Evaluation of Historical Control Liver and Duodenum Data from the Big Blue® TGR Assay - Is Sampling Day A Variant Of Concern?

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Introduction

OECD 488 compliant Big Blue® rat transgenic rodent (TGR) studies to support regulatory submissions have been running at Gentronix since May 2022. During this time reasonably large negative and positive historical control databases (HCDs) of mutant frequency (MF) data for the most frequently analysed tissues, liver and duodenum, have been generated across both TGR study designs - 28+28 day(d) and 28+3d.

A good HCD can be an invaluable data source to help with contextualising and differentiating between biologically/ non-biologically relevant genotoxicity data.

A recent publication by the IWGT (Dertinger et al., 2023) provides clear guidance on assessing the quality of negative control HCDs to ensure that a robust HCD is used to inform on data interpretation.

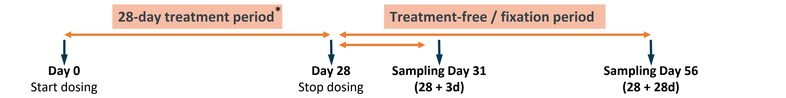
This poster presents the findings following evaluation of Gentronix historical control liver and duodenum data from the Big Blue® TGR assay. Analyses form a smaller HCD compiled with germ cell MF data from testes (28+28 d) is also presented for comparison.

Questions and Aims

- **Are Gentronix TGR data under control and stable over time?**
  - Generate control charts on liver and duodenum negative and positive control data.
  - Generate control charts on germ cell negative control data

    Control limits: mean ±3 standard deviations (SD); warning limits: mean ±2 SD (SD derived using the average moving range, i.e. calculate the average of the differences between successive mean MF, then divide by an unbiasing constant)
- **What proportion of the total variability within the TGR data is attributable to inter-study or inter-animal variability?**
  - Conduct variance component analyses (VCA) to understand the level of inter-animal and inter-study variability on MF data.
- **Is sampling day (+3d or +28d) a source of variance when assessing mutant frequency in Big Blue® rats?**
  - Conduct VCA on liver and duodenum MF data including sampling day as a study variable

Study Design and Datasets



*Groups:
- Negative control
- Positive control (ENU 20 mg/kg): Days 1, 2, 3, 10, 19, and 26 only

Gentronix Datasets		Number of Animals			Number of Studies		
		Total	28 + 3d	28 + 28d	Total	28 + 3d	28 + 28d
Liver	Negative control	188	92	96	35	17	18
	Positive control	183	87	96	35	17	18
Duodenum	Negative control	180	95	85	34	17	17
	Positive control	181	88	93	34	17	17
Germ cells	Negative control	47	-	47	9	-	9

Control Charts for Negative Control TGR Mutant Frequency Data and VCA Analyses

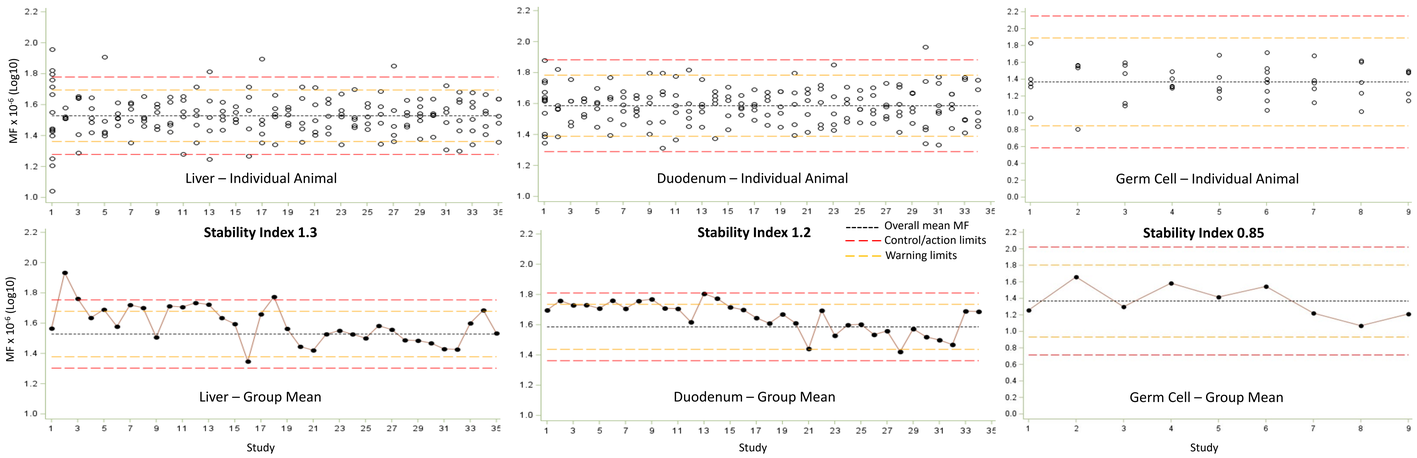


Figure 1 Individual animal and group mean control charts for liver, duodenum and germ cell negative control mutant frequencies (log_{10} transformed) by study

