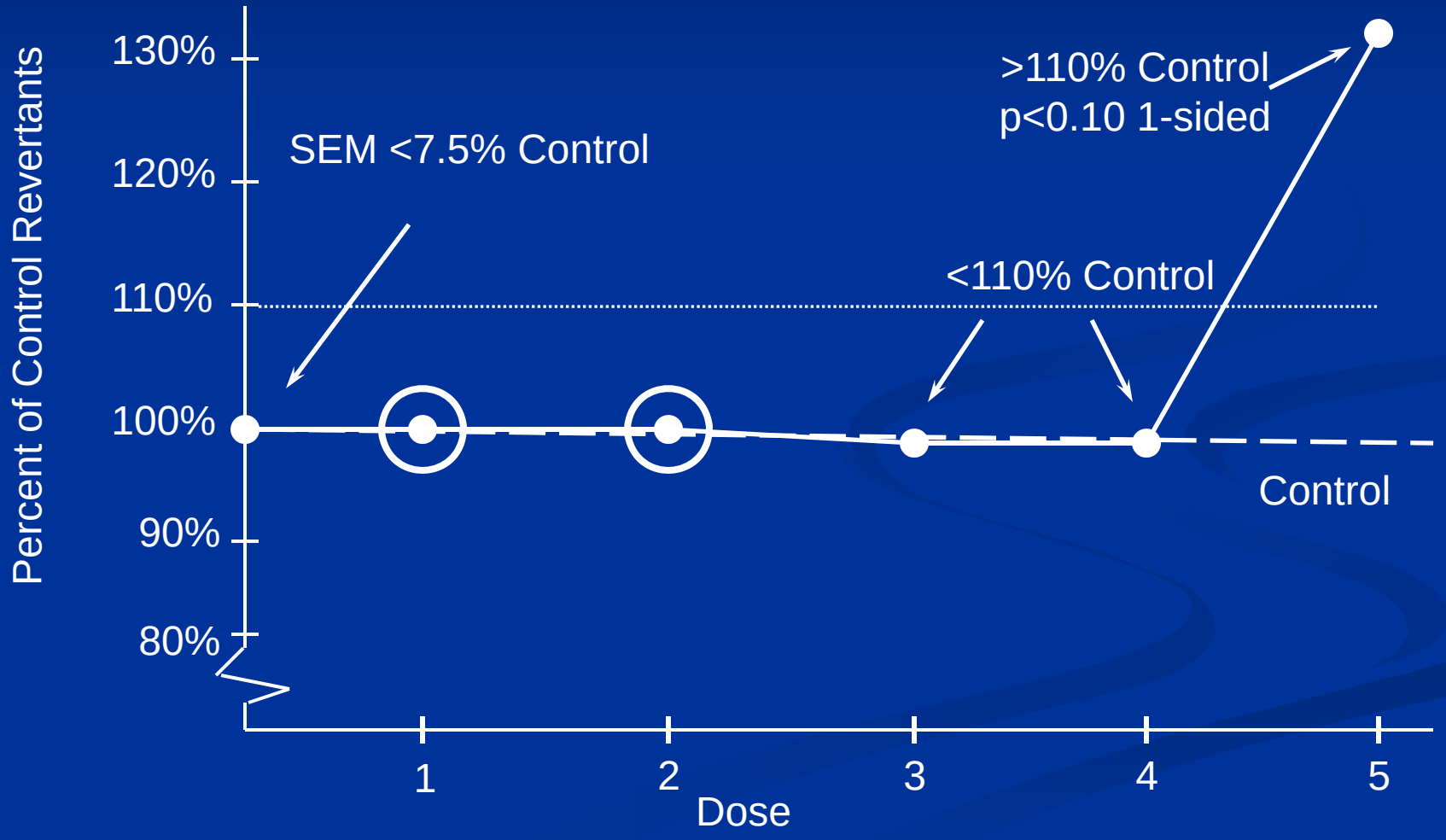


Figure 7b. Simulation Results (using all criteria) Dose 5 Response=110% Control Response



Conclusions

- Addition of Dose 5 Chemical Effect
  - Dose 5 Effect Dose 1 Response Dose 5 Response

105% Cntl	101% Cntl	120% Cntl
107.5% Cntl	100.5% Cntl	126 Cntl
110% Cntl	100.5% Cntl	131 Cntl

- Positive Bias at low doses disappears if there are 'small' dose 5 Chemical effects.

