

Assessing Genotoxic Risk: The *InVivo* Bone Marrow Chromosomal Aberration Assay

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Abstract

Genotoxicity, the potential of certain agents to cause damage to the genetic material within cells, is a critical concern in chemical safety evaluations due to its association with carcinogenesis and heritable genetic disorders. Pesticides, widely employed in agriculture, are among the major contributors to environmental genotoxins, necessitating rigorous toxicological assessment. The *in vivo* bone marrow chromosomal aberration assay is a well-established method endorsed by the OECD for evaluating the genotoxic potential of chemical substances. This assay utilizes rapidly dividing hematopoietic cells in rodent bone marrow to detect structural and numerical chromosomal abnormalities under physiological conditions, accounting for factors such as metabolism and DNA repair. Rodents are exposed to the test compound followed by colchicine treatment to arrest cells in metaphase. Bone marrow is then collected, processed, and stained for microscopic examination. Key endpoints include the mitotic index and frequency of aberrant metaphase cells. Despite its limitation in detecting damage in non-dividing tissues, this assay remains a cornerstone in genotoxicity testing due to its high sensitivity and biological relevance. When used in conjunction with *in vitro* methods, it contributes to a comprehensive assessment of genotoxic risk, thereby supporting regulatory frameworks and public health protection.

Introduction:

Genotoxicity, defined as the ability of certain agents to damage the genetic material within a cell, is recognized as a major contributing factor in the development of cancer. Genotoxic agents, referred to as genotoxins, include a wide variety of chemical substances (e.g., pesticides) as well as physical agents such as ionizing radiation. DNA damage in somatic cells may result in a range of pathological conditions, including carcinogenesis, whereas genotoxic effects on germ cells may lead to hereditary disorders. Therefore, evaluating the genotoxic potential of chemicals is a

critical component in toxicological risk assessment.

To standardize genotoxicity testing, the Organisation for Economic Co-operation and Development (OECD) prescribes a battery of assays, both *in vitro* and *in vivo*. Among these, the micronucleus assay, chromosomal aberration test, and comet assay are considered essential for identifying structural and numerical changes in chromosomes. These tests help ascertain whether a test substance can induce genetic damage and provide a holistic view of its genotoxic profile.

Significance of the *In vivo* Bone Marrow Chromosomal Aberration Test: The *in vivo* mammalian chromosomal aberration assay using bone marrow cells is a cornerstone method for evaluating genotoxic effects. Unlike *in vitro* systems, this assay incorporates critical biological variables such as metabolic activation, pharmacokinetics, and DNA repair pathways. These mechanisms can vary among species and significantly influence a chemical's genotoxic profile. This test not only complements *in vitro* findings but also provides essential data for understanding how a substance interacts within the physiological environment of a living organism. As such, it is a vital tool in regulatory toxicology and chemical safety evaluations.

Why Bone Marrow is Selected as Target Tissue: Bone marrow is the preferred target tissue in chromosomal aberration studies due to its high cellular turnover and rich vascular supply, which makes it a sensitive indicator of genotoxic damage. It contains rapidly proliferating hematopoietic cells that are readily accessible for microscopic analysis. However, for the bone marrow to serve as an appropriate target, the test compound or its active metabolites must reach this tissue within the specified exposure window. Failure of the compound to distribute to the bone marrow may render the sample inadequate for detecting chromosomal aberrations.

Test Principle and Experimental Procedure: In this assay, rodents, typically rats or mice, are administered the test compound via an appropriate route (e.g., oral, intraperitoneal). Before euthanasia, animals are treated with a metaphase-arresting agent such as colchicine to enrich dividing cells in metaphase. The bone marrow is then harvested, and the cells are processed for cytogenetic evaluation.

Key steps in processing include:

- **Centrifugation** of bone marrow suspension
- **Incubation** in a hypotonic solution to swell the cells
- **Fixation** with methanol-acetic acid fixative
- **Slide preparation and staining** (commonly Giemsa or similar stains)
- **Microscopic analysis** under oil immersion (100x) to evaluate metaphase cells for structural aberrations.

In rats, bone marrow collection is typically performed 2–5 hours post-colchicine administration, while in mice, the collection window is 3–5 hours.

Data Interpretation and Scoring Criteria

Mitotic Index: The mitotic index serves as an indicator of cytotoxicity and is calculated by assessing the proportion of dividing cells

Mitotic Index (%) = (Number of mitotic cells / Total cells counted) × 100

A minimum of 1,000 cells per animal must be evaluated across all treatment and control groups, including vehicle control, negative (untreated), and positive control.

Chromosomal Aberration Scoring: For genotoxic evaluation, 200 well-spread metaphase cells are analyzed for structural aberrations. Only cells with a chromosome count of $2n \pm 2$ (species-specific diploid range) are included in the analysis.

Percentage of Aberrant Cells = (Number of aberrant metaphase cells / Total metaphase cells examined) × 100

Aberrations are categorized as:

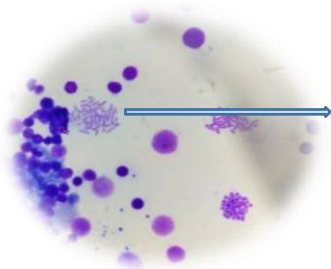
- **Chromatid-type** (e.g., single chromatid breaks, exchanges)
- **Chromosome-type** (e.g., dicentric, rings)

Types of Chromosomal Aberrations

Chromosomal abnormalities fall under two main categories:

1. **Numerical Aberrations:** Involve changes in chromosome number, such as trisomy or monosomy (e.g., trisomy 21 in Down syndrome).
 2. **Structural Aberrations:** Involve rearrangements such as deletions, duplications, inversions, and translocations of chromosome segments.
- **Clastogenic agents:** Induce structural changes in chromosomes.
 - **Aneugenic agents:** Alter the number of whole chromosomes or sets of chromosomes.

While structural changes that do not result in gene loss are often non-lethal, those causing genetic deletions are frequently cytotoxic and can lead to cell death. During mitosis, fragments excluded from the main nucleus may form micronuclei, which serve as markers of chromosomal damage. These are easily observable and quantifiable, making them valuable endpoints in genotoxicity screening.



Chromosomes of a Wistar albino rat under Giemsa staining (100x)

dividing cells, limiting their applicability to tissues with high mitotic activity. Consequently, slowly dividing or quiescent tissues may escape detection in such tests. Nonetheless, the in vivo bone marrow assay remains one of the most robust and biologically relevant methods for detecting clastogenic and aneugenic activity of test compounds.

Conclusion: The in vivo bone marrow chromosomal aberration test is an essential assay in genotoxicity testing protocols, providing insight into the ability of a chemical to induce chromosomal damage within a living organism. By taking into account critical physiological processes like metabolism and DNA repair, this assay ensures a more accurate assessment of genotoxic risks. When integrated with in vitro methods, it contributes to a thorough and reliable genotoxicity profile, guiding regulatory decisions and public health policies.

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Limitations and Conclusions: Traditional assays only detect chromosomal damage in