Usefulness of Integrating the Micronucleus Test with Repeat Dose Toxicity Studies

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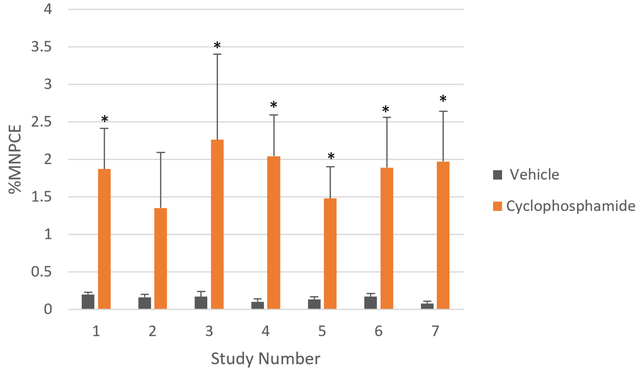
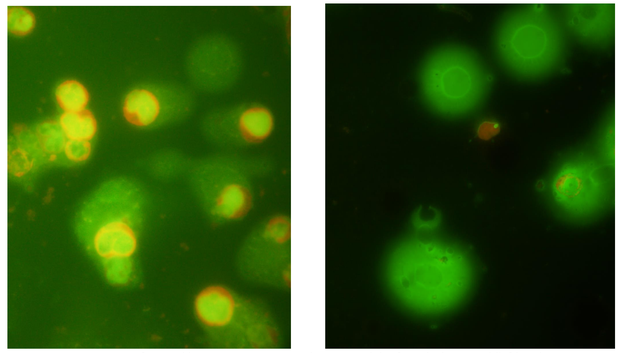
Figure 1. Group Mean %MNPCE Data





Representative images of stained bone marrow micronucleus assay slides for vehicle treated (left) and positive control treated (right) female rats

Table 1: Data from Vehicle Control Treated Female Animals

	Individual Animals		Studies	
	%PCE	%MNPCE	%PCE	%MNPCE
N	41	41	7	7
Mean	52.17	0.14	52.29	0.14
SD	3.63	0.05	1.27	0.04
95% UCL	59.43	0.25	54.83	0.22
95% LCL	44.91	0.03	49.74	0.07
Max	64.60	0.28	53.50	0.20
Min	45.80	0.05	49.50	0.08

Table 2: Data from Cyclophosphamide Treated Female Animals

	Individual Animals		Studies	
	%PCE	%MNPCE	%PCE	%MNPCE
N	32	32	7	7
Mean	48.76	1.88	49.19	1.84
SD	4.77	0.68	3.15	0.29
95% UCL	58.31	3.23	55.49	2.43
95% LCL	39.21	0.53	42.88	1.25
Max	57.60	4.03	53.10	2.26
Min	37.40	0.83	43.70	1.35

Figure 2. Comparison of Individual Vehicle Control Animal %MNPCE to Legacy Historical Control Values

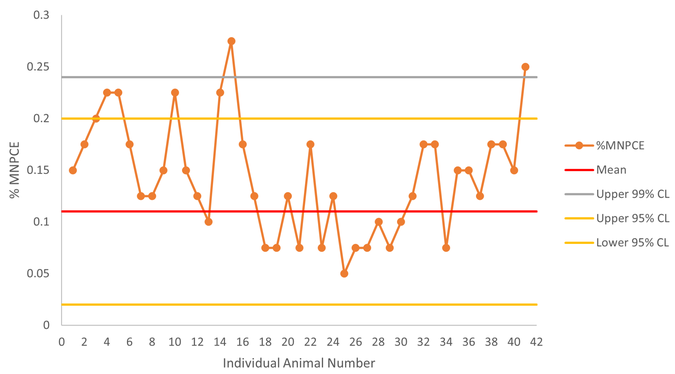
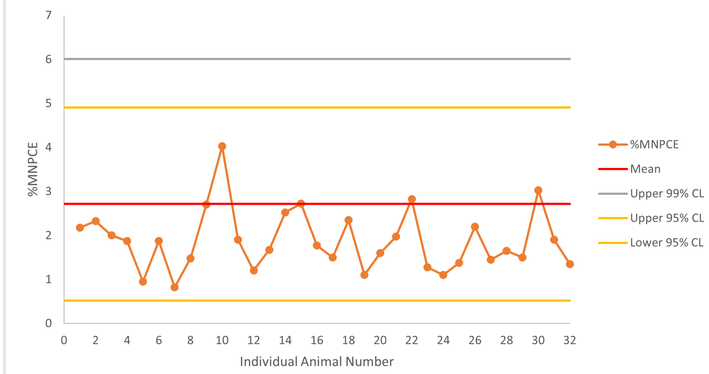


