NTP
National Toxicology Program

# Genetic Toxicology Studies in Mice and Rats Exposed to Radiofrequency Radiation

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## Outline

- Rationale for selecting the assays
- Description of the assay protocols
  - Erythrocyte micronucleus assay
  - Comet assay
- Data analysis
- Interpretation of the data
- Conclusion

# Genetic Toxicology Tests

## Subsets of mice and rats assessed after 14 (mice) or 19 (rats) weeks of exposure for genetic damage

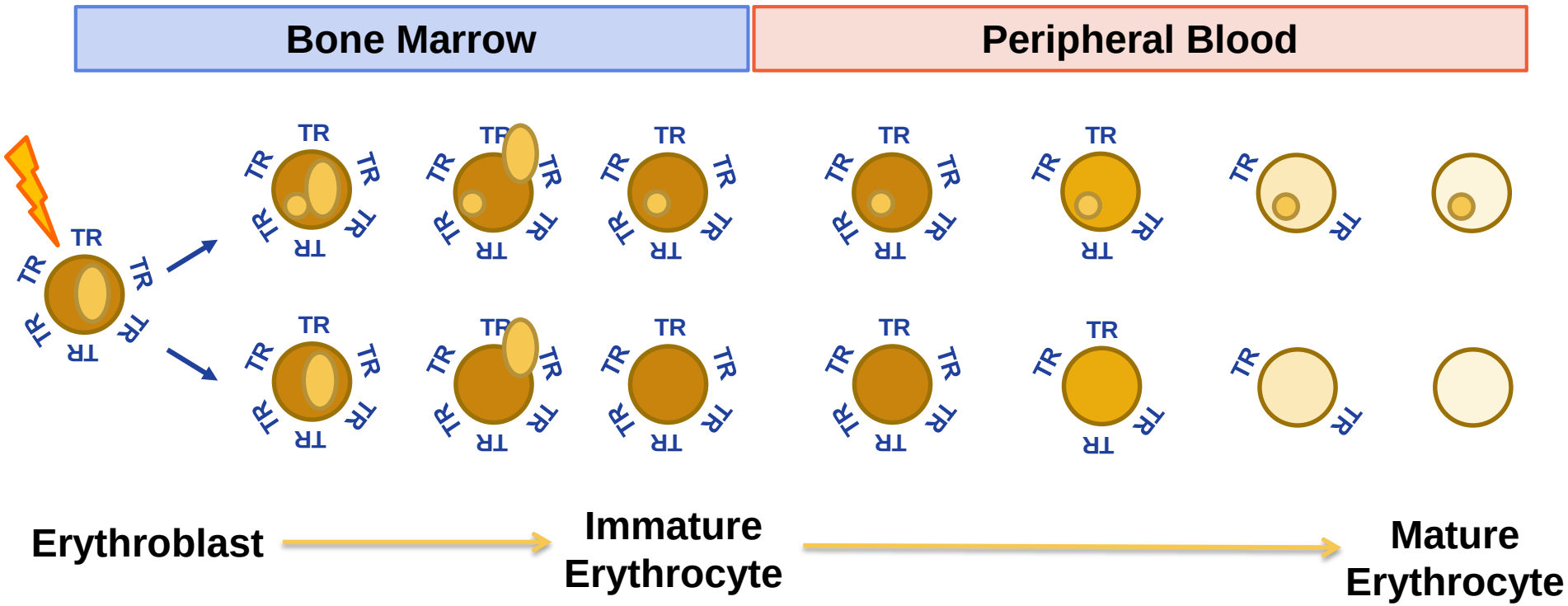
- Mice: 14 weeks of exposure, beginning at ~5 weeks of age
- Rats: 19 weeks of exposure beginning on GD5
- Five animals/treatment group; common sham control per sex
- Blood and tissue samples obtained for analysis in 2 independent assays:
  - Erythrocyte micronucleus assay for structural and numerical chromosomal damage
    - Peripheral blood
  - Comet assay (single cell gel electrophoresis) for DNA damage in multiple cell types
    - Frontal cortex, hippocampus, cerebellum, liver, and leukocytes

# Genetic Toxicology Tests: Rationale

- **In vivo rodent erythrocyte MN assay (OECD Test Guideline 474)**
  - Detects alterations in chromosomal number and structure in dividing cells
    - Cancer, birth defects, neurodegenerative diseases
  - Positive results are well correlated with cancer in rodents
  - Majority of known human carcinogens are positive in the rodent MN assay
  - Elevated levels of chromosomal damage are associated with elevated risk for cancer in large human population studies
- **In vivo rodent alkaline comet assay (OECD Test Guideline 489)**
  - Screen for DNA damaging potential; detects an early and transient event
  - Multiple fates of early DNA damage
    - Correctly repaired (considered the most frequent outcome)
    - Cell dies
    - Incorrectly repaired and a mutation (heritable change in DNA sequence) is introduced

