Exploration of Toxicity Cut-Offs in the *In Vitro* Micronucleus (MNT) Assay How Close is Close Enough?

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In vitro MNT (OECD 487) Cytotoxicity Limit is 55±5%

Has this limit ever caused you to question how to proceed with a study?

Has this limit ever caused you to ask yourself: “Is this close enough”?

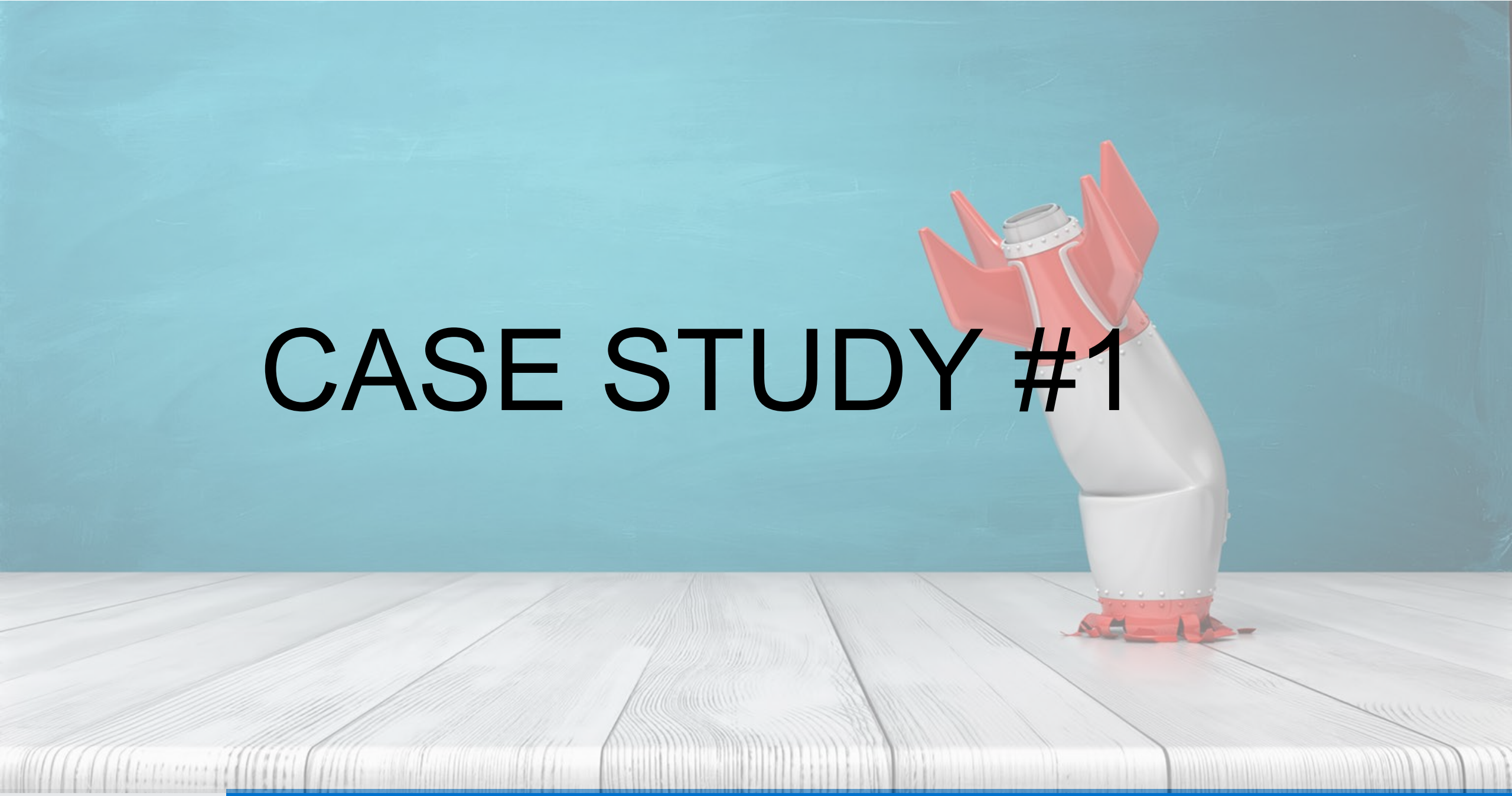
If so, you are not alone.

