

# Genotoxicity Testing and Impurity Qualification

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# Disclaimer

- The views presented today are those of the presenters and do not necessarily reflect the views of the entities with whom they are affiliated.



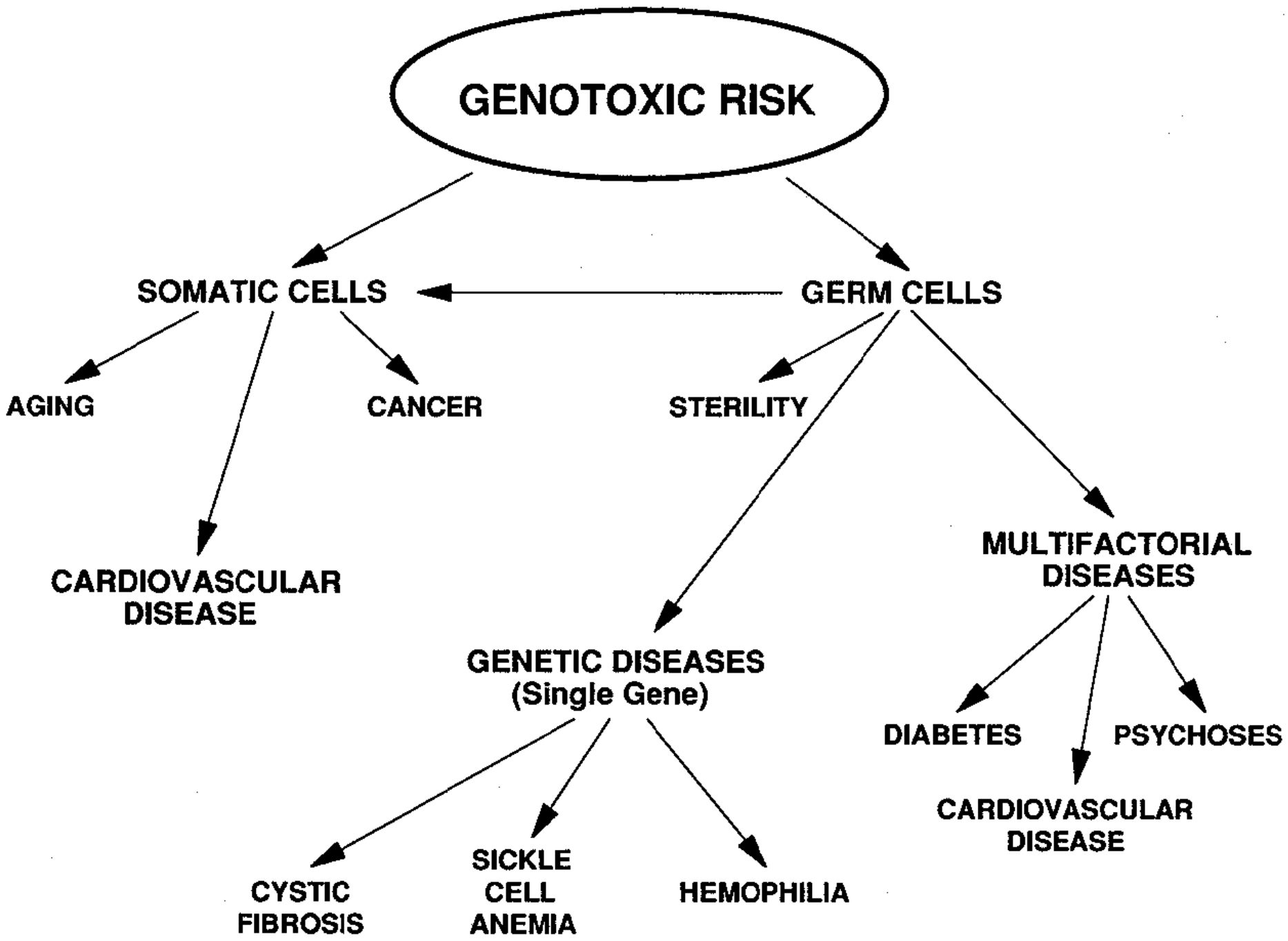
# Outline

- What is genotoxicity?
- Why are we concerned?
- Regulatory Guidances for Genotoxicity Testing
- Overview of the 4 genotoxicity assays
- What do you do with the results?
- Tertiary Genotoxicity Review Committee –  
FDA/CDER
- ICH-Q3A – Impurities in New Drug Substances
- ICH-Q3B – Impurities in New Drug Products
- ICH-Q3C – Residual Solvents