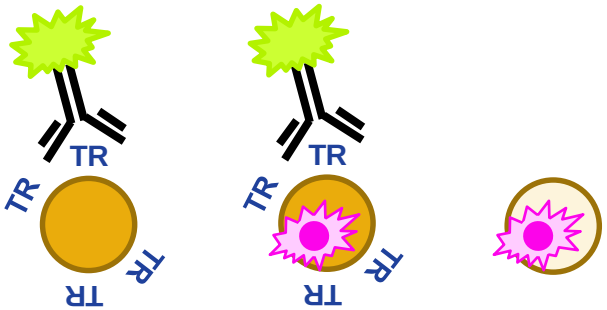
# Erythrocyte Micronucleus Test in Peripheral Blood

## Principle of the flow cytometric micronucleus assay in mice




**TR** = transferrin receptor

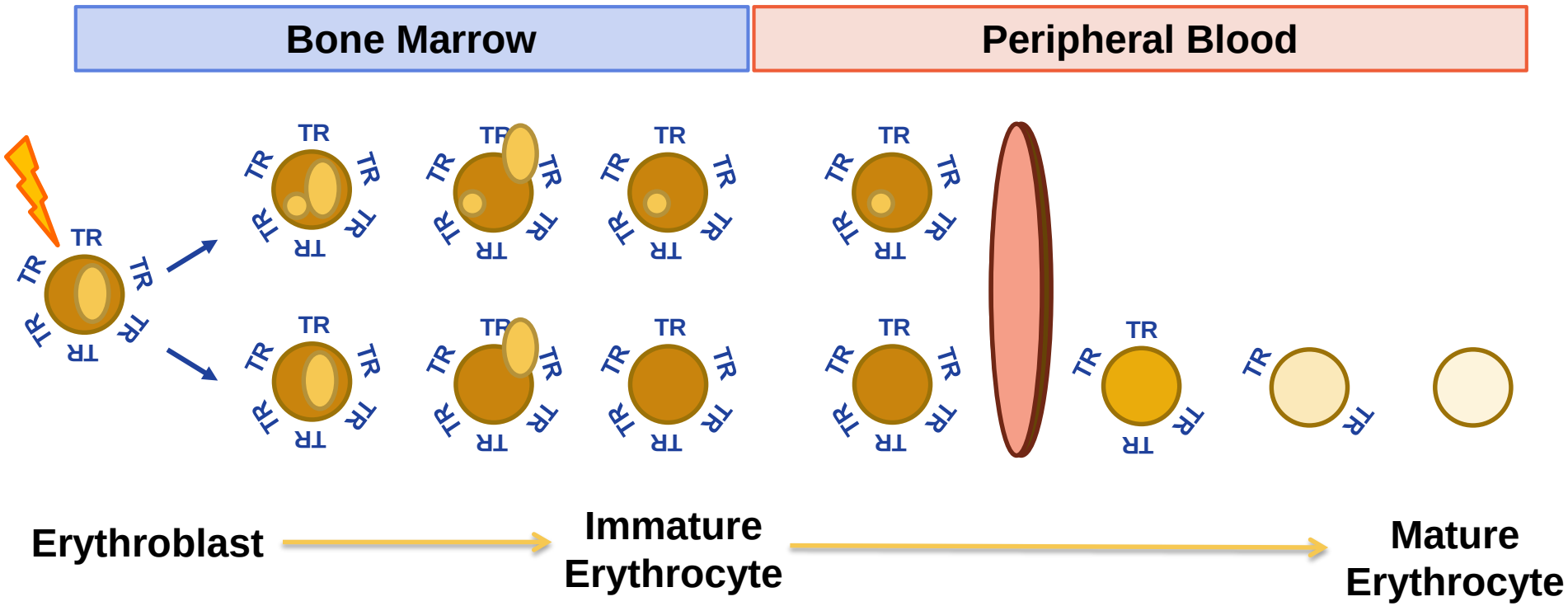
= RNA content (lost during maturation of erythrocytes)




