

Risk Management Strategies for Ames Mutagens

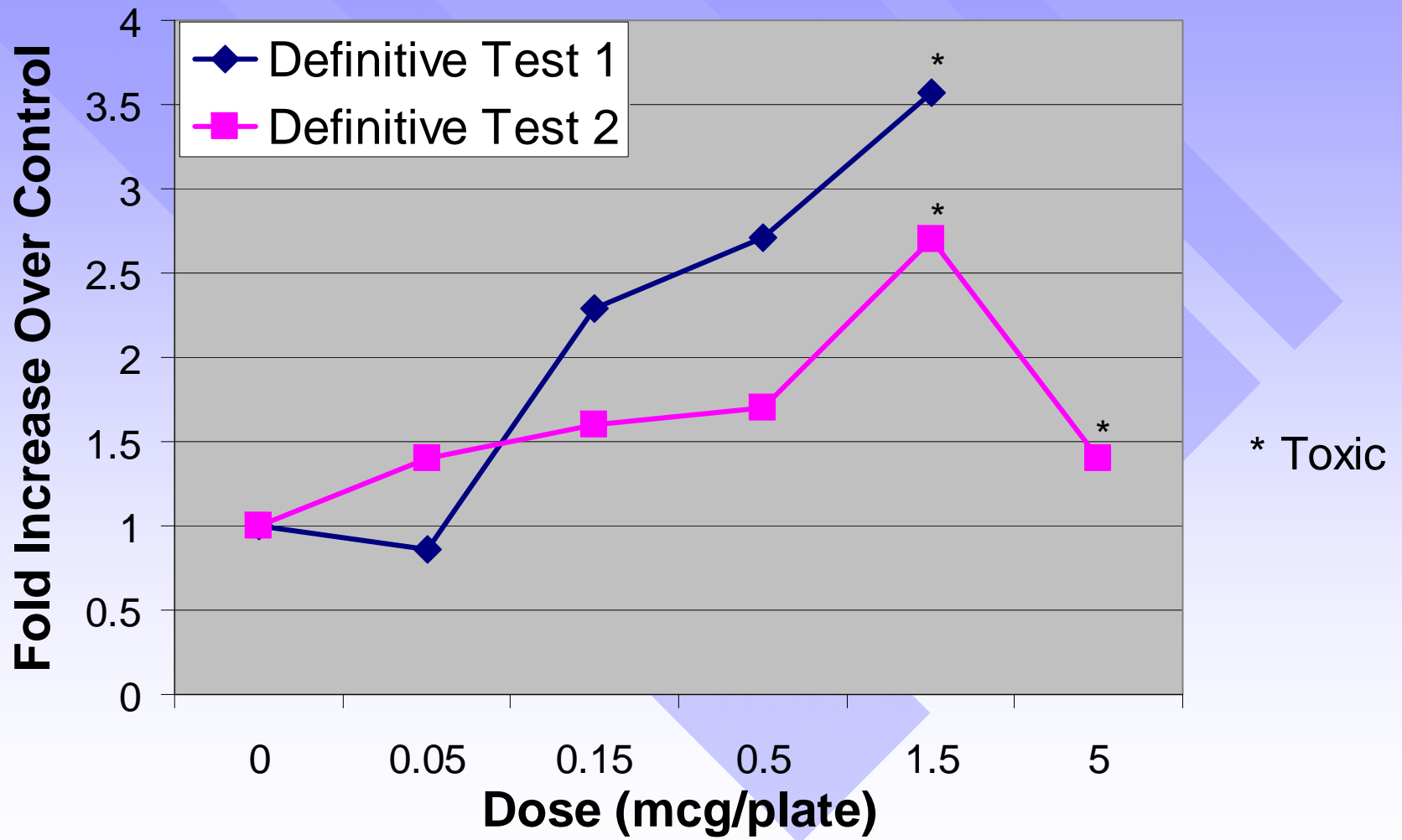
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Compound 1

- Class: Antibacterial DNA Gyrase Inhibitor
- In vitro micronucleus assay and Biolum Ames (TA98 and TA100) - negative up to cytotoxic concentrations with and without S9
- Ames testing in TA1537, TA1537, TA98, TA100 and WP2uvrApKM101
 - » Mutagenic only in TA1537 starting at the lowest cytotoxic concentration
- Levofloxacin is a marketed DNA gyrase inhibitor that has been demonstrated to be a bacterial specific mutagen at doses that do not inhibit Human Topoisomerase II
 - » Mutagenicity only observed in DNA repair proficient strains such as TA102 (not TA1537)

Compound 1 Ames Data: TA1537 +S9



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Compound 1: Hypothesis

- Compound 1 is a bacterial specific mutagen. Genotoxicity should not be observed in mammalian assays unless concentrations that inhibit huTopo II are achieved

Compound 1: Hypothesis Testing

- Ames test of Compound 1 in TA102
- Tested several DNA gyrase inhibitors, including Levofloxacin with TA1537 and TA102
- MLA and 24h direct in vitro cytogenetics assay
- Generated TA1537 DNA gyrase resistant mutant (~4X increase in MIC) by low dose exposure to Compound 1
 - Repeated mutagenicity testing of Compound 1 and other DNA gyrase inhibitors.

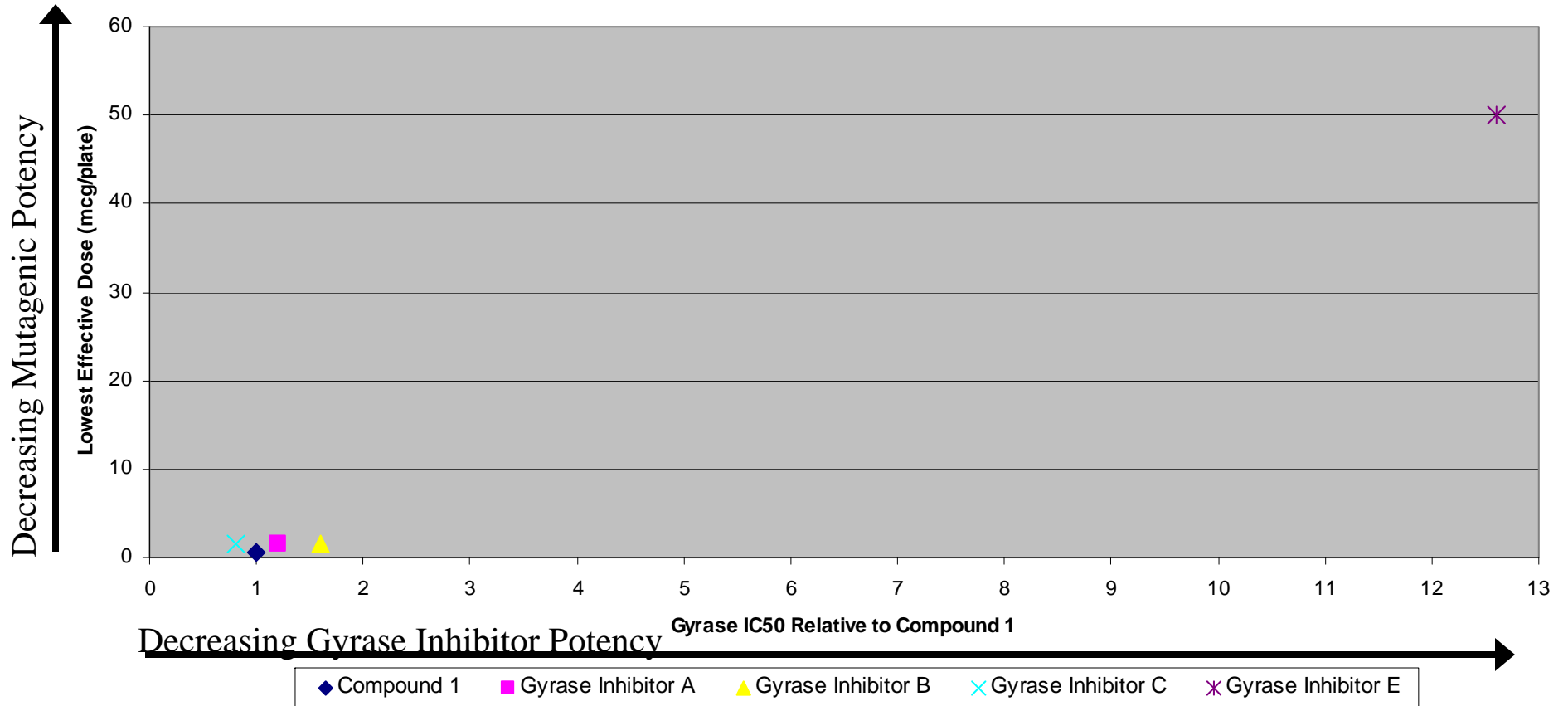
Comparison of Mutagenic Potencies

TA102 +S9				
<u>Compound</u>	<u>IC50 Gyrase*</u> <i>(relative to Cpd1)</i>	<u>LED</u> <i>(mcg/pl)</i>	<u>MED</u> <i>(mcg/plate)</i>	<u>Max. fold- increase</u>
Levofloxacin		0.15	0.5	2.8
Compound 1	1	0.50	5	3.5
Gyrase Inhibitor A	1.2	1.50	15	4.3
Gyrase Inhibitor B	1.6	1.50	15	3.5
Gyrase Inhibitor C	0.8	1.50	15	3
Gyrase Inhibitor D		5.00	15	2.8
Gyrase Inhibitor E	12.6	50.00	150	2.6