# What is Genetic Toxicology?

- Study of an agent's ability to induce genetic damage
- Subspecialty of toxicology
- Current paradigm developed over last 30 years
- Short-term *in vitro* and *in vivo* genetic toxicology tests



**GENOTOXIC RISK**

**SOMATIC CELLS**

**GERM CELLS**

**AGING**

**CANCER**

**STERILITY**

**CARDIOVASCULAR  
DISEASE**

**MULTIFACTORIAL  
DISEASES**

**GENETIC DISEASES  
(Single Gene)**

**DIABETES**

**PSYCHOSES**

**CARDIOVASCULAR  
DISEASE**

**CYSTIC  
FIBROSIS**

**SICKLE  
CELL  
ANEMIA**

**HEMOPHILIA**



# Regulatory Genetic Toxicology

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)
  - Regulatory and pharmaceutical representatives from US, Europe & Japan
  - Labor intensive process for completion of genetic toxicology guidances (1992 - 1997)
- Office of Economic and Cooperation Development (OECD)
- FDA/CDER



# Regulatory Genetic Toxicology

- ICH M3 – Guidance for Industry: Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals
  - Prior to first human exposure
    - In vitro tests for the evaluation of mutations and chromosomal damage are generally needed. If an equivocal or positive finding occurs, additional testing should be performed.
  - Prior to the initiation of Phase II studies
    - The standard battery of tests for genotoxicity should be completed



# Regulatory Genetic Toxicology

## FDA/CDER

- S2A Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals.
  - Federal Register 61:18199, 1996.
- S2B Guidance for Industry: A Standard Battery for Genotoxicity Testing of Pharmaceuticals.
  - Federal Register 62:62472, 1997.



# Purpose of Genotoxicity Test Assays

- Assays allow detection of a drug's potential for genotoxicity early in drug development
- Assays are inexpensive, have high statistical power, are generally reproducible and detect a variety of genotoxic end-points
- Assays designed to be more sensitive to damage in order to enhance hazard identification



# Standard Genotoxicity Test Battery

- An **in vitro** test for gene mutation in bacteria.
- An **in vitro** test with cytogenetic evaluation of chromosomal damage with mammalian cells or an **in vitro** mouse lymphoma thymidine kinase (tk) assay.
- An **in vivo** test for chromosomal damage using rodent hematopoietic cells.



# Bacterial Mutation Assay

- DNA Damage Detected
  - Base pair substitutions (-ATGC-→-ACGC)
  - Frameshift mutations (-ATGC-→-ATTGC)
- Ames or Salmonella Assay
  - Developed by Bruce Ames early 1970s
  - Most widely used, fast, easy and cost-effective
- 5 Bacterial strains engineered to be more sensitive to damage (rfa, uvrB-, pKM101)
  - Alteration to cell wall, increased permeability
  - Removal of excision repair & introduction of error-prone repair

# Bacterial Mutation Assay

- Detects reverse mutation in histidine gene in his- Salmonella auxotroph strains (cannot produce histidine).

Mutagenic agent

↓ mutation

Auxotroph



Prototroph

(his- strain)

(his+ strain)

Mutated Codon -CCC-

WT Codon -GCC-

Prototroph (revertant) grows in absence of histidine

TA100

# Ames Assay Plates



Negative  
Controls

Positive  
Controls





# In Vitro Mouse Lymphoma Thymidine kinase (tk) Assay

## ● Damage Detected

- Detects broadest spectrum of DNA alterations
  - base pair substitutions
  - frameshifts
  - small and large chromosomal deletions & translocations
  - mitotic recombination/gene conversion
  - aneuploidy



# In Vitro Mouse Lymphoma Thymidine Kinase (tk) Assay

- Detects forward mutations at the thymidine kinase locus of mouse lymphoma cells (L5178Y cell line).

