

Helix3 Inc.

Comet Assay

Case Study
Interpretation Problem

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Compound X

Ames Assay

Negative

- No statistically significant increases in revertant numbers
- No dose related increase
- Within HCD range
- Dosed up to solubility limit

Strain	S9	VC	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	PC
TA1535	-	11.2	14.6	11	9	12.2	10 [‡]	569
TA1537	-	10.6	4.6	13	11	9	3.2 [‡]	142.6
TA98	-	28	31.6	27	28	33.6	23.6 [‡]	89.6
TA100	-	161	156.2	147.2	139	133.6	139 [‡]	574.6
WP2 uvrA	-	48	46	49	49.6	44.2	41.6 [‡]	962.2
TA1535	+	9	9.2	11	12	10.2 [‡]	7.2 [‡]	105
TA1537	+	7	7.2	8	12.6	7.2 [‡]	7.6 [‡]	192.2
TA98	+	28.2	31.6	37.2	26	31.6 [‡]	29 [‡]	704.2
TA100	+	179.2	155.2	155.6	164.2	165.2 [‡]	159.2 [‡]	2006.2
WP2 uvrA	+	60	51.6	64	50.6	67.2	61 [‡]	145.6

[‡] Precipitation

Compound X

Mouse Lymphoma Assay

Negative

- Statistically significant increase in mutation frequency at highest dose 3hr +S9
- Dose related increase
- Outside of HCD range
- Dosed up to cytotoxic dose
- **No increase detected in repeat study**

Dose	RTG % of VC	Mutant frequency $\times 10^{-6}$	Mean small colony mutant frequency $\times 10^{-6}$
VC	-----	128	29
1	76.4	152	28
2	75.5	146	19
3	58.3	144	30
4	39.8	188	41
5	33.8	183	35
6	24.3	265*	71*
7	12.8 [‡]	-----	-----
PC	38.8	1197*	261*

*Mutant frequency > VC + Global eval factor

[‡] invalid dose due to low Relative total growth

Compound X

In Vivo PBL MN Assay

Negative

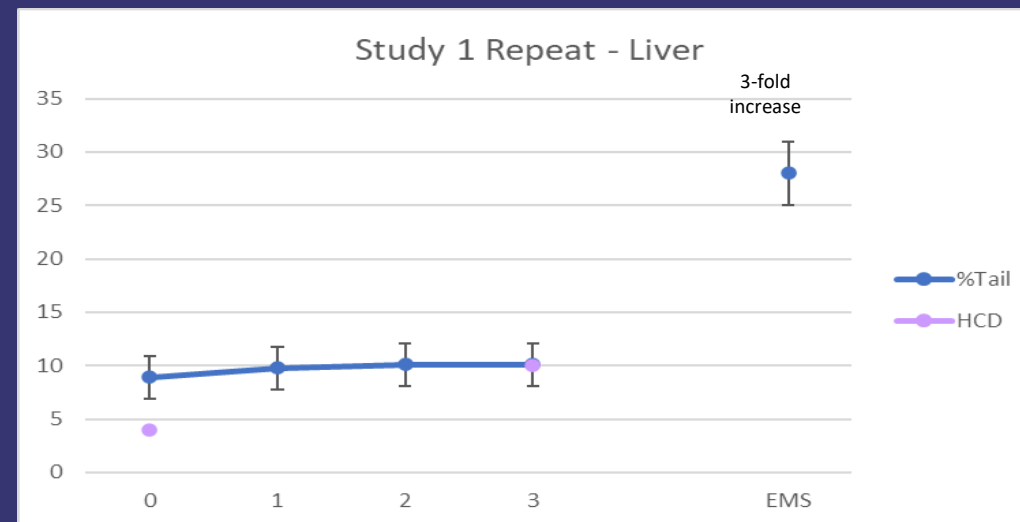
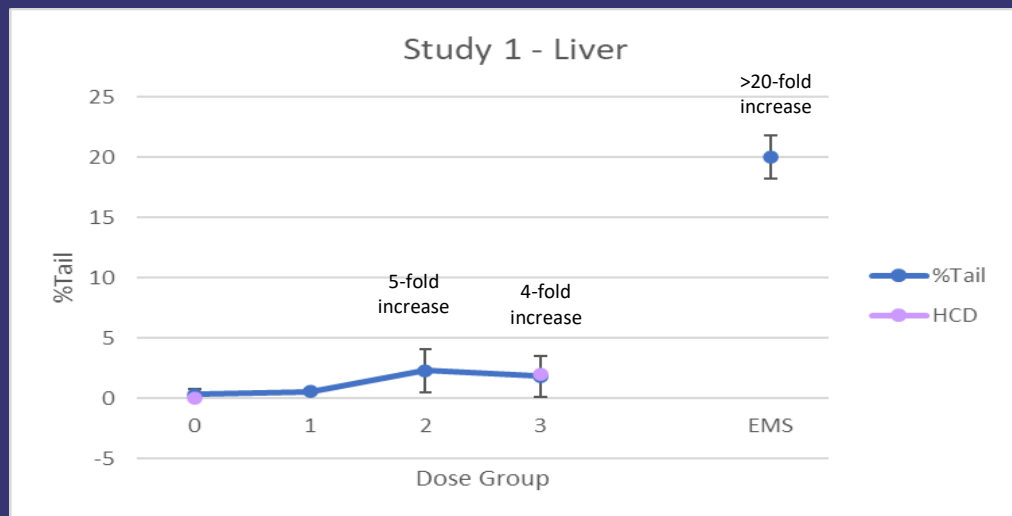
- Statistically significant increases in MN-PCEs at all doses
- No dose related increase
- All values within HCD range and below HCD mean value
- Dosed up to MFD with detected plasma concentrations