TA1537 +S9				
<u>Compound</u>	<u>IC50 Gyrase*</u> <i>(relative to Cpd1)</i>	<u>LED</u> <i>(mcg/pl)</i>	<u>MED</u> <i>(mcg/plate)</i>	<u>Max. fold- increase</u>
Levofloxacin				Negative
Compound 1	1	0.15	1.5	3.6
Gyrase Inhibitor A	1.2	0.15	5	2.7
Gyrase Inhibitor B	1.6	1.50	15	3.2
Gyrase Inhibitor C	0.8	5.00	5	2.5
Gyrase Inhibitor D		1.25	5	3.3
Gyrase Inhibitor E	12.6			Negative

* Measured in *S. aureus*

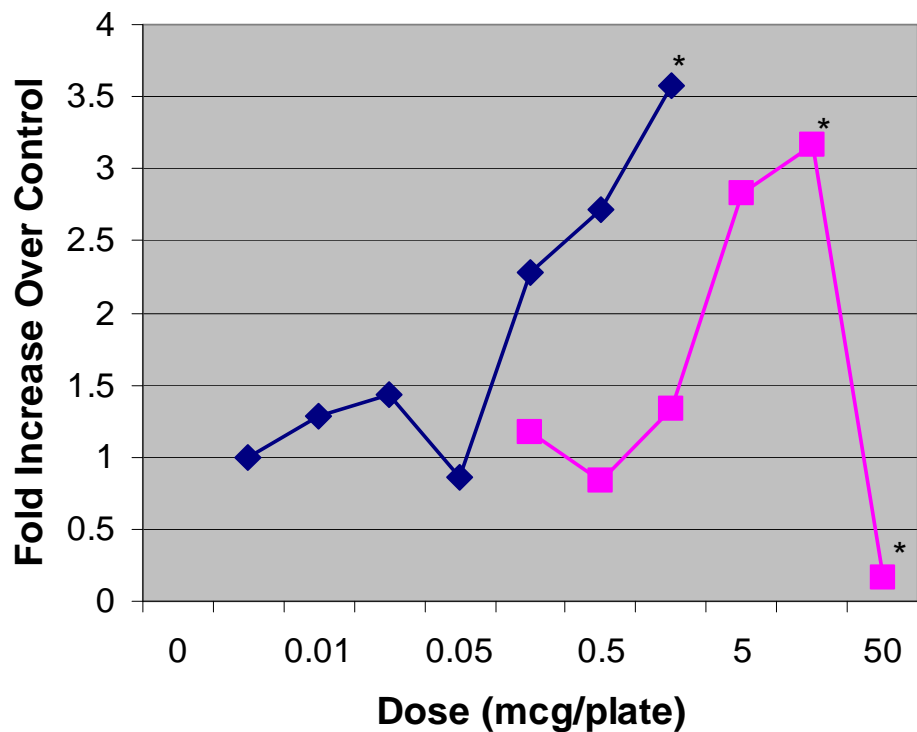
Comparison of Gyrase Inhibition to Mutagenic Lowest Effective Dose (TA102 +S9)



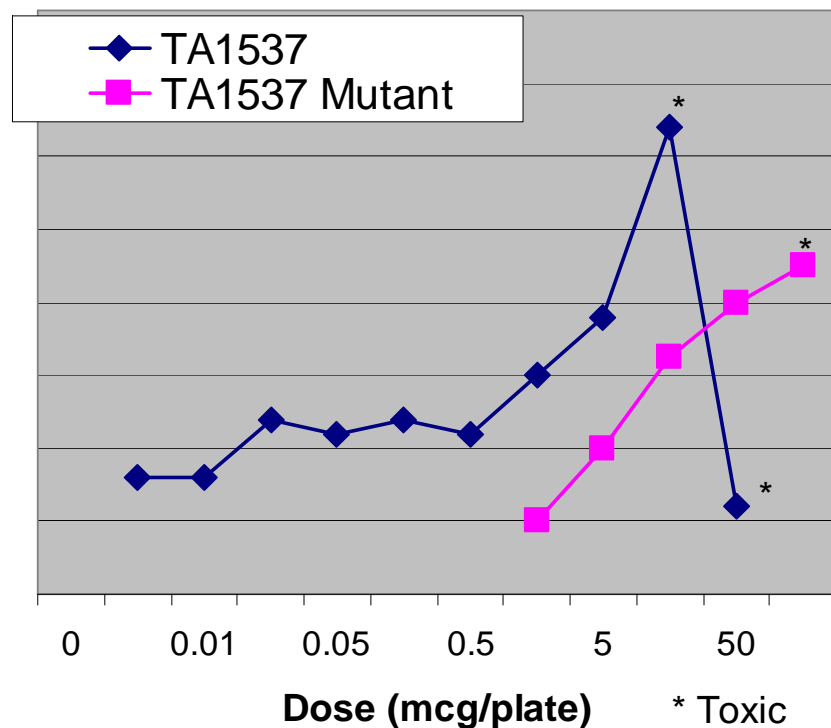
DNA Gyrase Inhibitor E, which is a weak inhibitor, has a 100-fold lower Lowest Effective Dose (LED) than Compound 1, which is a potent DNA gyrase inhibitor.