VCA Analyses – Negative Control Data

Tissue	Source	Variance Component	% of Total
Liver	Between Study (study to study)	0.01159	36%
	Sampling Day (+3d or +28d)	0	0%
	Within Study (animal to animal)	0.02036	**64%**
Duodenum	Between Study (study to study)	0.00740	30%
	Sampling Day (+3d or +28d)	0	0%
	Within Study (animal to animal)	0.01690	**70%**
Germ cells	Between Study (study to study)	0.02789	33%
	Within Study (animal to animal)	0.05624	**67%**

Negative Control Data Summary

- Inter-animal variability was the major source of variance in liver (64%), duodenum (70%) and germ cells (67%)
- Negative control MF data considered stable over time in all tissues: liver (SI: 1.3), duodenum (SI: 1.2), germ cells (SI: 0.85)
- Sampling day was not a source of variance in liver or duodenum (p=0.135 in liver and p=0.720 in duodenum)

Control Charts for Positive Control TGR Mutant Frequency Data and VCA Analyses

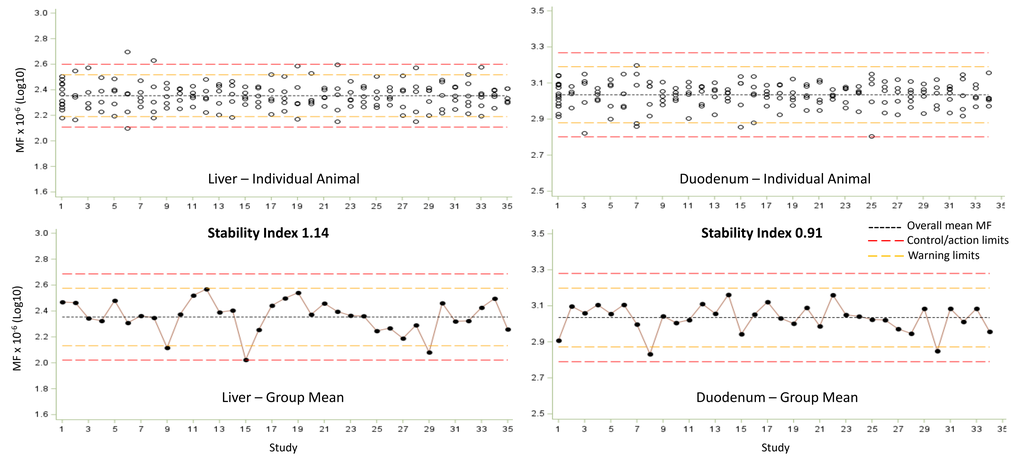


Figure 2 Individual animal and group mean control charts for liver and duodenum positive control mutant frequencies (log_{10} transformed) by study

Positive Control Data Summary

- Sampling day was a major source of variance in liver (p=0.0003), not in duodenum (p=0.512)
  - Recommend to have separate positive control HCDs by sampling day for slowly dividing tissues
- Positive control MF data is stable over time in liver (SI: 1.14) and duodenum (SI: 0.91)

VCA Analyses – Positive Control Data

Tissue	Source	% of Total Variance: All	+3d	+28d
Liver	Between Study (study to study)	25%	50%	23%
	Sampling Day (+3d or +28d)	37%	-	-
	Within Study (animal to animal)	38%	50%	77%
Duodenum	Between Study (study to study)	48%		
	Sampling Day (+3d or +28d)	0%		
	Within Study (animal to animal)	52%		

CONCLUSION

Analyses confirmed inter-animal variability was the major source of variance in both liver, duodenum and germ cell negative control mutant frequency data and this was evident even in tissues with smaller HCDs; sampling day was not a source of variance. Having a combined (including 28+3d and 28+28d study data) negative control HCD is therefore justified for both rapidly and slowly proliferating tissues in the Big Blue® rat TGR assay and is beneficial for laboratories trying to build robust HCDs of ≥ 20 studies and ≥100 animals. Based on these analyses, Gentronix has a strong degree of confidence in using its negative control HCD data in contextualising and differentiating between biologically/non-biologically relevant TGR study data.

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