Fluorescent Ab to TR to identify immature erythrocytes & DNA dye to detect MN

# Erythrocyte Micronucleus Test in Peripheral Blood

## Principle of the flow cytometric micronucleus assay in rats

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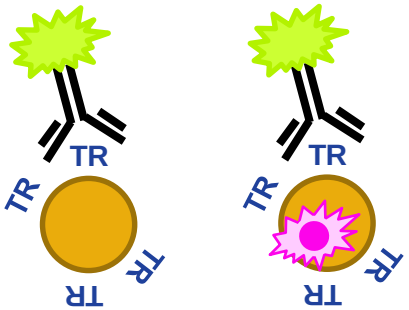
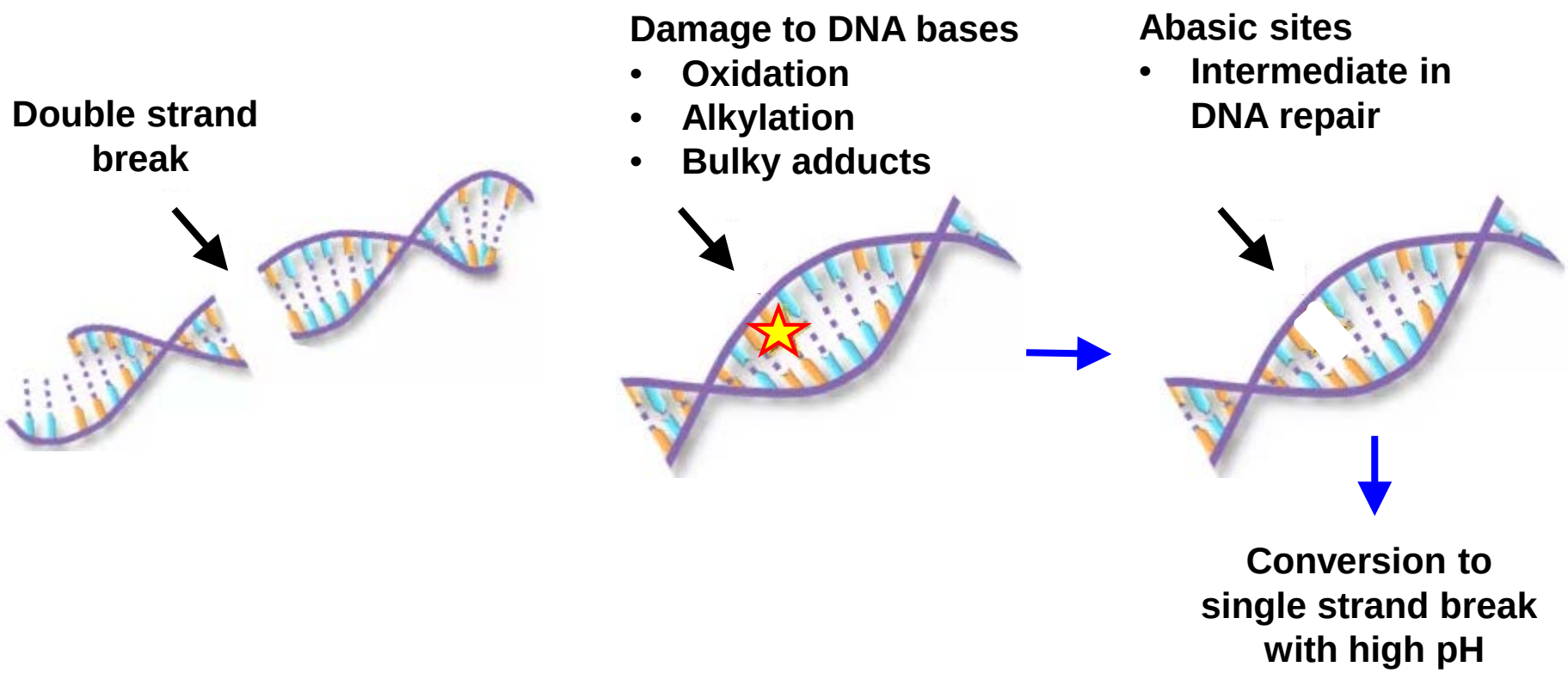



Fluorescent Ab to TR to identify immature erythrocytes & DNA dye to detect MN

# Principle of The In Vivo Alkaline Comet Assay

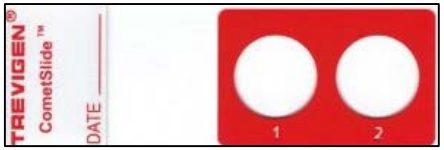
## Detecting DNA damage in individual cells



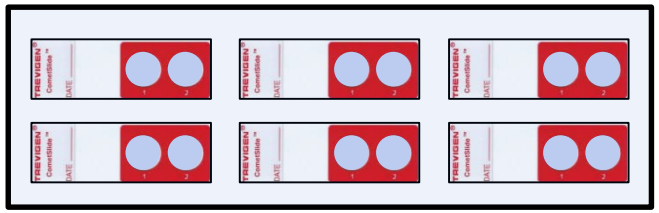

- DNA is normally in a supercoiled state
- Breaks in the DNA sugar-phosphate backbone relax the supercoils and allow it to migrate out of the nucleus when the nucleus is immobilized in an agarose gel and an electrical field is applied to the gel