Is 'normal assumption' a limitation?

- Margolin (1981) suggested control response has hyper-Poisson variability.
- Data are given for response on replicate controls at 4 labs for TA100
- We consider these data as a population of 'responses' for control.
- Simulations are repeated using simple random samples (with replacement) of 3 responses using Margolin's data.

Figure 7a. Simulation Results (using all criteria) Dose 5 Response=100% Control Response Assuming Response Normally Distributed

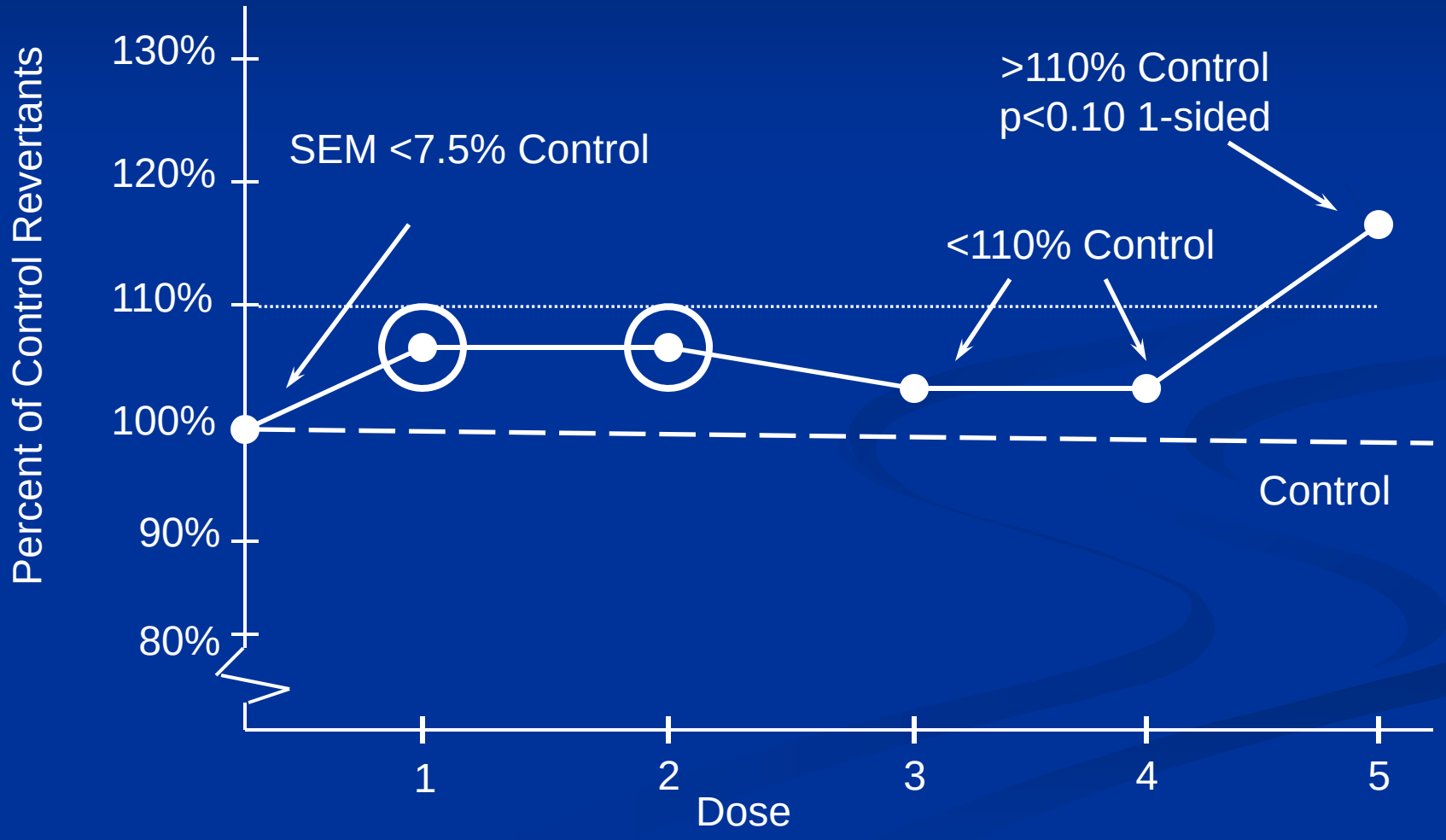


Figure 9a. Simulation Results (using all criteria) Dose 5 Response=100% Control Response Using Margolin's Response Data (lab 1)