Mutagenic agent



**mutation**

tk+/- cell



tk-/- cell

TK enzyme present

Nonfunctional TK

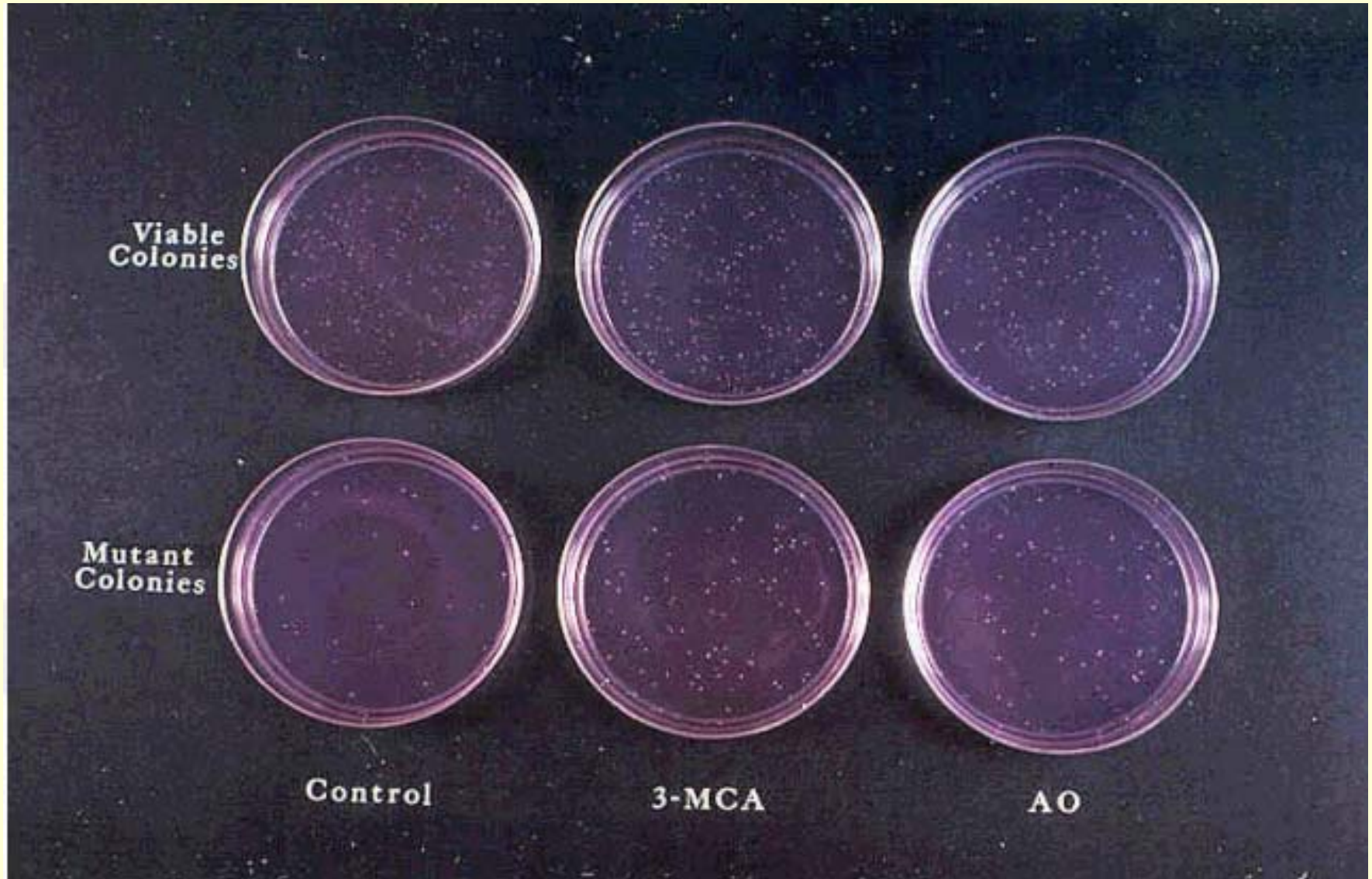
Cells sensitive to TFT

Cells insensitive to TFT

No growth

**Only mutants grow**

# Mouse Lymphoma Colonies Soft Agar Plates

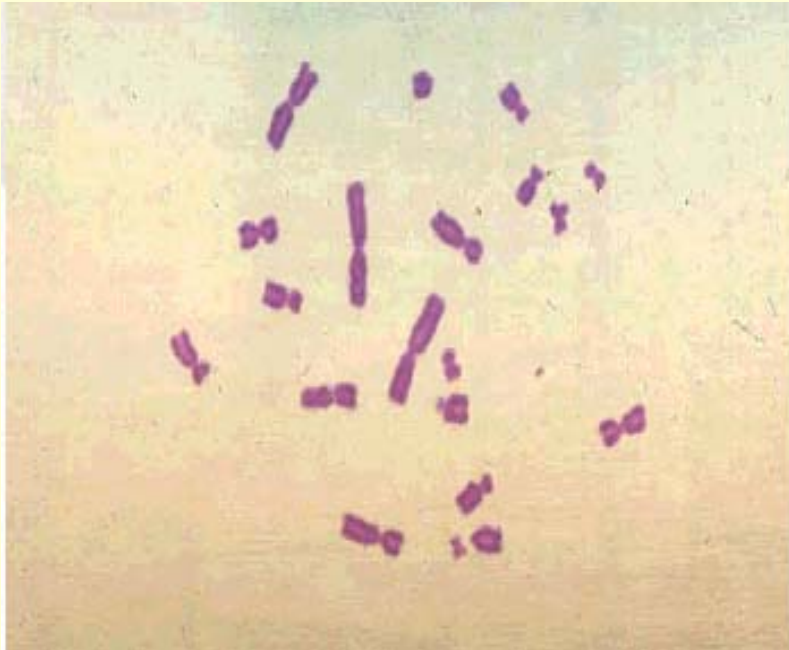




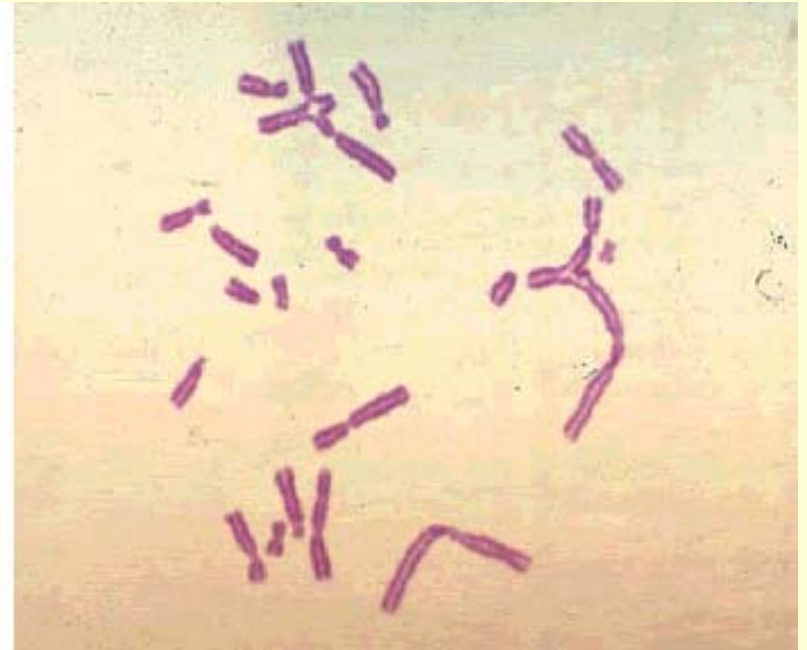
# In Vitro Cytogenetics Assay

- **Damage Detected – chromosomal aberrations observable at the light microscope level.**
  - Deletions
  - Rearrangements
  - Numerical changes
- **Established Cell Lines**
  - Chinese hamster ovary cells (CHO) or lung cells (CHL)
  - Monolayer
- **Primary cells**
  - Human peripheral blood lymphocytes (HPBL)
  - Suspension culture.