Effect of Gyrase Resistance on Mutagenicity in TA1537 +S9

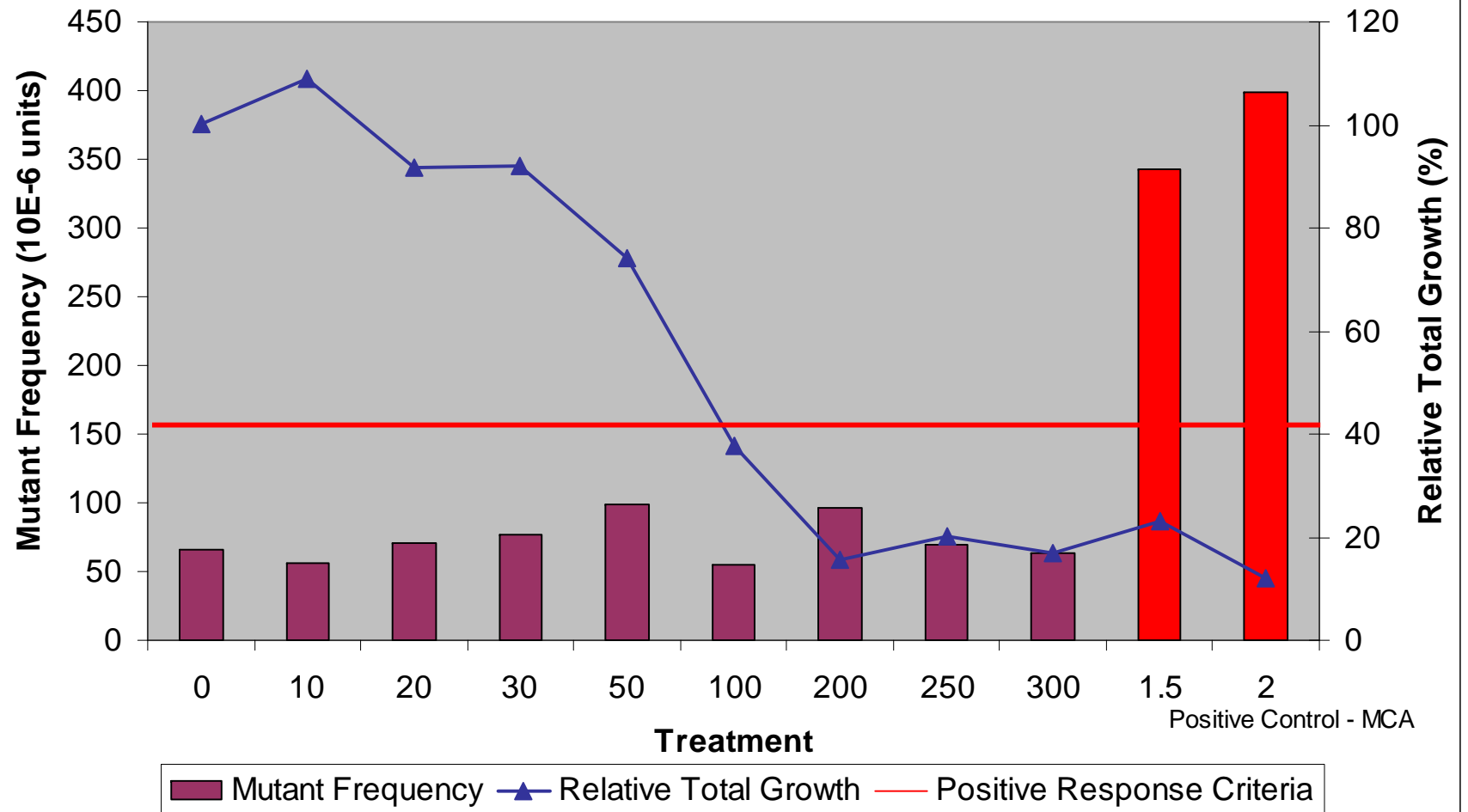
Compound 1



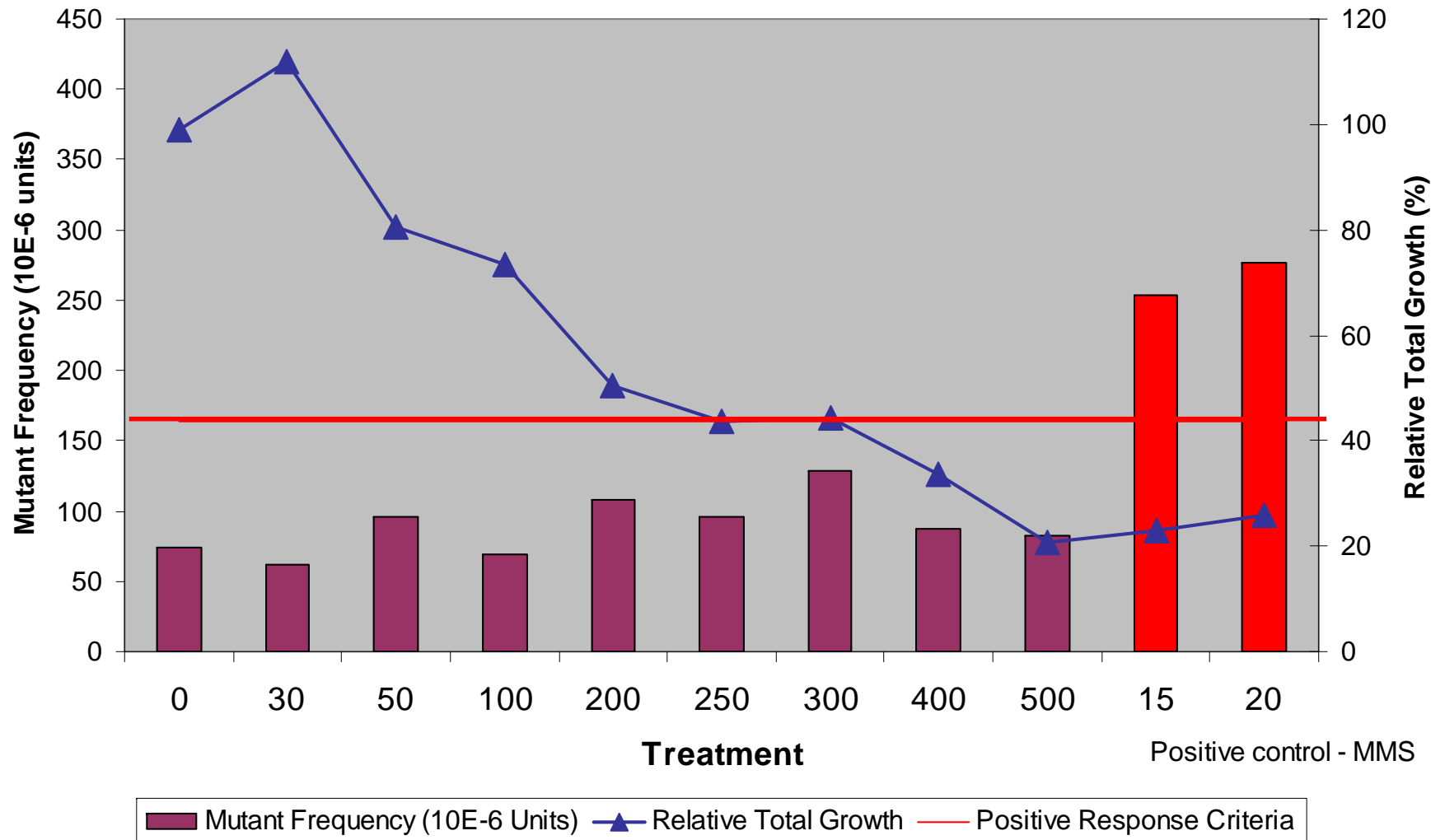
Gyrase Inhibitor B



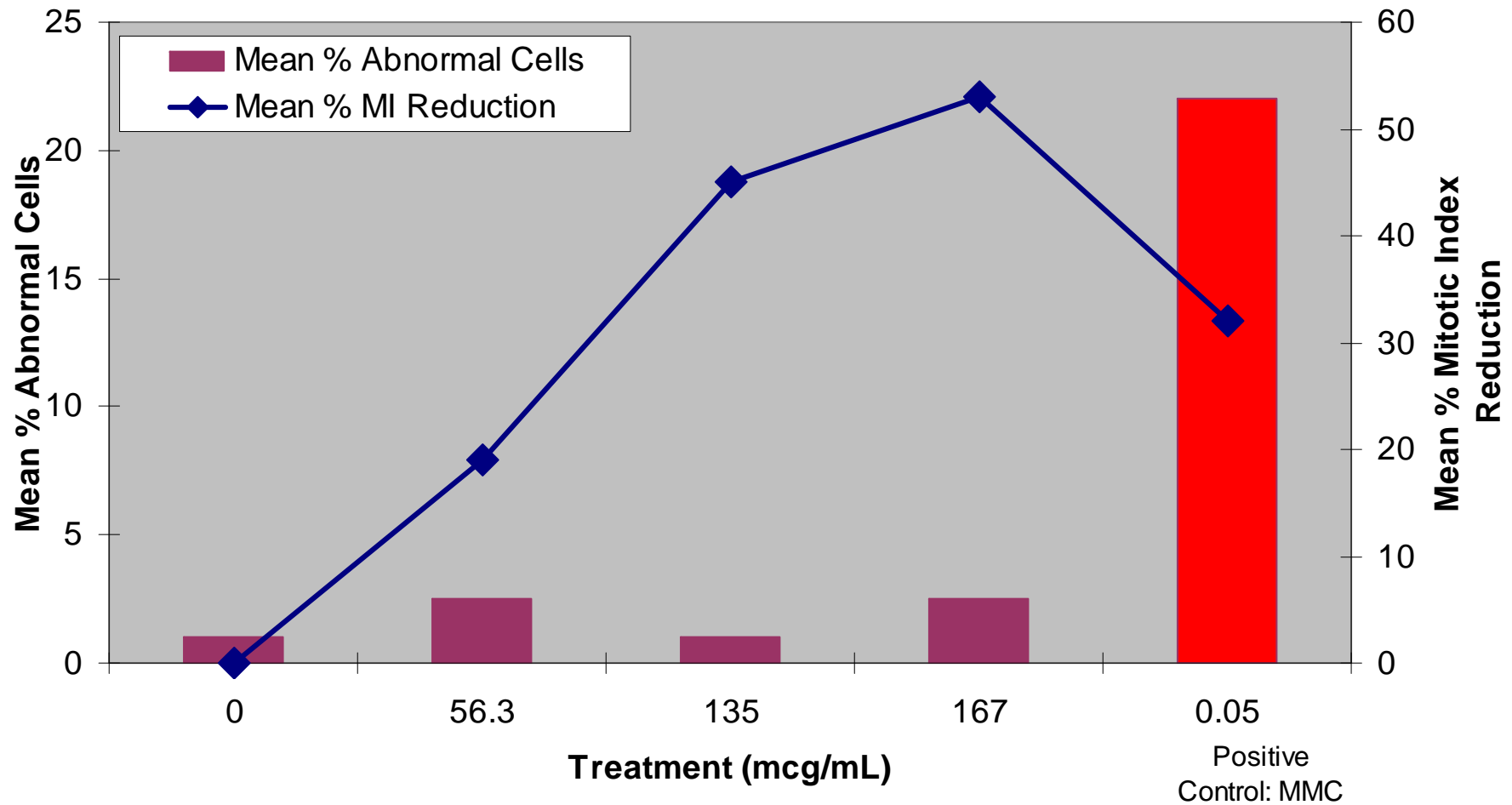
Compound 1: MLA With Activation



Compound 1: MLA Without Activation



Compound 1: In Vitro Human Lymphocyte Cytogenetics Assay: 24h Direct



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Compound 1: Summary of results