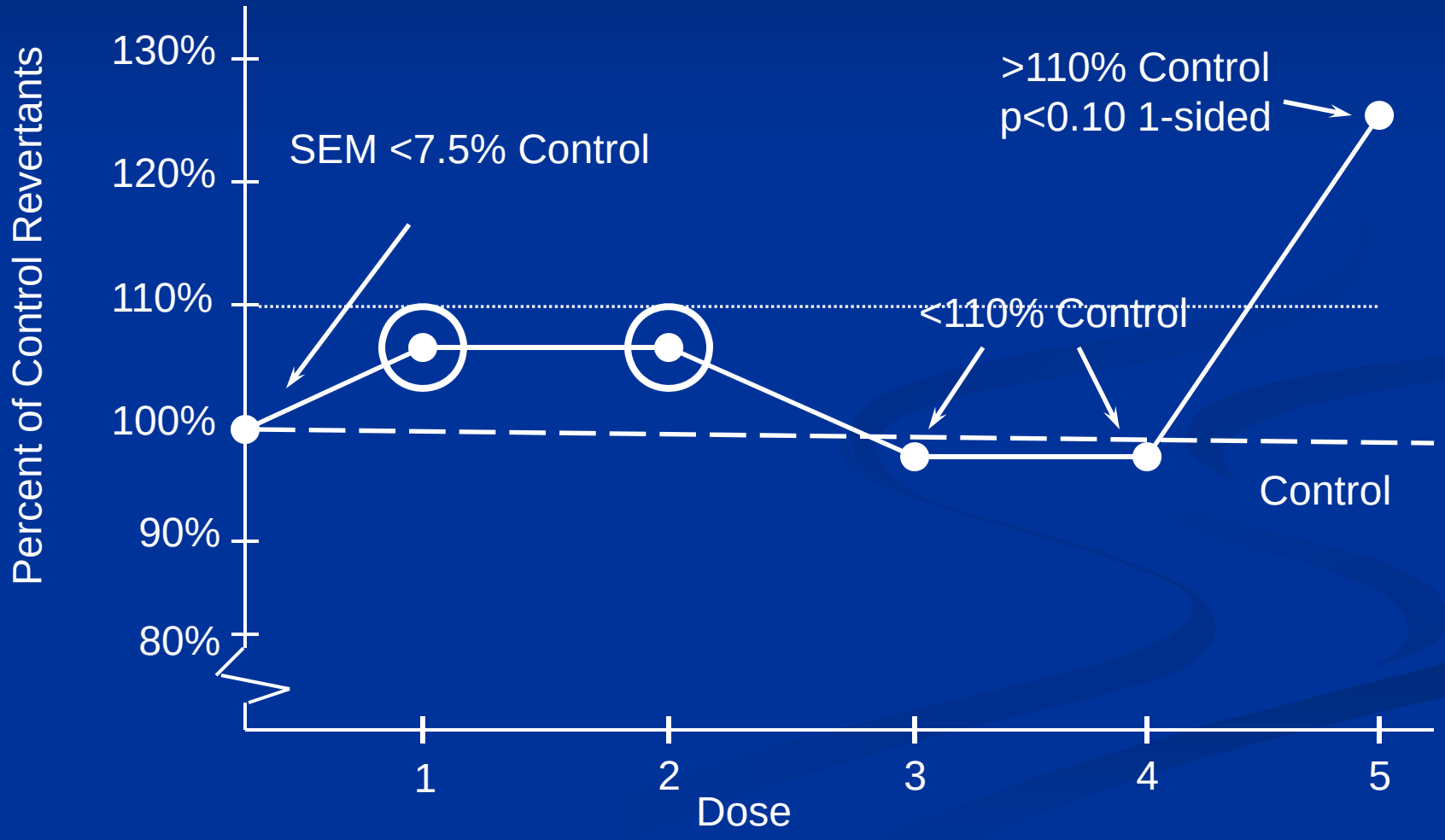


Figure 9b. Simulation Results (using all criteria) Dose 5 Response=105% Control Response Using Margolin's Response Data (lab 1)