# CHO-WBL Cell Karyotypes



**Control**



**Positive**



# In Vivo Cytogenetic Assay

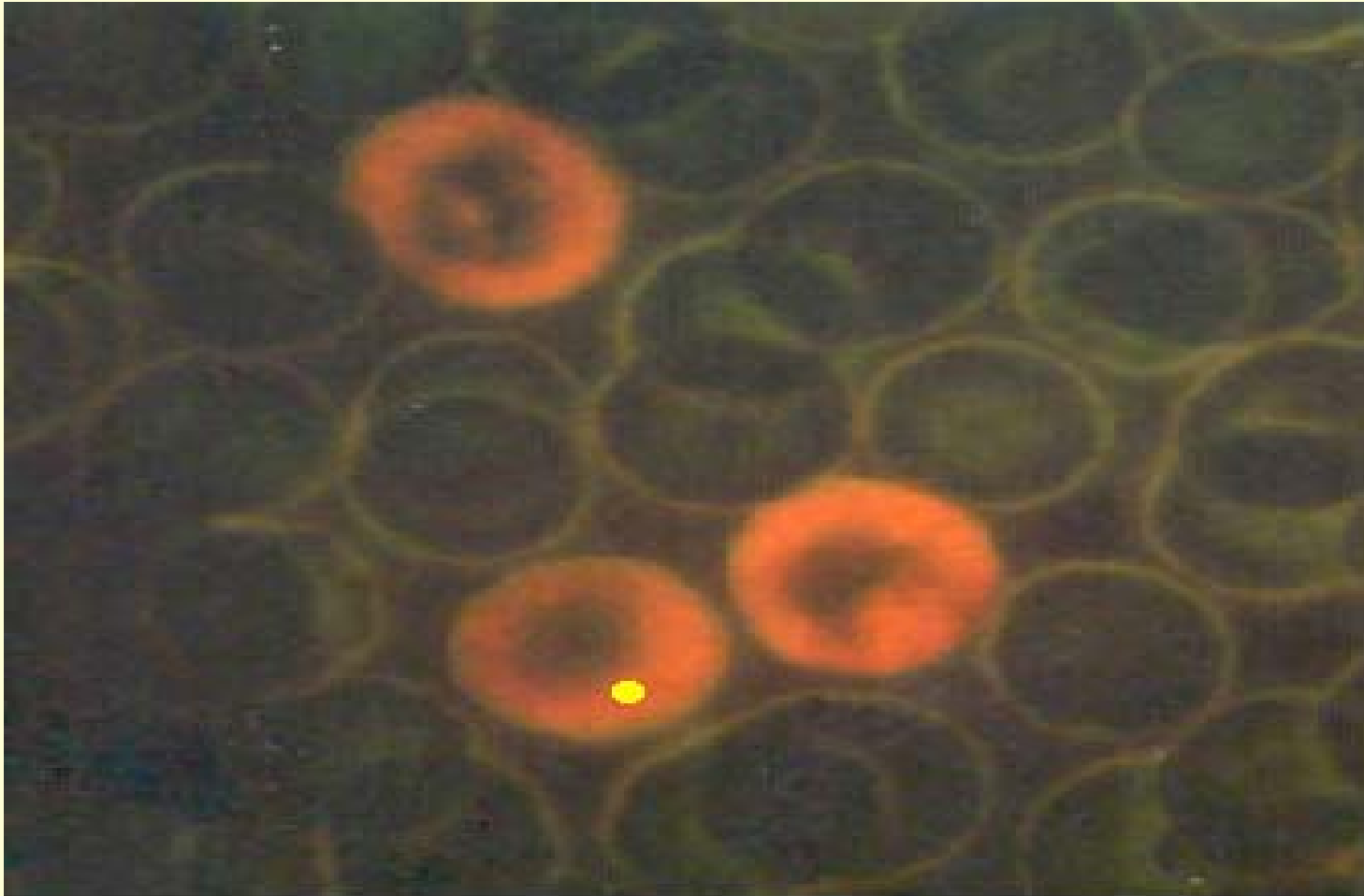
- **Damage Detected - Macrolesions, Chromosomal damage.**
- **Detects chromosomal damage in rodents (generally mice and rats) as:**
  - Either chromosomal aberrations in bone marrow cells (analyze metaphase spreads) (In Vivo Chromosomal Aberrations Assay)
  - Or micronuclei in bone marrow or peripheral blood erythrocytes. (In Vivo Micronucleus Assay)
- **Importance of an in vivo assessment**
  - Endogenous metabolic activation and detoxification of test article.
  - Evaluation using route of administration relevant to humans.
  - Included in battery because this assay detected a small number of genotoxic carcinogens which tested negative in in vitro tests.



# *In Vivo* Micronucleus Assay

- Micronuclei (MN): Cytoplasmic chromatin containing bodies formed when acentric chromosome fragments or whole chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division.
- Genetic damage that causes chromosome breaks, structurally abnormal chromosomes or spindle abnormalities leads to MN formation
- Therefore, incidence of MN serves as index of these types of damage.

# Micronucleated PCE Acridine Orange Stain





# Evaluation of Battery Results - General Principles

- Battery tests are complementary.
  - No one test can detect all types of alterations.
  - Not all tests in the battery expected to be positive.
- Battery tests enable hazard identification, not risk assessment
  - One positive identifies potential hazard
  - IVth IWGT - movement to change from "hazard identification" to "risk assessment".
- When used for hazard identification battery tests do not represent different levels of hierarchy.
  - Results from in vitro tests using mammalian cells do not cancel those from tests using bacterial cells.
  - In vivo test results do not cancel in vitro test results.



# Assessing a Positive Genetic Toxicology Finding During Clinical Development - Draft Guidance

- Draft Guidance for Industry - Integration of Genetic Toxicology Study Results into Regulatory Decisions
  - Purpose is to inform industry how CDER views positive findings in genotoxicity assays and provide recommendations on how to proceed in assuring safety of healthy subjects or patients when positive genotoxicity results suggest a potential hazard.




