In Vivo

Pig-a Gene Mutation Assay using N-Nitroso-N-Ethylurea and Benzo(a)Pyrene

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Introduction

The rodent Pig-a assay is being increasingly recognized in the scientific community for its unique advantages. Notably, it requires minimal blood volume, as even a microlitre contains millions of red blood cells and a small proportion of reticulocytes. This assay is particularly useful for repeated blood sampling without euthanizing the animal, making it an ethical choice that aligns with the 3Rs principles (Replacement, Reduction, and Refinement) in animal testing. Its compatibility with standard rodent models and its application in regulatory safety assessments enhance its appeal for *in vivo* mutation studies. The Pig-a assay is effective for investigating somatic gene mutations and complements bacterial mutation assays in regulatory safety evaluations. It integrates well into various toxicology studies, as outlined by OECD Test Guidelines, making it a vital tool for assessing mutagenicity and exploring genotoxic potential identified in other *in vitro* systems. Our study focuses on evaluating the Pig-a gene mutation in Wistar rats exposed to N-Nitroso-N-Ethylurea and Benzo(a)Pyrene following three days of consecutive dosing.

Principle

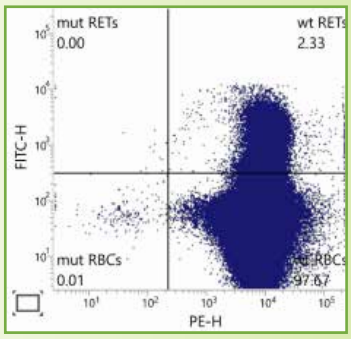
The assay is centered around the Pig-a gene, crucial for producing N-acetylglucosaminyl phosphatidylinositol (GlcNAc-PI), the foundational compound in GPI anchor biosynthesis. The GPI anchor is vital for attaching proteins to blood cell surfaces. While GPI anchor synthesis involves around 30 genes, the Pig-a gene, located on the X-chromosome, is unique in being a single functional copy. Mutations in the Pig-a gene primarily cause the absence of GPI anchors and associated proteins. The assay employs fluorescent antibodies to detect these proteins on reticulocytes and red blood cells, measuring mutant cell frequencies using flow cytometry.

Materials and Method

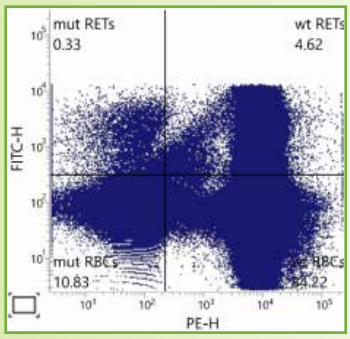
We sourced N-Nitroso-N-Ethylurea and Benzo(a)Pyrene from Sigma-Aldrich, and materials for the Pig-a assay from Muta Flow® PLUS 25-R kit (Litron Laboratories), including specific antibodies and reagents. Additional equipment like the Octo-MACS™ separator and MS-Columns were obtained from Miltenyi-Biotech. Male and female Wistar rats, sourced from Jai Research Foundation's animal breeding facility, were used in experiments adhering to OECD TG 470 guidelines and the Muta-Flow PLUS kit manual.

Result and Conclusion

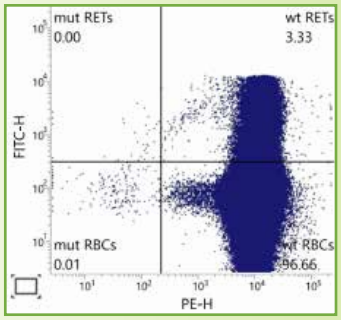
The study observed a statistically significant increase in RBC^{CD59-} per 10^6 total RBCs and RET^{CD59-} per 10^6 total RETs in both male and female rats treated with 20 mg/kg body weight of ENU and 125 mg/kg body weight of benzo(a)pyrene for three days, compared to the control group. This finding underscores the assay's effectiveness in detecting mutagenic effects and its potential in broader regulatory safety assessments.



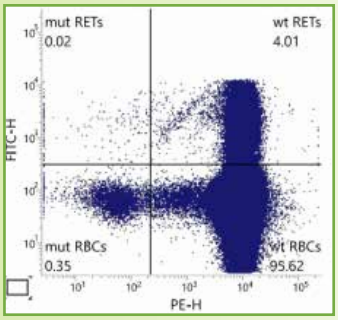
Vehicle Control (PBS)



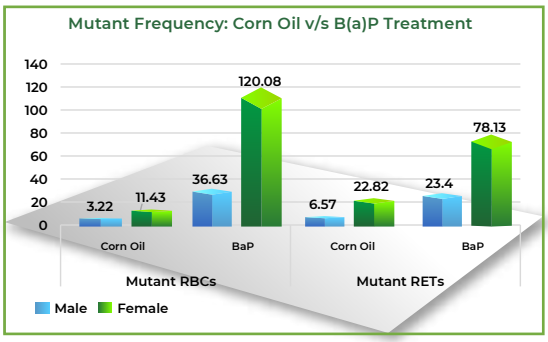
20 mg/ kg body weight of ENU

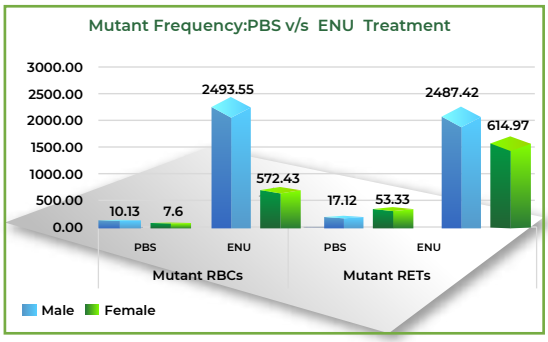


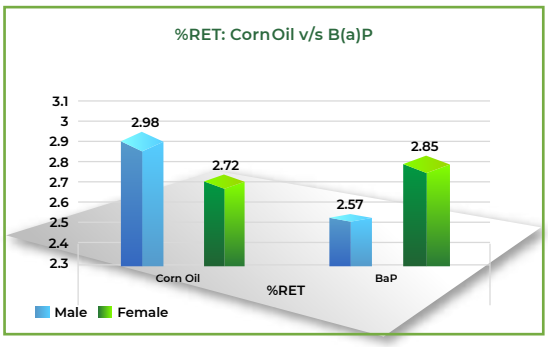
Vehicle Control (Corn Oil)

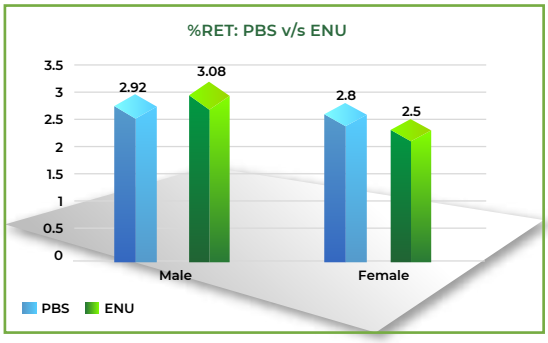


125 mg/kg body weight of B(a)P









The results obtained in this experiment are promising and highlight the robustness of the rodent *in vivo* Pig-a assay under our laboratory conditions. Therefore, this assay can be utilized as an independent or follow-up test for *in vitro* genotoxicity assessments, as well as an integrated test alongside routine sub-acute or sub-chronic toxicology studies.

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