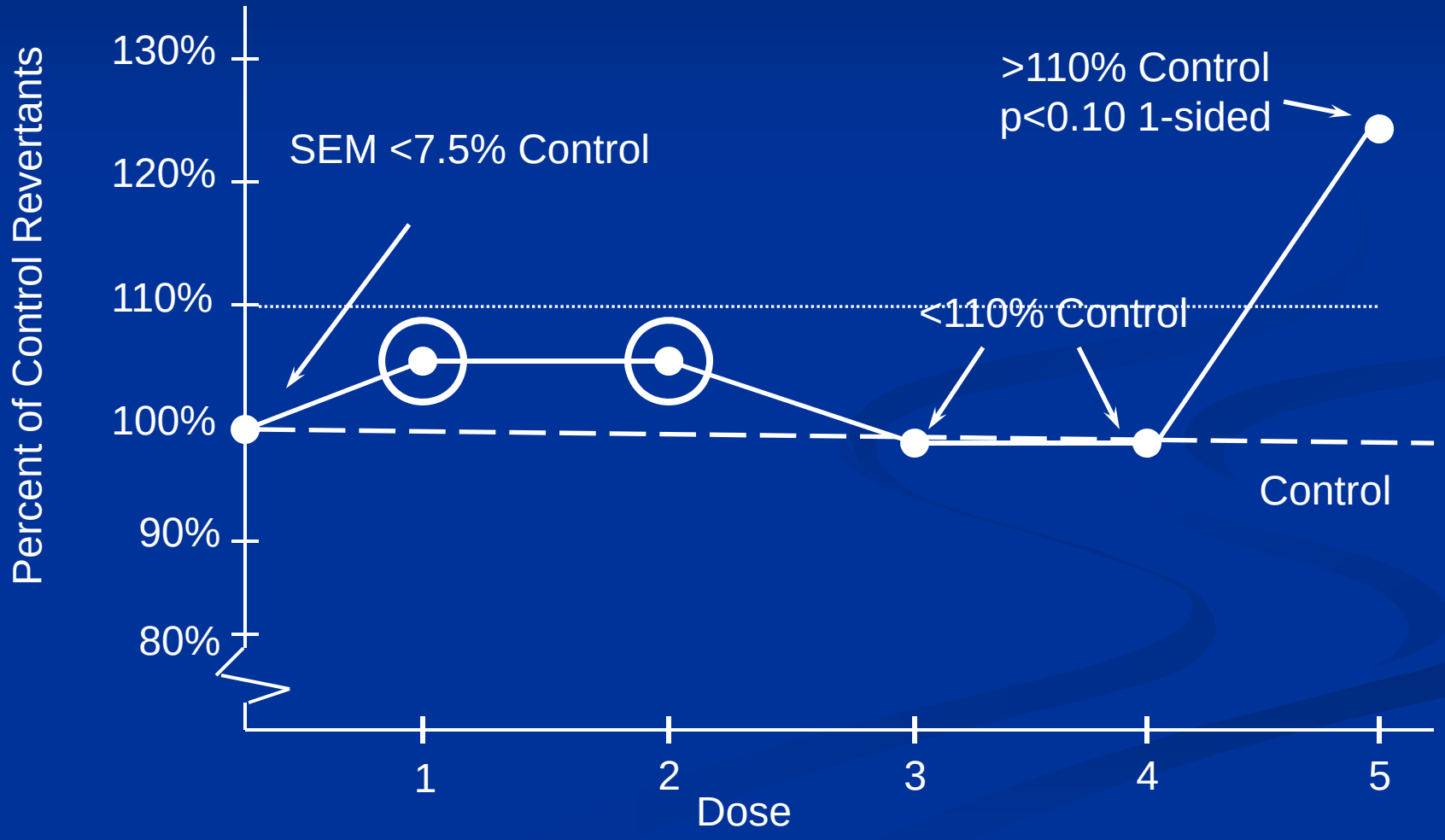


Figure 9c. Simulation Results (using all criteria) Dose 5 Response=107.5% Control Response Using Margolin's Response Data (lab 1)