In this presentation we will explore 2 independent case studies.

In all case studies:

- Cytokinesis Block Proliferation Index (CBPI) was used to calculate cytotoxicity.
- The CBPI are presented as the average of duplicate cultures.
- Cytotoxicity is presented as percentage.

CASE STUDY #1



Treatment	Average CBPI	% Cytotoxicity
Vehicle	1.59	NA
0.02 µg/mL	1.65	-10.4
0.05 µg/mL	1.61	-3.2
0.1 µg/mL	1.56	4.4
0.15 µg/mL	1.52	11.9
0.2 µg/mL	1.58	0.9
0.25 µg/mL	1.39	33.0
0.3 µg/mL	1.32	45.3
0.35 µg/mL	1.22	63.0
0.4 µg/mL	1.11	80.6
0.45 µg/mL	1.08	86.2
0.5 µg/mL	1.04	93.5

If you obtained the results in the green box, would you:

Repeat with intermediate concentrations?

Choose 0.3 µg/mL as the top concentration for MN evaluation?

Include both 0.3 and 0.35 µg/mL in the MN evaluation?

Other?

Exploring the Choices

Our choice :

- Repeat with intermediate concentrations?
- Choose 0.3 µg/mL as the top concentration for MN evaluation?
- Include both 0.3 and 0.35 µg/mL in the MN evaluation?
- Other?

Formulation, technical and biological variability may lead to the targeted concentrations not being obtained due to the close spacing of the selected intermediate concentrations.

Since this concentration was below the 55±5% cytotoxicity window, it could be questioned if the MN evaluations were conducted at a high enough concentration, especially if a negative result is obtained.

Evaluating concentrations that exceed 60% are not recommend by the guideline. Cytotoxicity could lead to unclear responses.

What other avenues do you think could be explored?

CASE STUDY #2

What do you think the path was for this data set?

Treatment	Average CBPI	%Cytotoxicity
Vehicle	1.46	NA
0.001 µg/mL	1.53	-15.4
0.002 µg/mL	1.52	-12.2
0.005 µg/mL	1.63	-37.2
0.007 µg/mL	1.54	-16.3
0.01 µg/mL	1.53	-15.2
0.03 µg/mL	1.49	-6.1
0.05 µg/mL	1.58	-25.2
0.07 µg/mL	1.47	-2.8
0.1 µg/mL	1.48	-3.3
0.3 µg/mL	1.12	73.5
0.5 µg/mL	1.03	94.6
0.7 µg/mL	1.00	99.3
1 µg/mL	1.00	99.3

Repeated with Intermediate Concentrations

Treatment	Average CBPI	%Cytotoxicity
Vehicle	1.51	NA
0.01 µg/mL	1.57	-11.9
0.025 µg/mL	1.55	-7.2
0.05 µg/mL	1.64	-24.3
0.1 µg/mL	1.55	-8.4
0.12 µg/mL	1.53	-3.7
0.14 µg/mL	1.46	10.8
0.16 µg/mL	1.54	-5.1
0.18 µg/mL	1.34	32.7
0.2 µg/mL	1.34	32.7
0.24 µg/mL	1.21	59.1
0.26 µg/mL	1.16	69.5
0.28 µg/mL	1.12	76.5
0.3 µg/mL	1.11	79.5
0.5 µg/mL	1.02	95.3

Concluding Thoughts

When selection of concentrations for micronuclei evaluation are based on the cytotoxicity profile of a test material, hitting the targeted range prescribed by the OECD 487 guideline can prove to be challenging.

There is not a one size fits all approach that can be taken unless that approach is to perform repeat experiments until the appropriate cytotoxicity level is achieved, but is that really feasible?

If you do perform repeat testing with intermediate concentrations, is there a point where you would say the spacing between concentration levels is too close?

Thoughts

Questions