# Alkaline (pH > 13) comet assay

Single cell suspension

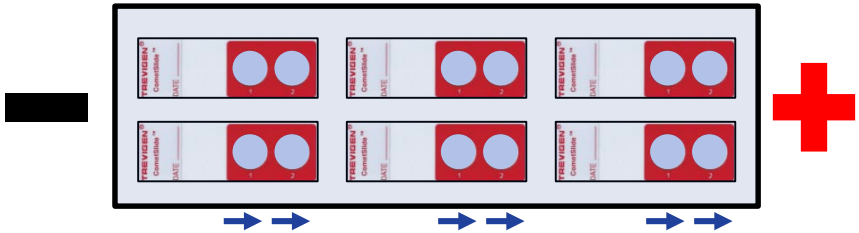



Combine cells with agarose, layer onto slides



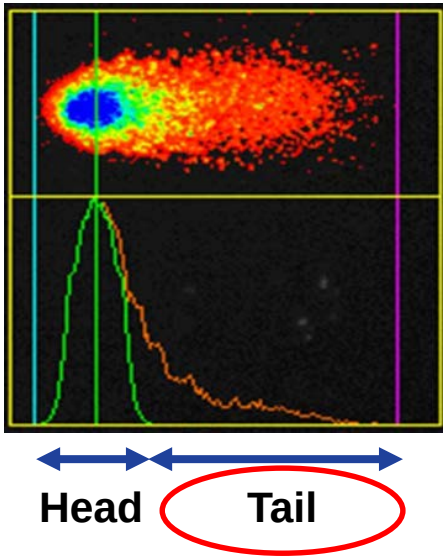
Lyse cells in buffer with high salt concentration and detergent

DNA unwinds in alkaline (pH > 13) buffer followed by application of electrical field



Negatively charged relaxed DNA and fragments migrate toward the anode

Comet

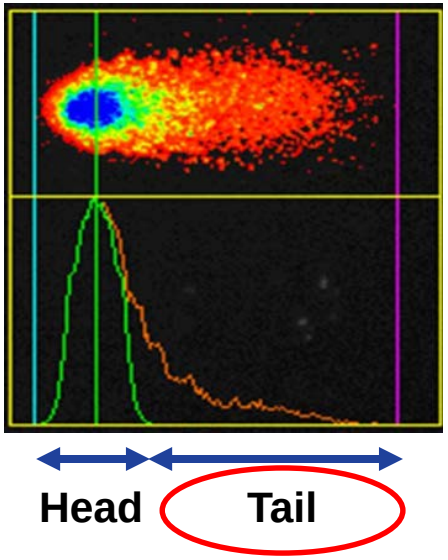



Stain DNA and score comets using software

Evaluate % Tail DNA (DNA migration)

# Comet Assay: Scoring Comets

**Comet software analysis**




**Endpoint evaluated:**
**% tail DNA**
**(DNA migration)**

**Software calculates % tail DNA (tail intensity)**

**<u>Increased migration of DNA from the "head" into the "tail" of the comet figure represents increasing levels of DNA damage/fragmentation:</u>**

**% Tail DNA, images from male rat hippocampus slide**

**5% 11% 26% 36% 56% 65% 71% Hedgehog**

# Genetic Toxicology Tests: Data Analysis

- For the MN assay: 20,000 immature and ~1 x $10^6$ mature erythrocytes evaluated per animal
- For the comet assay: 100 cells* scored per tissue per animal
- Evaluation criteria: significance set at $P \leq 0.025$ for trend and pairwise comparisons
  - **Positive**
    - Significant trend <u>and</u> at least 1 significant dose group; or at least 2 significant dose groups
  - **Equivocal**
    - Either a significant trend or 1 significant dose group
  - **Negative**

***A second scoring, evaluating 150 cells, was conducted on all rat samples; selected samples from mice were also re-evaluated in this manner.

# Genetic Toxicology Tests: Interpretation

- **In vivo erythrocyte peripheral blood MN assay**
  - Biomarker of chromosomal damage, which is associated with a number of adverse health outcomes
  - Positive results correlated with cancer in rodents and elevated risk of cancer in humans (positive predictivity is high)
  - Negative results do not predict negative results in cancer tests (sensitivity is low)
- **In vivo rodent comet assay**
  - Screen for genotoxicity potential
  - Multiple fates of DNA damage
    - Damage correctly repaired (considered the most frequent outcome)
    - Damaged cell dies
    - Damage incorrectly repaired
      - Mutation (permanent, viable, heritable change) or chromosomal damage

## Conclusions

- Exposure-induced genetic damage is an adverse effect, but the health consequences of the induced damage are not necessarily predictable.
- Conversely, lack of genotoxicity, as detected in standard assays, does not necessarily imply that exposure to an agent carries no risk for adverse health consequences.

# Questions?