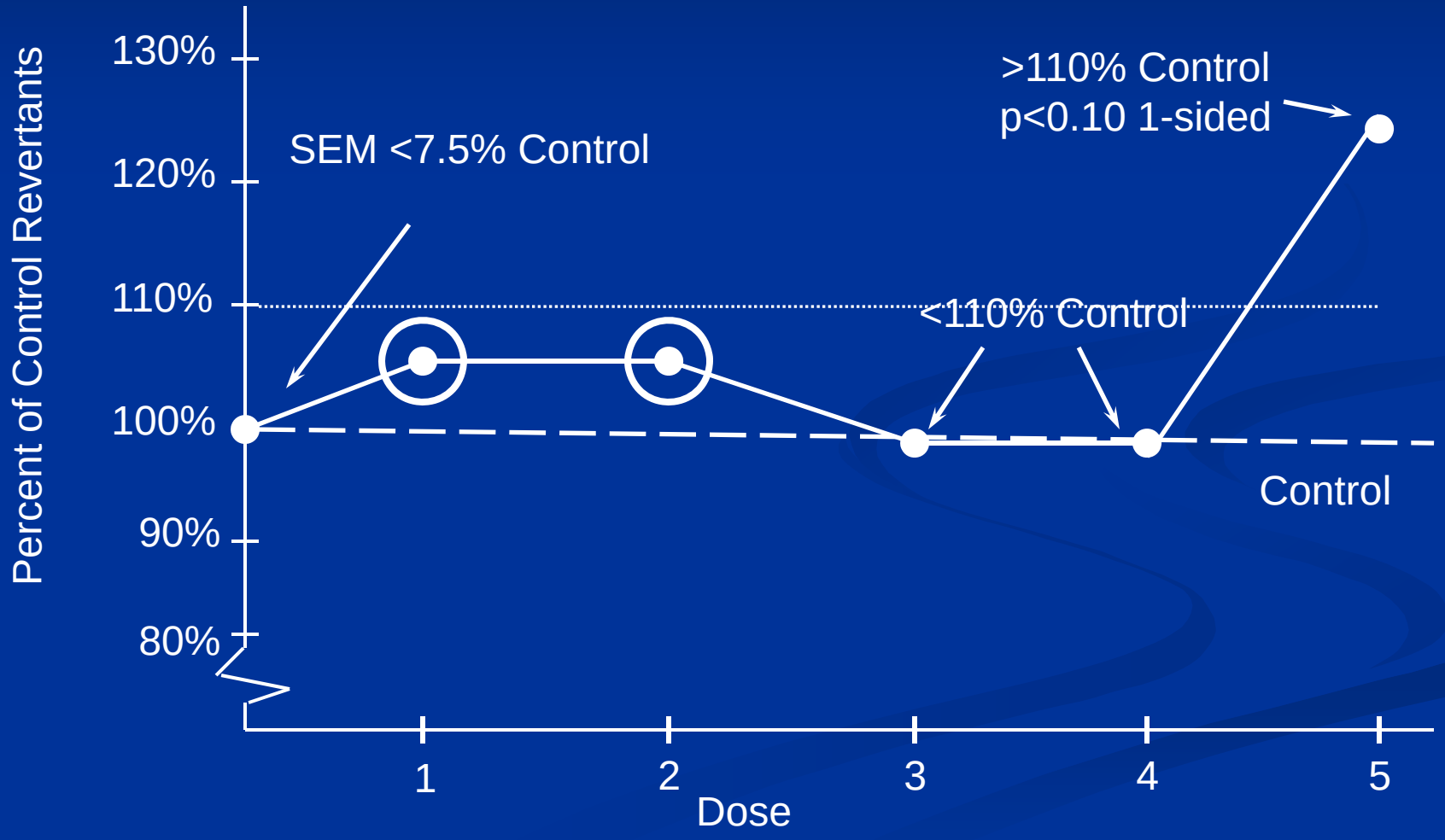


Figure 9d. Simulation Results (using all criteria) Dose 5 Response=110% Control Response Using Margolin's Response Data (lab 1)