Figure 3. Comparison of Individual Cyclophosphamide Treated Animal %MNPCE to Legacy Historical Control Values



Introduction

In vivo genotoxicity assessment in rodents plays a critical role in the evaluation of hazard, safety, and subsequent risk assessment of test compounds, thus, enabling comprehensive safety data for regulatory submissions. Although an independent testing guideline exists for micronucleus assay (OECD 474), integration of this endpoint within a general toxicity study may provide additional information in the same study setup. This approach not only minimizes the number of animals used (3R concept), but it also considers aspects of a living system which are more relevant for risk assessment. We have evaluated multiple proprietary compounds using this integrated screening approach. In each study, female rats were subcutaneously or orally administered test compound for 5 days. Cyclophosphamide was administered intraperitoneally or orally 24 hours prior to sacrifice. Bone marrow smears were prepared and scored using standard Acridine orange method. Slides were evaluated for toxicity (%PCE) per 500 cells and for the presence of micronuclei (MNPCE) per 4000 cells. In the vehicle control group, the %PCE was approximately 50% for both vehicle and positive controls. The number of MNPCE observed was ≤ 10 for vehicle control and > 20 for positive control. The positive control group showed significant induction of MNPCE compared to vehicle control. This study demonstrates significant value of integrating micronucleus evaluation in bone marrow with repeat dose toxicity studies, in a variety of scenarios, to obtain genotoxicity information. This study also demonstrates our capability of conducting in vivo genotoxicity assessment at our newly established genetic toxicology division within Inotiv.

Materials

Female Sprague-Dawley rats (8 weeks, 150 grams at initiation) were obtained from Charles River Breeding Labs (Raleigh, NC).

Cyclophosphamide (CP), methylcellulose (400 cPs), Tween 80, sodium citrate, citric acid, 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD), 0.9% saline, sterile water for injection, 200 proof ethanol, fetal bovine serum, and Acridine Orange were purchased from Sigma Aldrich (St. Louis, MO). All other reagents and media were of the highest available grade.

Methods

Vehicle control groups consisted of 5 to 6 female animals. Positive control groups consisted of 2 to 5 female animals. CP was administered at 30 mg/kg body weight. The vehicles were 30% w/v 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) in water, pH 8.0 ± 0.2 or 0.5% methylcellulose and 0.1% Tween 80 in 30mM citrate buffer, pH 3.5 ± 0.2. The dose volume was 5 mL/kg. Vehicles were subcutaneously or orally administered for 5 days. CP was intraperitoneally or orally administered once approximately 24 hours prior to necropsy. Vehicle controls were administered subcutaneously in studies 1 to 3 and were administered orally in studies 4 to 7. Positive controls were administered intraperitoneally in studies 1 to 3 and were administered orally in studies 4 to 7.

All animals were euthanized by CO_2 inhalation and femoral bone marrow was aspirated into a syringe containing fetal bovine serum. The bone marrow was transferred to tubes containing additional fetal bovine serum, centrifuged, and resuspended cells were spread onto clean glass slides. Slides were stained with Acridine Orange, coded, and evaluated by fluorescent microscopy.

Results

Figure 1: Group mean %MNPCE values in vehicle an CP treated animals in the 7 studies performed. All but one study had a statistically significant increase between the vehicle and positive control treated groups (student's t-test, $p<0.01$). The positive control group in study number 2 had two animals and only demonstrated a non-statistically significant increase in MNPCE.

Figure 2: Comparison of individual vehicle control animal %MNPCE to legacy historical control values. The legacy historical vehicle control mean is 0.11% with upper 95% and 99% control limits of 0.2% and 0.24%, respectively.

Figure 3: Comparison of individual Cyclophosphamide treated animal %MNPCE to legacy historical control values. The legacy historical positive control mean is 2.72% with upper 95% and 99% control limits of 4.91% and 6.01%, respectively.

Conclusions

- Data collected at Inotiv's newly established genetic toxicology laboratory was found to be largely within legacy historical control limits for %MNPCE in both vehicle and positive control treated animals
- The significant response of the positive control was observed irrespective of the route of administration used in the assay when suitable numbers of animals were included in each group.
- A micronucleus test can be incorporated into general toxicity studies to concurrently detect damage to the chromosomes or mitotic apparatus of erythroblasts.

References

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