- All DNA gyrase inhibitors tested are mutagenic in both TA1537 and TA102
 - Exception one compound with limited DNA gyrase inhibitory activity (DNA Gyrase Inhibitor E) was only mutagenic in TA102, like Levofloxacin, and was a much less potent mutagen than Compound 1
- All tested DNA gyrase inhibitors showed a shift in mutagenic potency and cytotoxicity when tested with a gyrase resistant mutant
- MLA: Negative with and without activation
- In vitro cytogenetics: Negative 24h direct

Compound 2

- Neuroscience compound for non-life-threatening indication
- Intended for sub-chronic treatment
- Spiral Ames (HCL salt) and In vitro micronucleus assay - negative
- Regulatory Ames testing identified Compound 2 (tartrate salt) as a bacterial mutagen
 - Mutagenic only in TA100 +S9 at 5 mg/plate

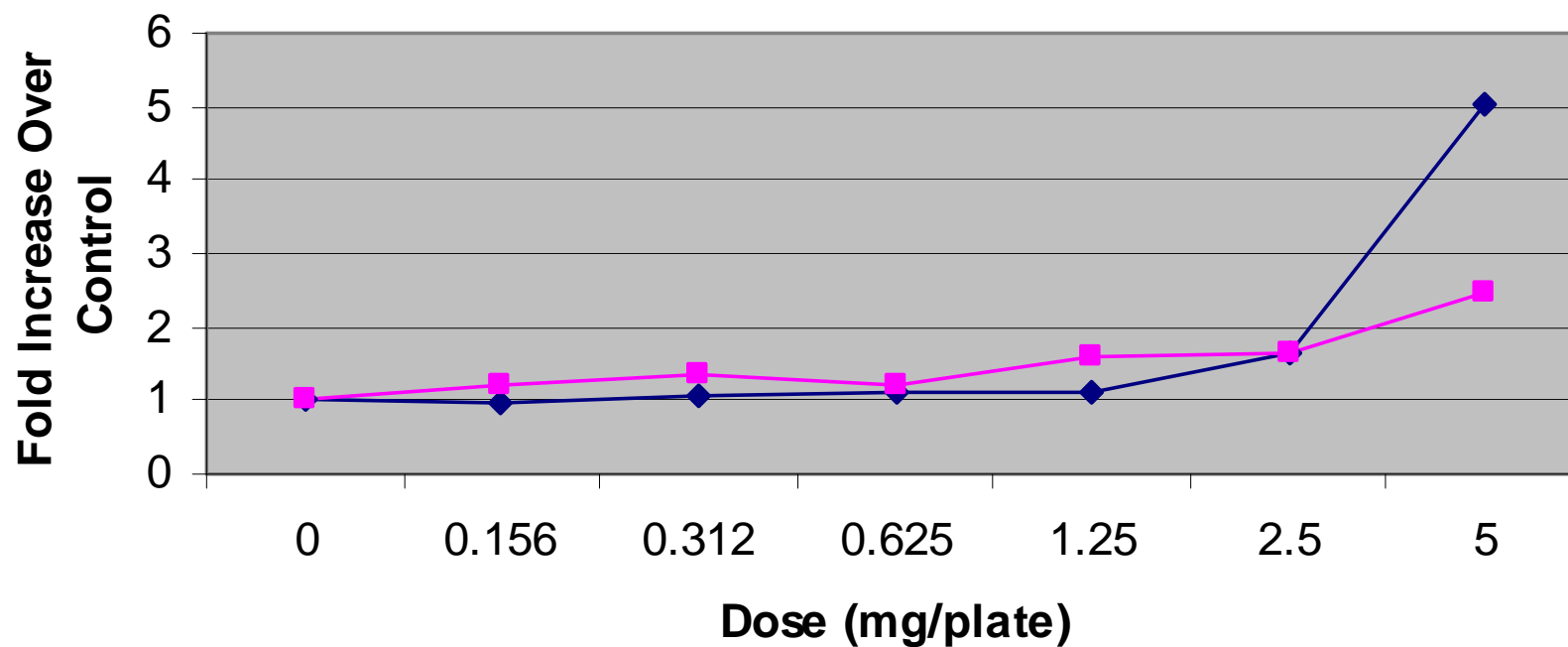
Compound 2 Bacterial Mutation Response: Platform vs. Impurity

Platform	Salt Form	
	HCl	Tartrate
Spiral Ames	Negative	Negative
Plate Ames	Positive	Positive
Biolum Ames	Negative	Negative
Modified Biolum Ames	Positive	Positive

Data suggests that the response difference is due to platform and not an impurity.