Dose	%PCEs	% MN-PCEs
VC	2.13	0.09
1	2.14	0.12*
2	2.35	0.13*
3	2.22	0.14*
PC	0.74*	1.94*

*Statistically significant at $p < 0.001$

Compound X

In Vivo Liver Comet Assay



Positive

- Statistically significant increases in %Tail
- Dose related increase
- All values within HCD range
- Dosed up to MTD with detected plasma concentrations
- Dosed and processed in order of dose

Negative

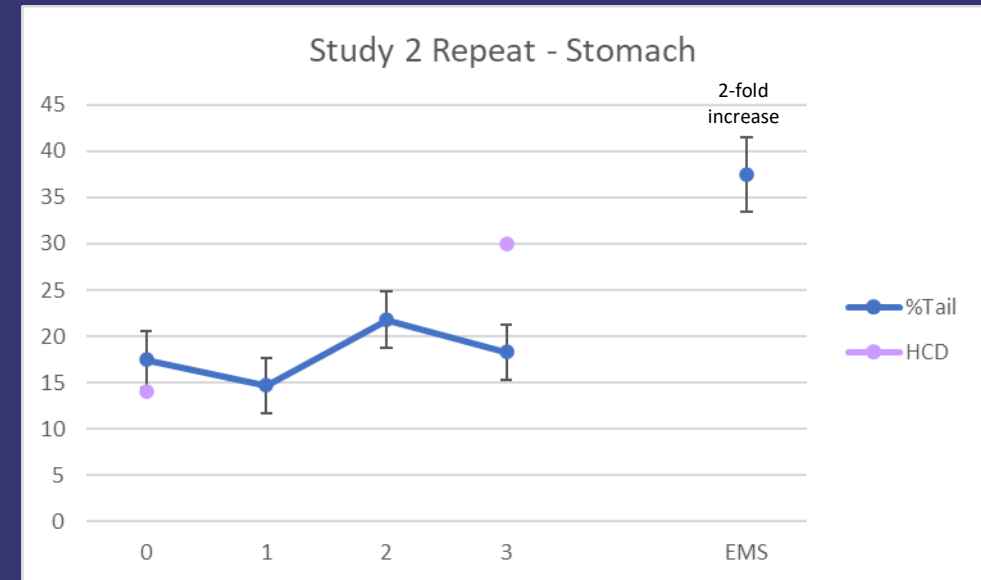
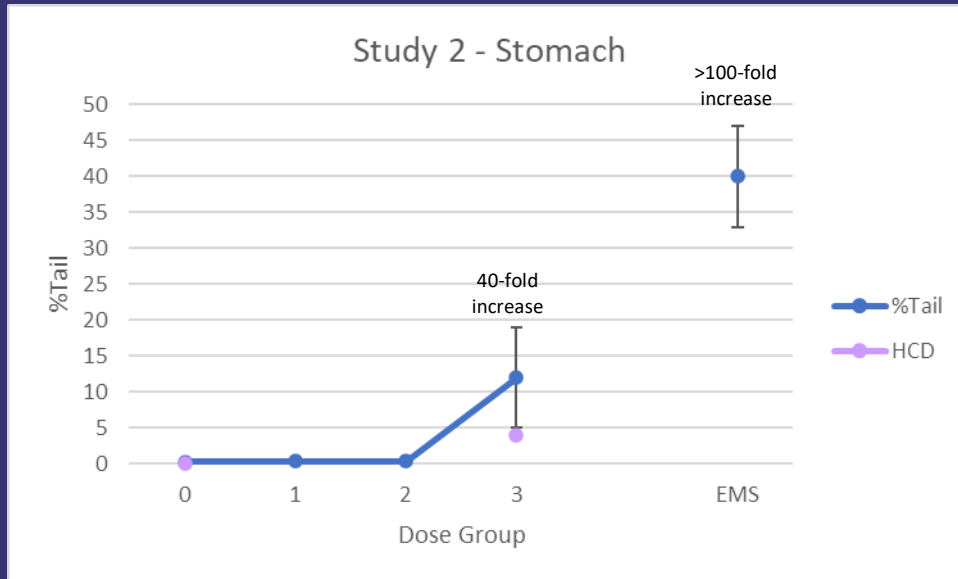
- No statistically significant increases in %Tail
- No dose related increase
- All values within HCD range
- Dosed up to MTD with higher plasma concentrations
- Dosed and processed in rotating order or block design

What is the conclusion?



Compound Y

In Vivo Stomach Comet Assay



Genetox profile

- Ames negative
- In vitro Chrom Ab positive but only at high dose with ppt
- In vivo BMMN negative

Notes

“One complication with %Tail DNA is that the presence of zero values would complicate statistical analysis...There are suggestions that negative control cells should have between 10 and 20% DNA in [the] tail which would obviate statistical problems.”

Lovell, David P., and Takashi Omori. (2008) Statistical Issues in the use of the comet assay. *Mutagenesis* Vol 23 (3) 171-182

“The best test of whether cells are in a satisfactory condition for comet assay analysis is that control, untreated cells should give comets with a background level of breaks (i.e., mostly class 0 [for visually scored cells] or ~10% of DNA in the tail [for image analysis scored cells].”

Collins, Andrew (2004) The comet assay for DNA damage and repair. *Molecular Biotechnology* Vol 26: 249-257