# Assessing a Positive Genetic Toxicology Finding During Clinical Development - Draft Guidance

- A positive result in any assay does not necessarily mean that the test compound poses a genotoxic/carcinogenic hazard to humans.
  - Due to extreme culture conditions or cytotoxicity in in vitro tests?
  - Absence of SAR alerts. Due to a contaminant?
  - Due to in vitro specific metabolism?
  - Other mechanistic explanations show results not relevant? e.g. Threshold mechanism.



# FDA/CDER Tertiary Review of Genetic Toxicology Studies – Solution to Inconsistencies of Result Interpretation

- Tertiary Review of Genetic Toxicology Studies Resulting in a Recommendation for a Clinical Hold or Conduct of Additional Studies (MAPP 7400.4)
  - When a hold is imminent
  - Establishes Genetic Toxicology Review Committee consisting of Associate Director for Pharm/Tox and ODE Associate Directors for Pharm/Tox plus supervisor and reviewer for submission in question
  - Provides review at the Center level of positive genetic toxicology findings that might give rise to a clinical trial hold and recommendations for additional studies.



# Guidance for Industry: ICH-Q3A Impurities in New Drug Substance

## ● Products covered by guidance

- Organic-usually a by-product of synthesis
- Extractables and leachables from containers and/or in manufacturing
- Residual solvents from manufacturing process,
- Extraneous contaminants such as pesticides, other drugs

## ● Products not covered:

- Biological, biotechnological
- Peptides, oligonucleotides
- Radiopharmaceuticals, fermentation and semi-synthetic products as derived
- Botanicals and crude products of animal or plant origin.

# ICH-Q3A (cont.)

Maximum Daily Dose <sup>1</sup>	Reporting Threshold <sup>2,3</sup>	Identification Threshold <sup>3</sup>	Qualification Threshold <sup>3</sup>
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

<sup>1</sup> The amount of drug substance administered per day

<sup>2</sup> Higher reporting thresholds should be scientifically justified

<sup>3</sup> Lower thresholds can be appropriate if the impurity is unusually toxic



# ICH-Q3A (cont.)

- Qualification is the process of acquiring and evaluating data to establish the safety of an individual impurity or a given impurity profile at the level(s) specified.
- An impurity is qualified if its specification limit is below the threshold concentration:
  - 0.1% or 1 mg whichever is lower if the daily dose of drug substance is 2000mg or less,
  - 0.05% if daily dose is more than 2000mg/day.
- The level of impurity in a drug substance that was adequately tested in non-clinical and/or clinical studies is considered to be qualified at that level.
- If the impurity has not been adequately qualified then additional non-clinical studies are necessary

# ICH-Q3A (cont.)

- Decision Tree for Safety Studies
  - Is impurity level above threshold?
  - Is the molecular structure known?
  - Is the toxicity documented/sufficient?
  - Related to others of known toxicity?
  - Consider patient population and use?
- Additional non-clinical studies that will be required is
  - Minimally the in vitro genotoxicity studies, the in vivo micronucleus assay may also be required
  - Repeat dose studies in one species (14-day to 90-day)
  - Other specific studies as requested



# Guidance to Industry: ICH-Q3B Impurities in New Drug Products


- Guidance limited to:
  - Impurities that are degradation products of the active drug substance
  - Reaction products of the active drug substance with an excipient and/or immediate container/closure system
- Impurities from excipients present in drug product are excluded as well as those exclusions from the ICH-Q3A guidance.

# ICH-Q3B (cont.)

## ● Qualification Thresholds (% of drug substance)

Maximum Daily dose of DS	Threshold (total daily intake)
< 10 mg	1.0% or 50 mcg (whichever is lower)
10 mg - 100 mg	0.5% or 200 mcg (whichever is lower)
> 100 mg - 2 g	0.2% or 2 mg (whichever is lower)
> 2 g	0.15%

● Decision tree is equal to that in Q3A



# ICH-Q3C - Residual

## Solvents

- Pertains to organic volatile chemicals not deliberately used as excipients
- Guidance recommends use of:
  - Less toxic solvents (Class 3)
  - Recommends acceptable levels of more toxic solvents (Class 2)
  - Avoidance of extremely toxic solvents with exceptions (Class 1)
  - Discussion of solvents with poor data (Class 4)
  - Discusses exceptions when use unavoidable (subpart H drug)