Compound 2

Bacterial Mutagenicity Data: TA100 With S9

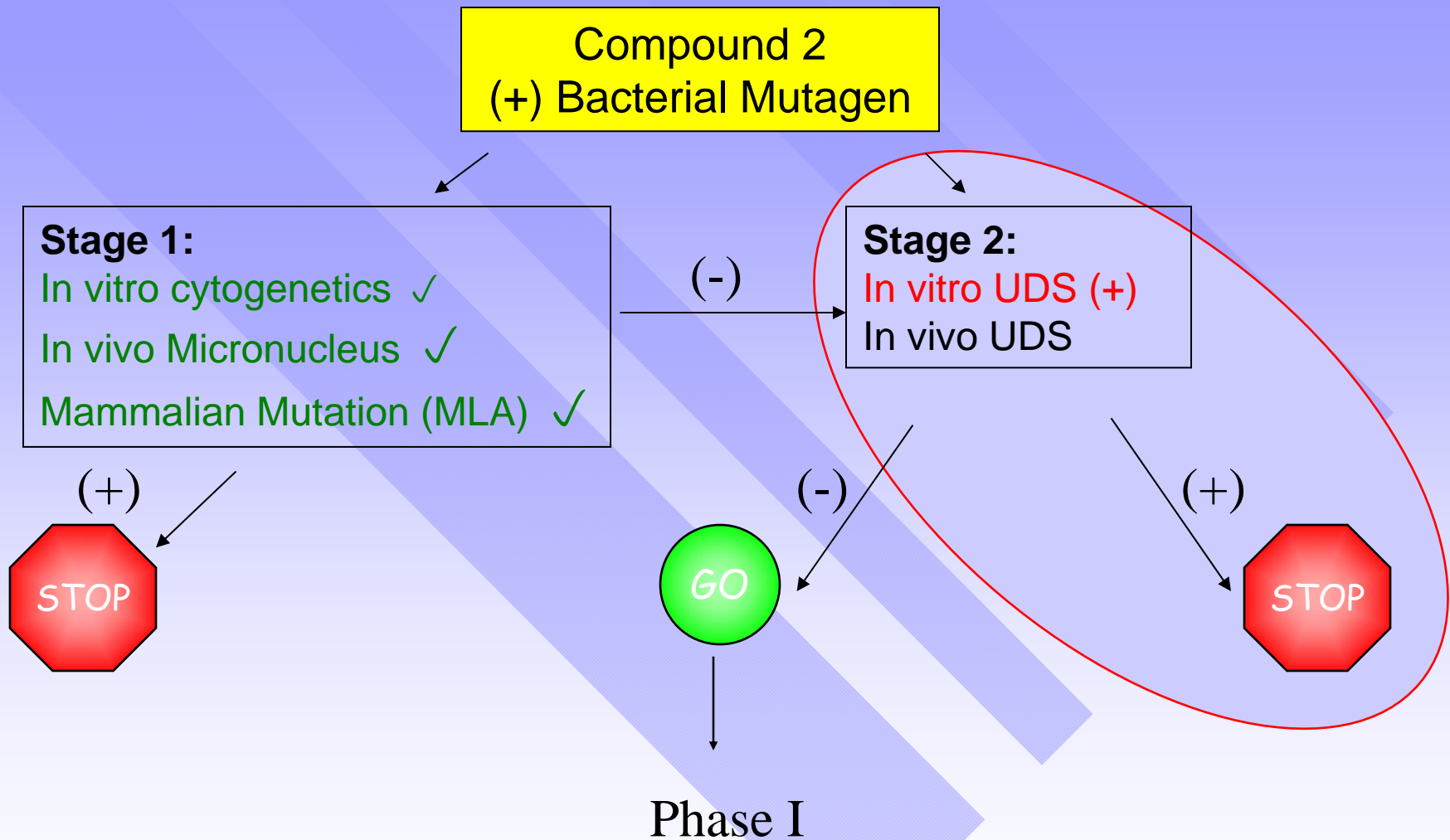


—◆— Compound 2 HCl Salt —■— Compound 2 Tartrate Salt

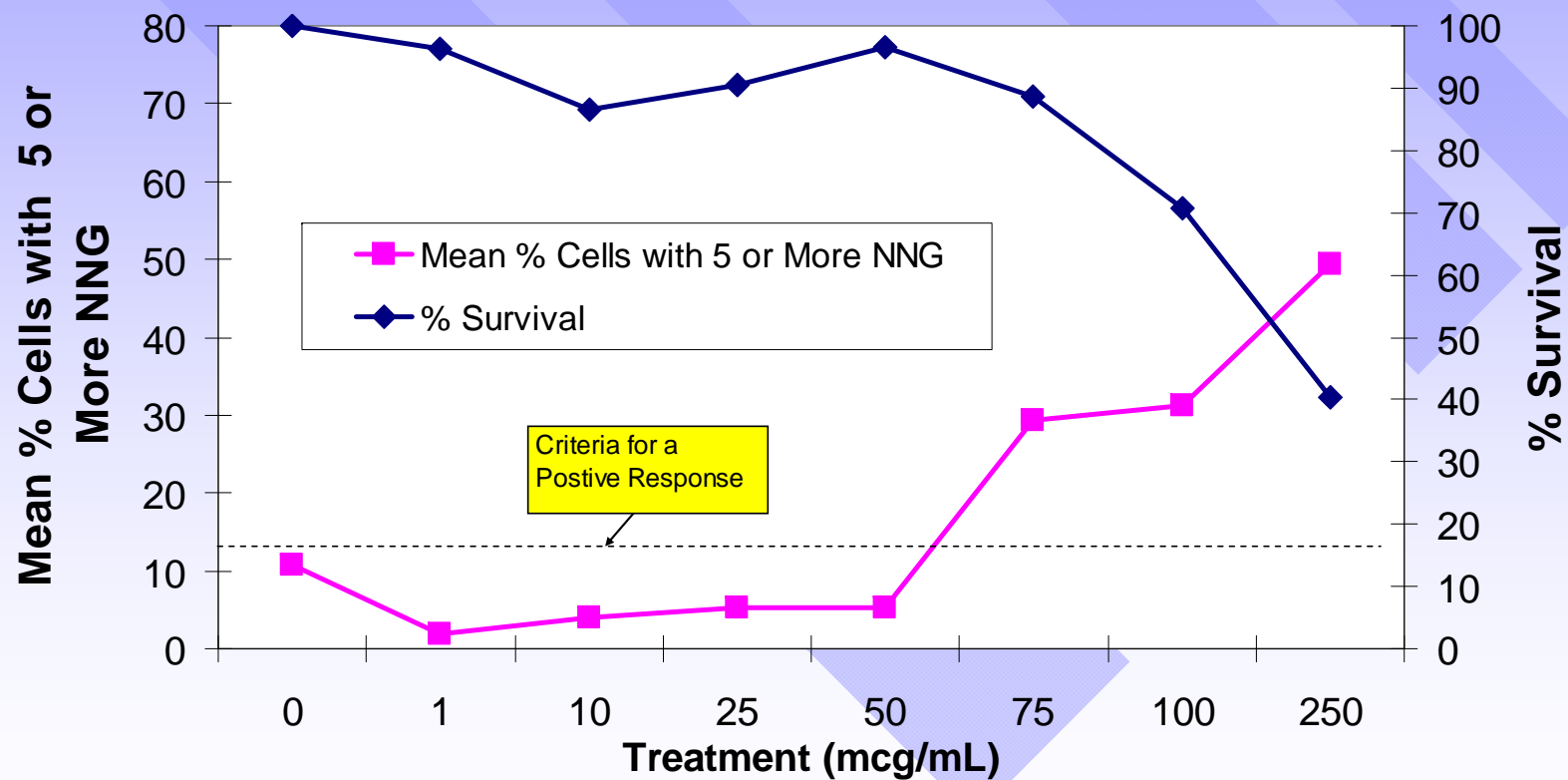
Follow-up Strategy for Compound 2

- Desire to bring compound to Phase I clinical trial for toleration and limited POC
 - Replace with a compound without mutagenic liability
 - Bring Compound 2 to full development
 - » Frontload reproductive and 2 year carcinogenicity studies or consider alternative approaches for an earlier readout
- Devised a staged follow-up strategy to get to Phase I
 - Establish WOE for lack of genotoxicity

Strategy to move to Phase I

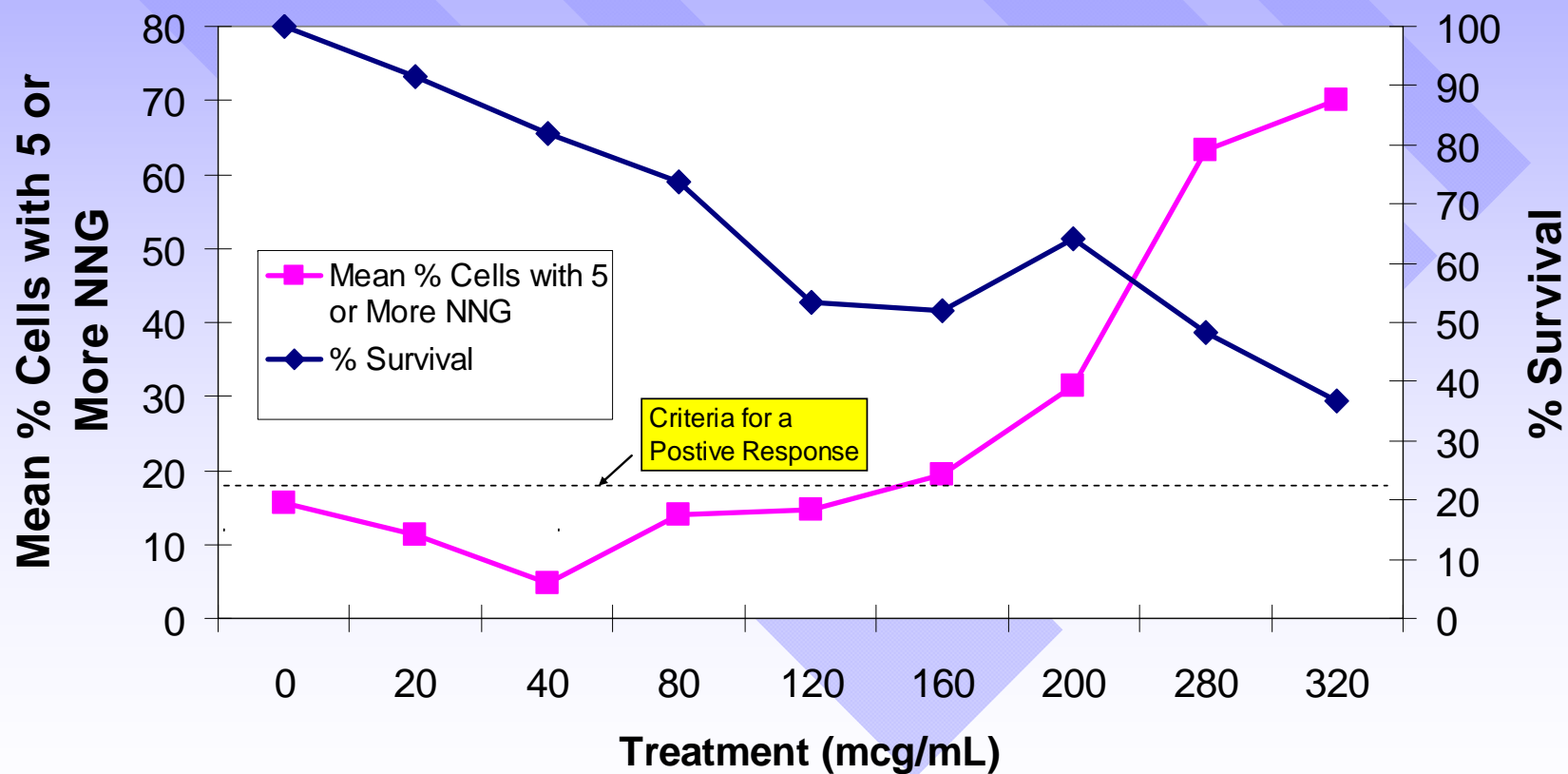


Compound 2 In vitro UDS Results – Trial 1



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Compound 2 In vitro UDS Results – Trial 2