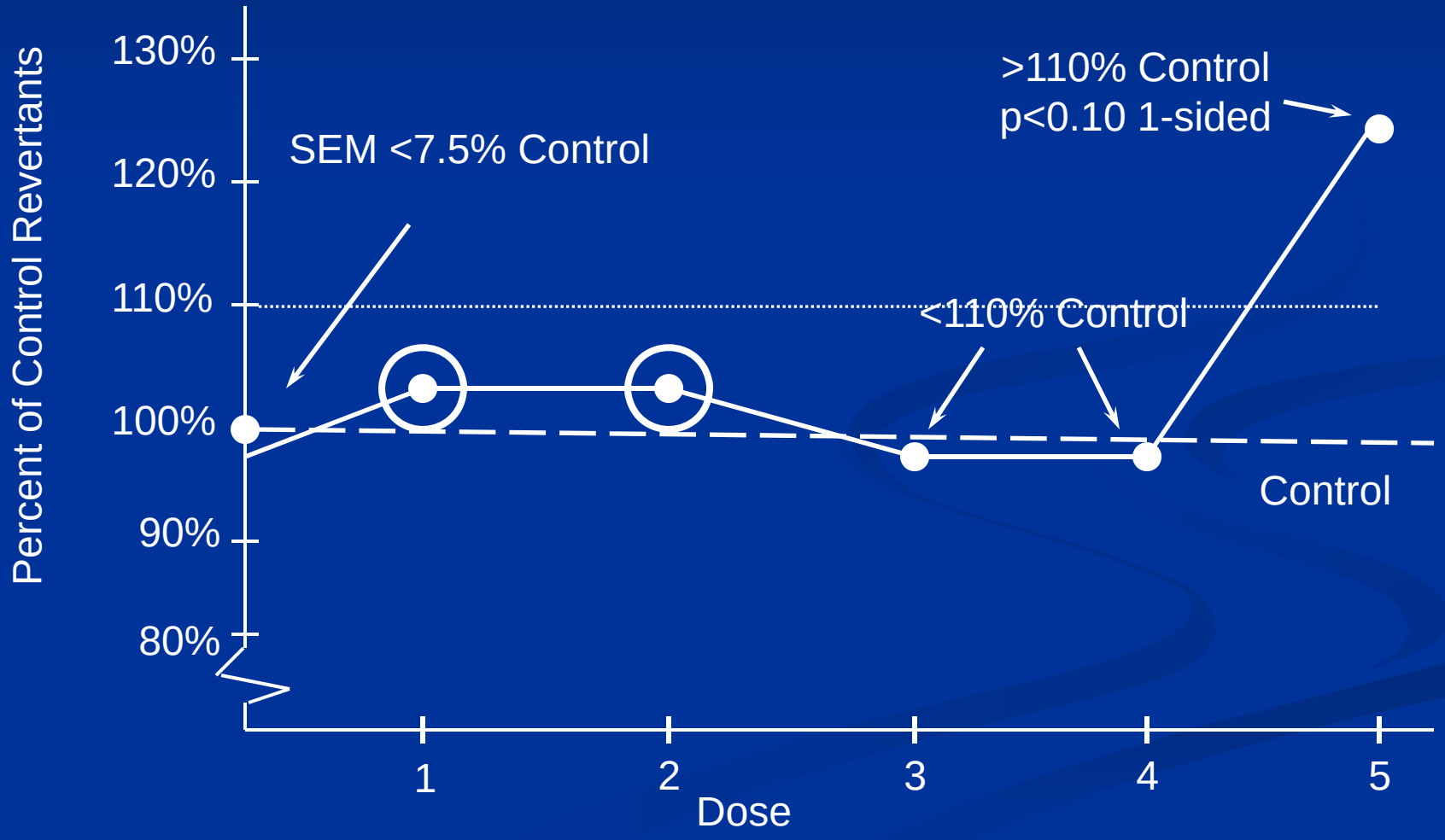


Figure 9e. Simulation Results (using all criteria) Dose 5 Response=125% Control Response Using Margolin's Response Data (lab 1)