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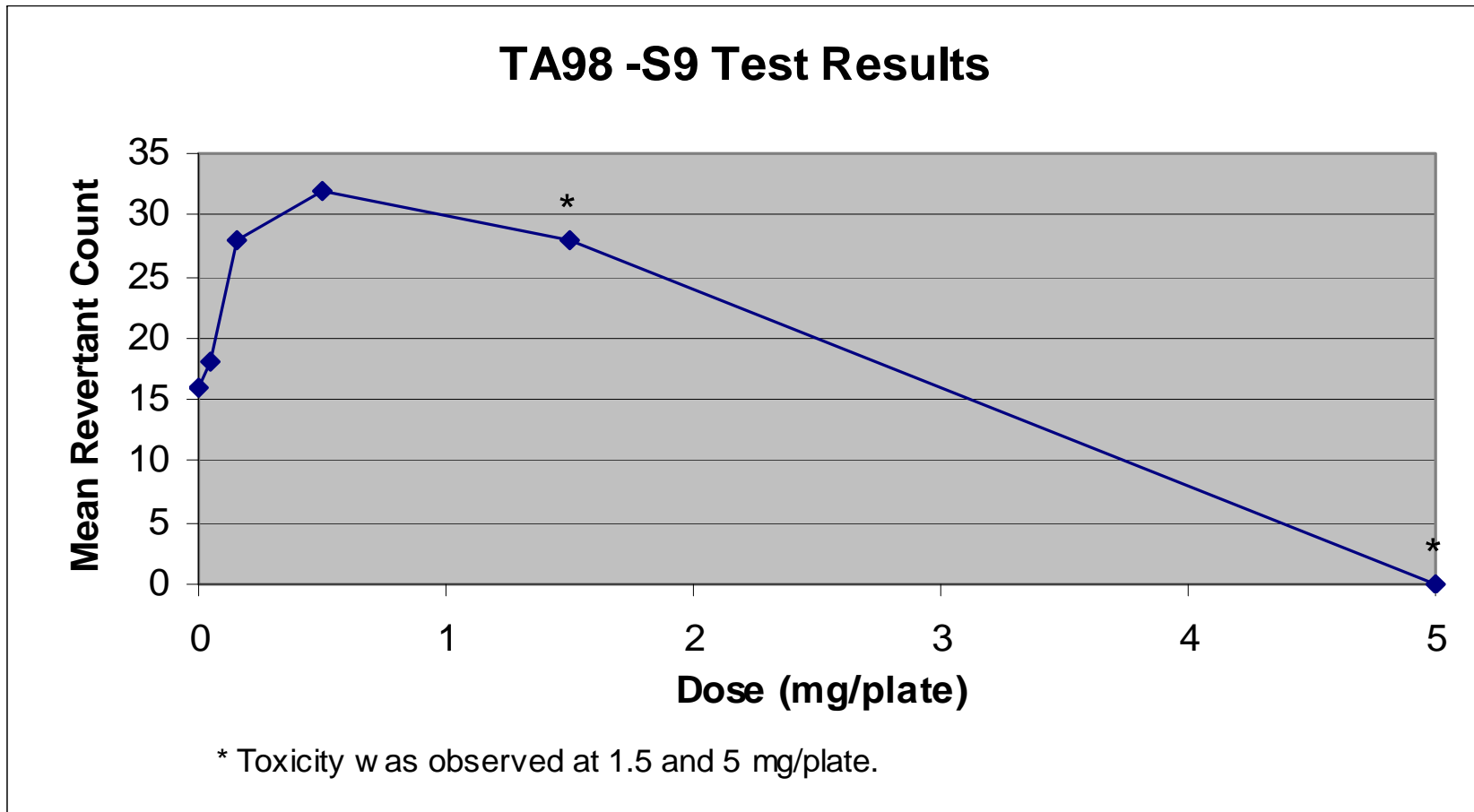
Compound 2 – Development Halted

- **Implications of Positive Response in In Vitro UDS Assay**
 - Positive predictivity for induction of cancer in rodent bioassay is 93 to 99% (Based on a data set of 275 Compounds).
 - “Observation of activity in both hepatocytes of rats and bacteria would be predicted as carcinogenic and mechanistically classified as genotoxic carcinogen” (Probst, et al., 1981)
 - » Based on study of 218 compounds for which both in vitro UDS and Ames data were available.
 - Data indicate that Compound 2 acts through a direct genotoxic mechanism, relevant to mammalian cells, for which no safe level of exposure can be established.

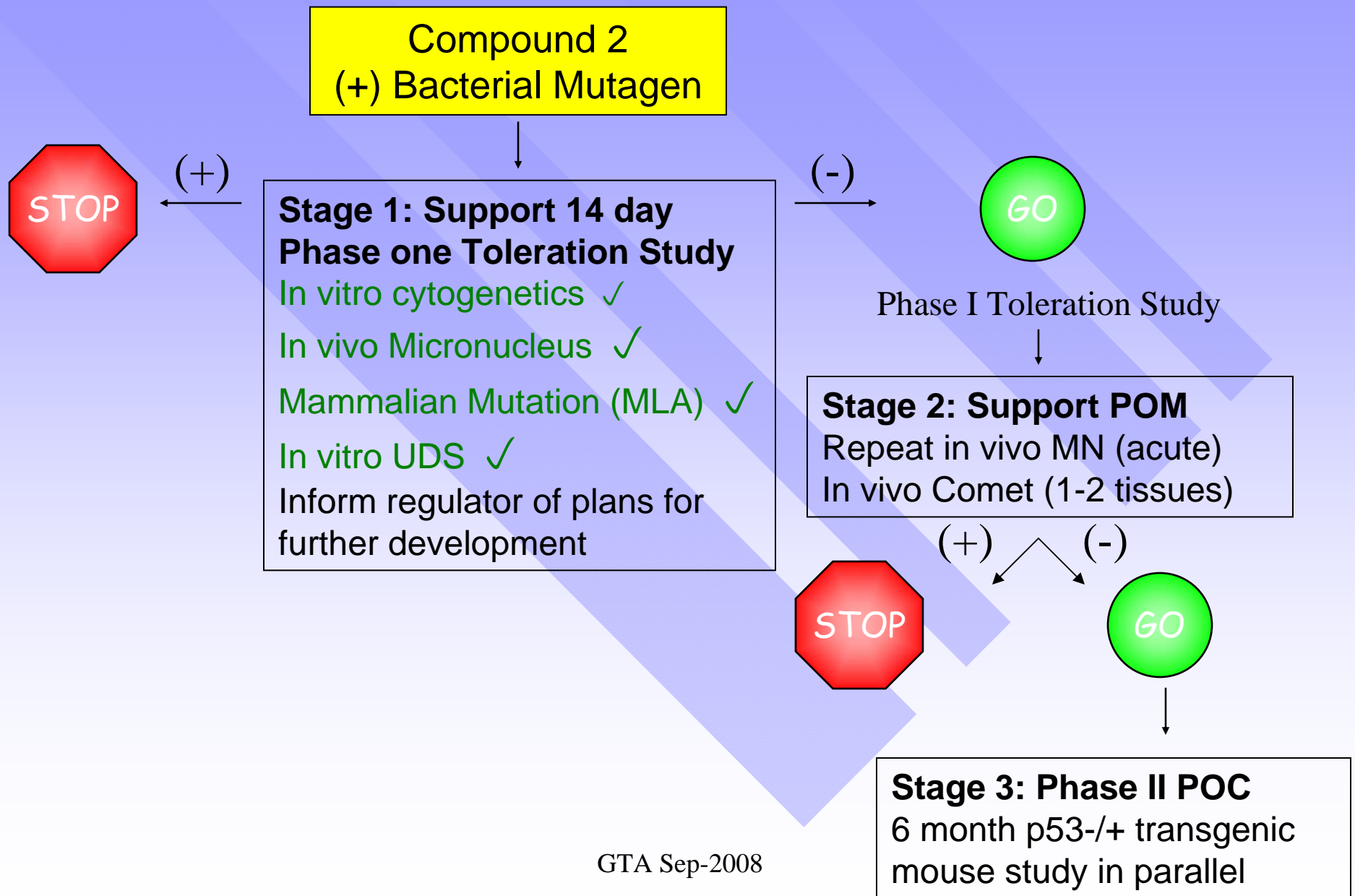
Compound 3

- Neuroscience compound for a non-life-threatening indication
- Intended for chronic use
- Negative BiolumAmes (TA98 & TA100)
- Reproducible weak increases (1.7 to 2-fold) in Ames Assay w/o S9 in TA98 at first non-toxic dose; Toxicity observed
- Negative *in vitro* micronucleus
- Devised a follow-up strategy to get to Phase I toleration and beyond to better understand target viability

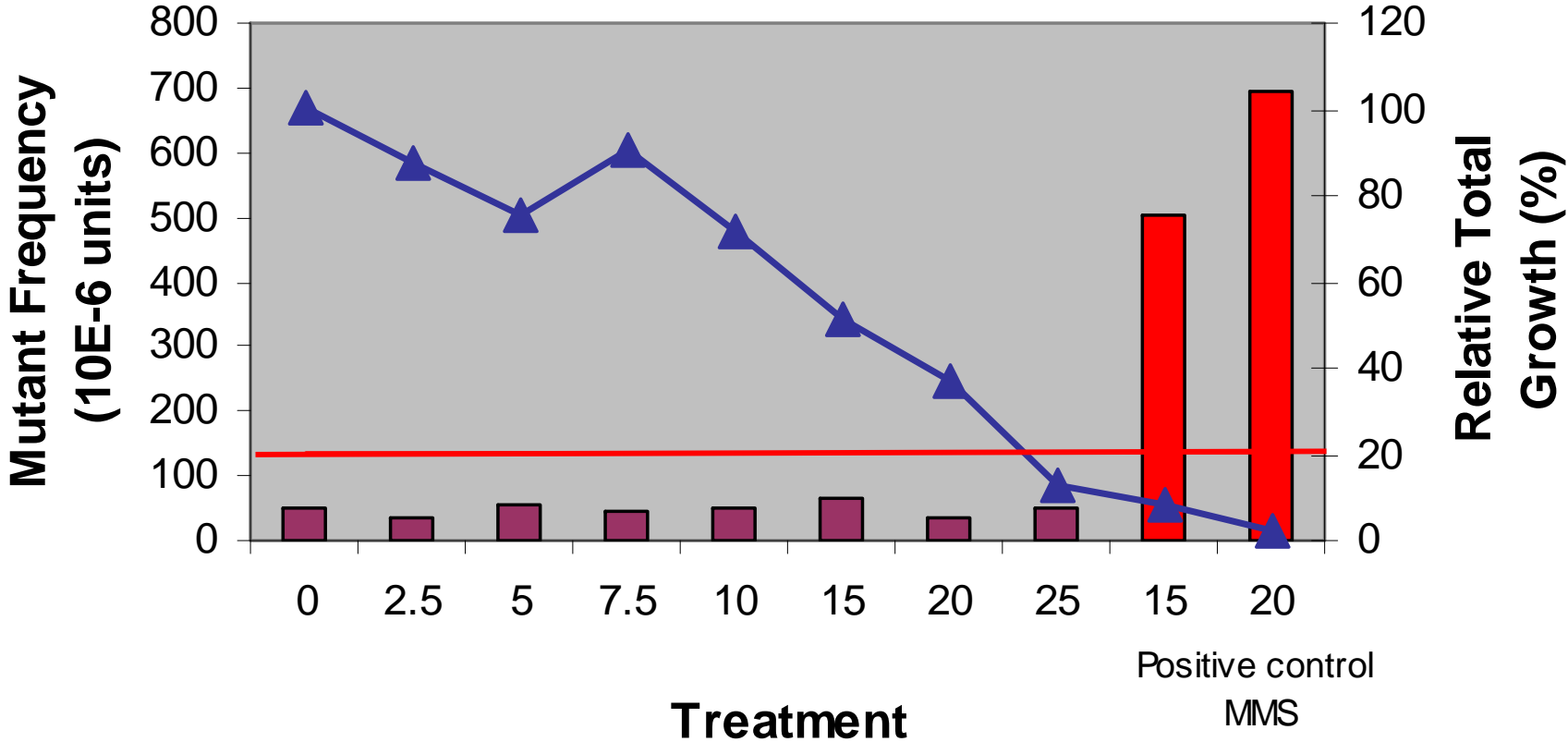
Compound 3: Ames results



Compound 3: Strategy for Clinical Development

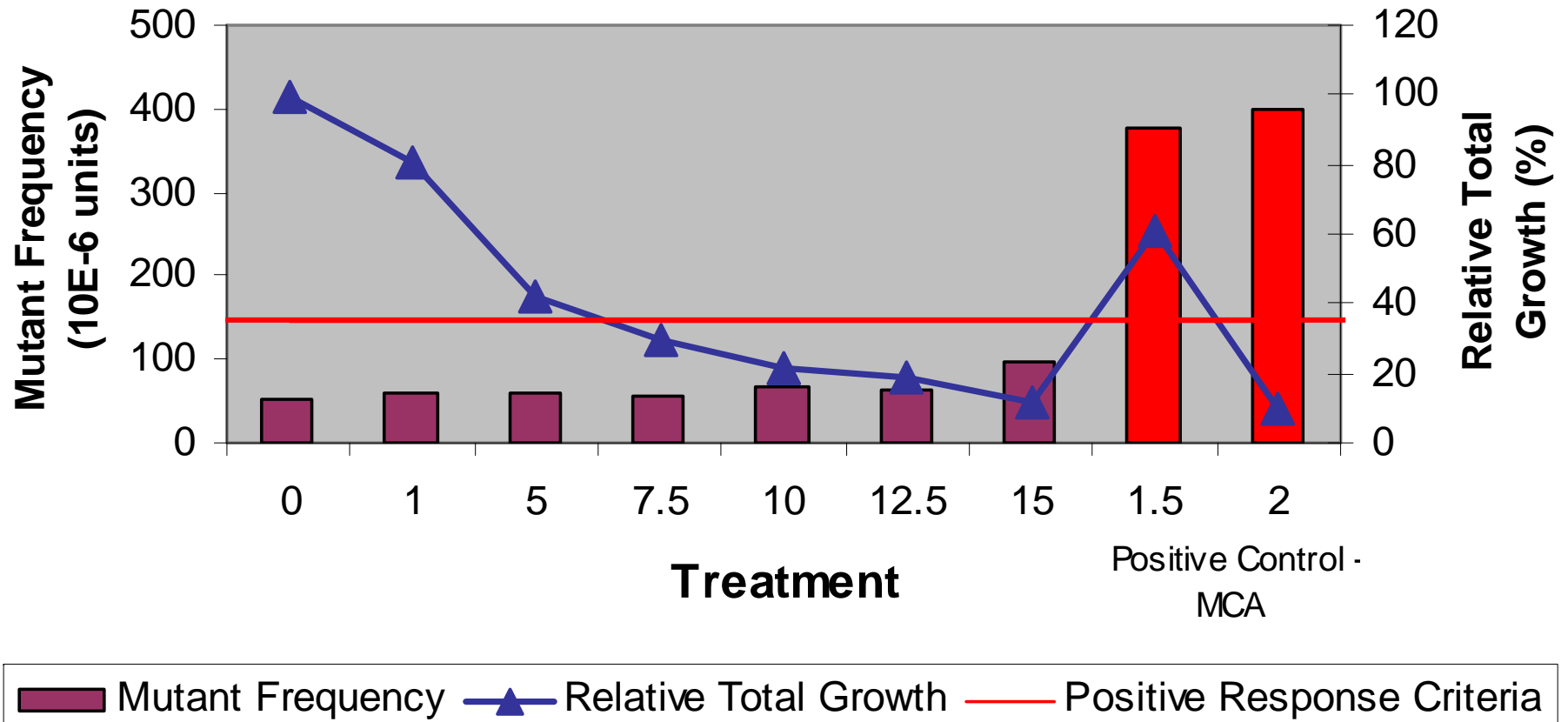


Compound 3: MLA Without Activation

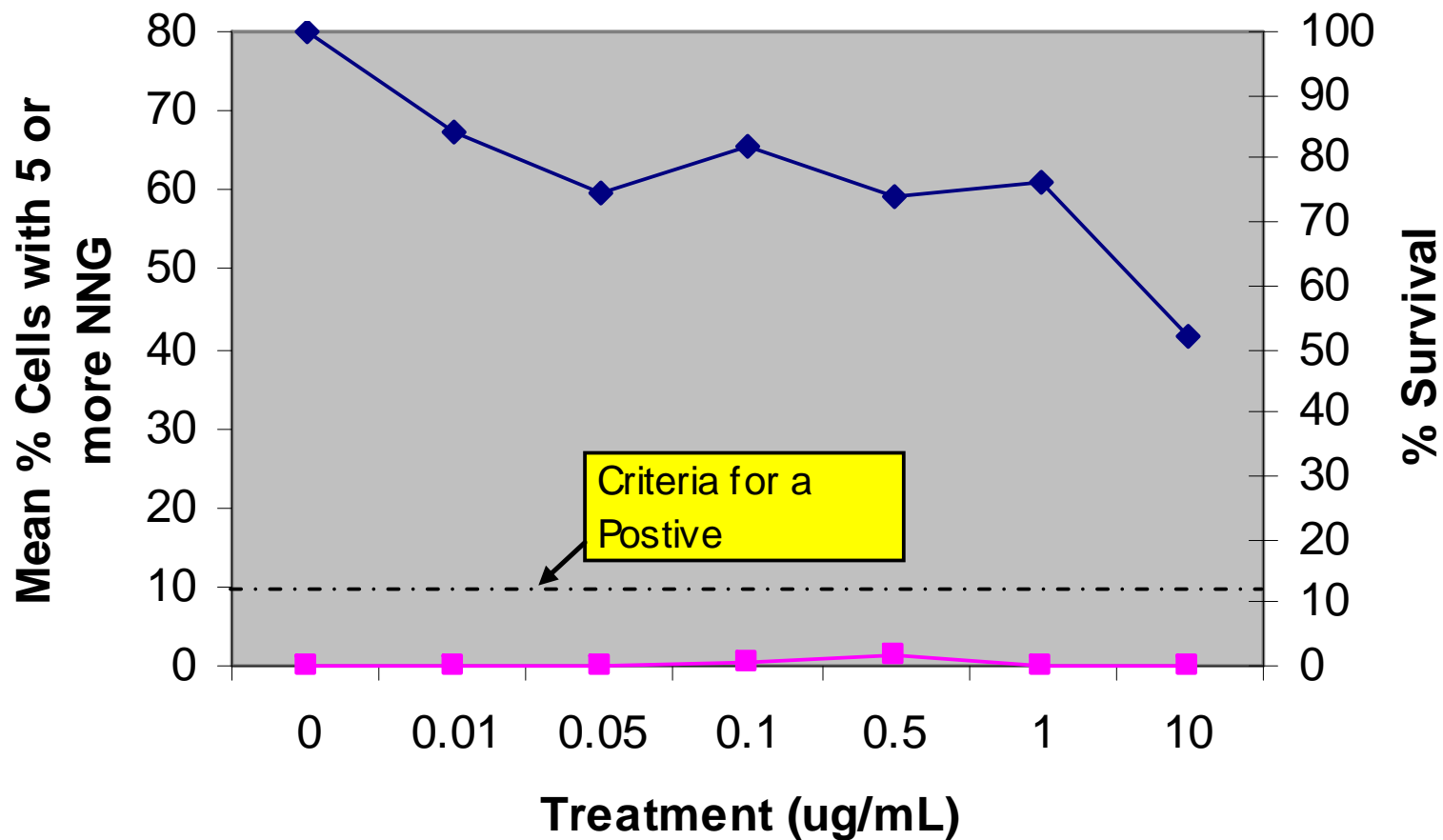


■ Mutant Frequency ▲ Relative Total Growth — Positive Response Criteria

Compound 3: MLA With Activation

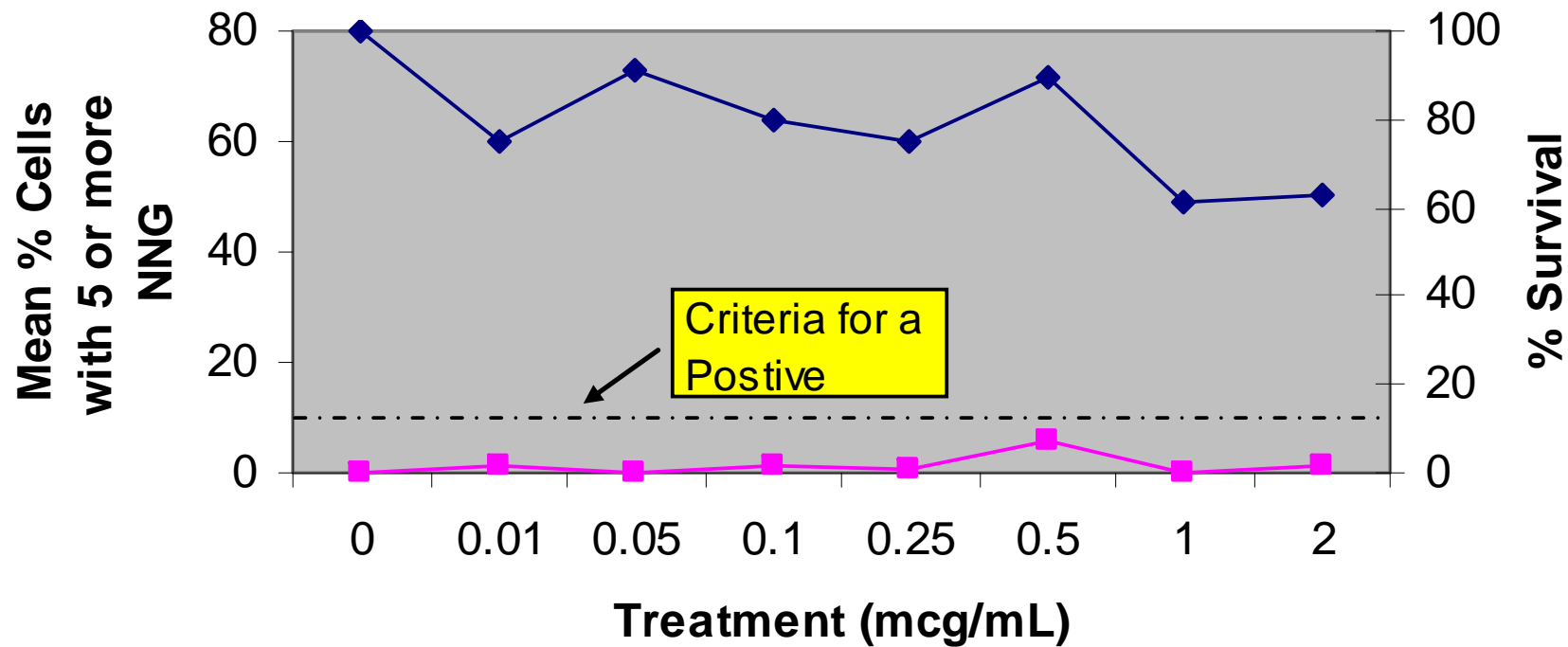


Compound 3 In vitro UDS Results - Trial 1



—◆— % Survival —■— Mean % Cells with 5 or more NNG

Compound 3 In vitro UDS Results - Trial 2



—◆— % Survival —■— Mean % Cells with 5 or more NNG

Questions for discussion

- What follow-up testing is necessary to demonstrate that a compound is a bacterial specific mutagen (e.g Compound 1 – gyrase inhibitor)? Is mechanistic data and the standard battery of testing sufficient (i.e. no second in vivo test)?
- Is the in vitro UDS a more appropriate follow-up for bacterial mutagens than other in vitro or in vivo mammalian assays? For compound 2, it provided evidence for genotoxicity despite negative results in the standard battery of in vitro and in vivo tests, including the MLA, while it was negative for compound 3.
 - » Was there any other testing that could have been done to support development of Compound 2 despite the positive UDS? For example, would a negative in vivo UDS have provided confidence for moving forward?
- Is one negative in vivo test sufficient to support a 14 day Phase I clinical study for a compound with a weak signal in the Ames Assay when several other in vitro tests and a single in vivo test are negative (e.g. compound 2)?