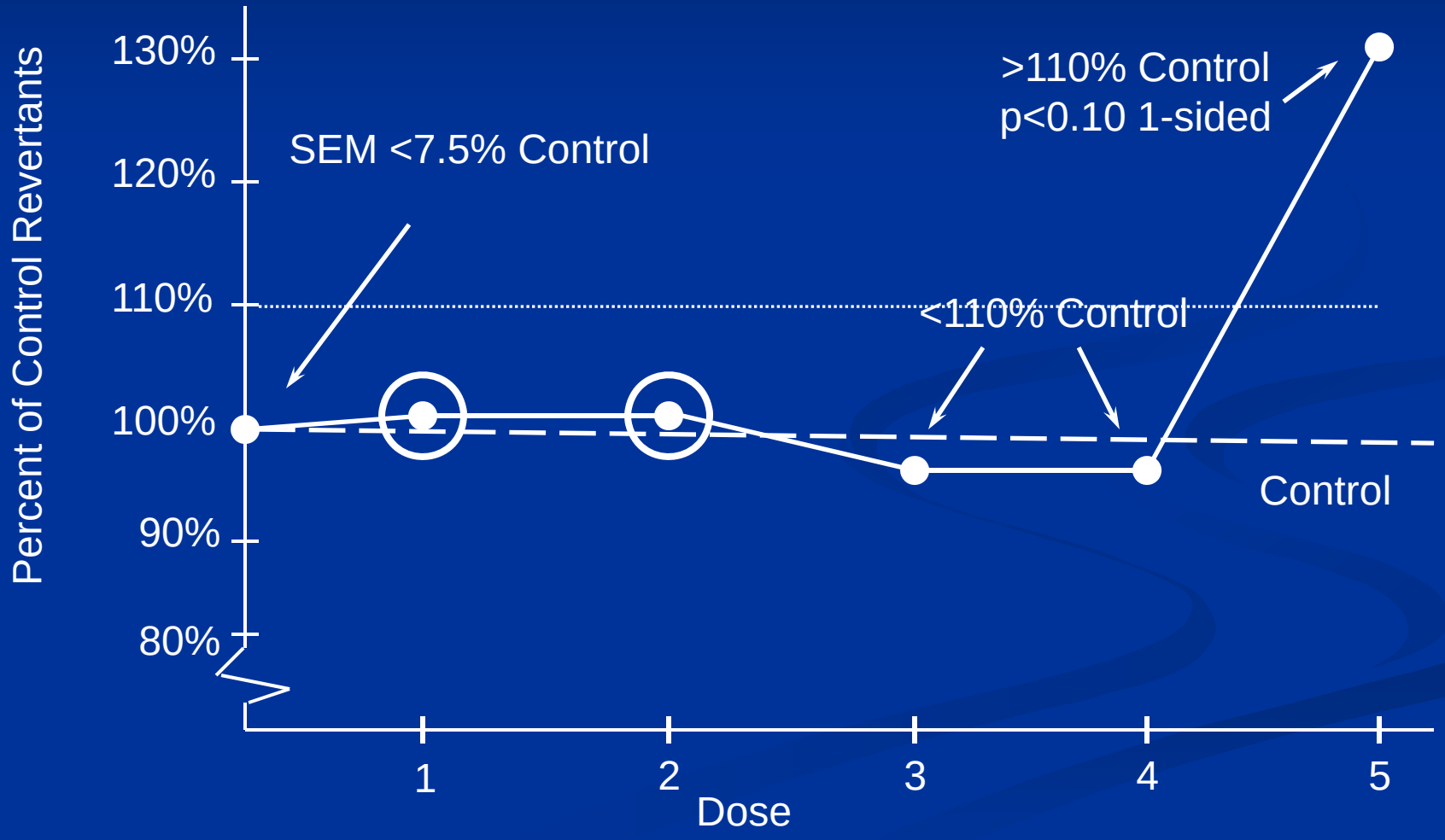
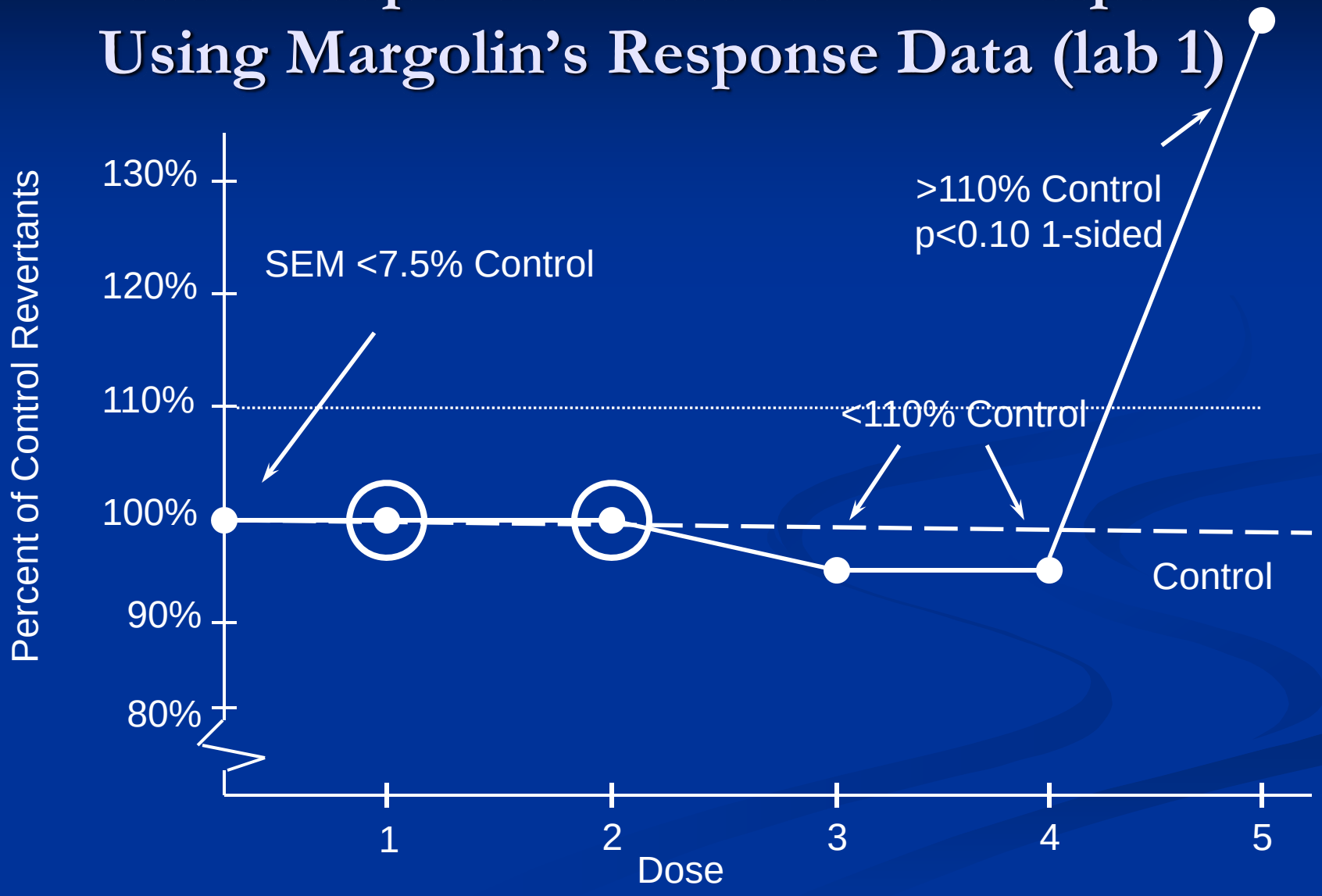


Figure 9f. Simulation Results (using all criteria) Dose 5 Response=150% Control Response Using Margolin's Response Data (lab 1)



Conclusions

- The same 'general' patterns occur-
  - Response at 'low doses' is positively biased
  - Bias decreases as Dose 5 Response increases
  - Larger Dose 5 Response needed for bias to be < 1% using Margolin's response data
  - Similar results occur using other Lab data
- Among 107 Selected Assays (dose 1 &2):
  - TA100 (61 assays) Excess of Responses < Control (like hormesis)
  - TA 97/98 (46 assays) No excess positive/negative response (like threshold)

Conclusion

- Basic Scientific method applied to observational data:
  - Retrieve data
  - Re-organize to examine theories and hypotheses
  - Check for selection biases using Simulation
  - Summarize and Compare
  - develop other possible theories and hypotheses
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Thanks

- Questions?