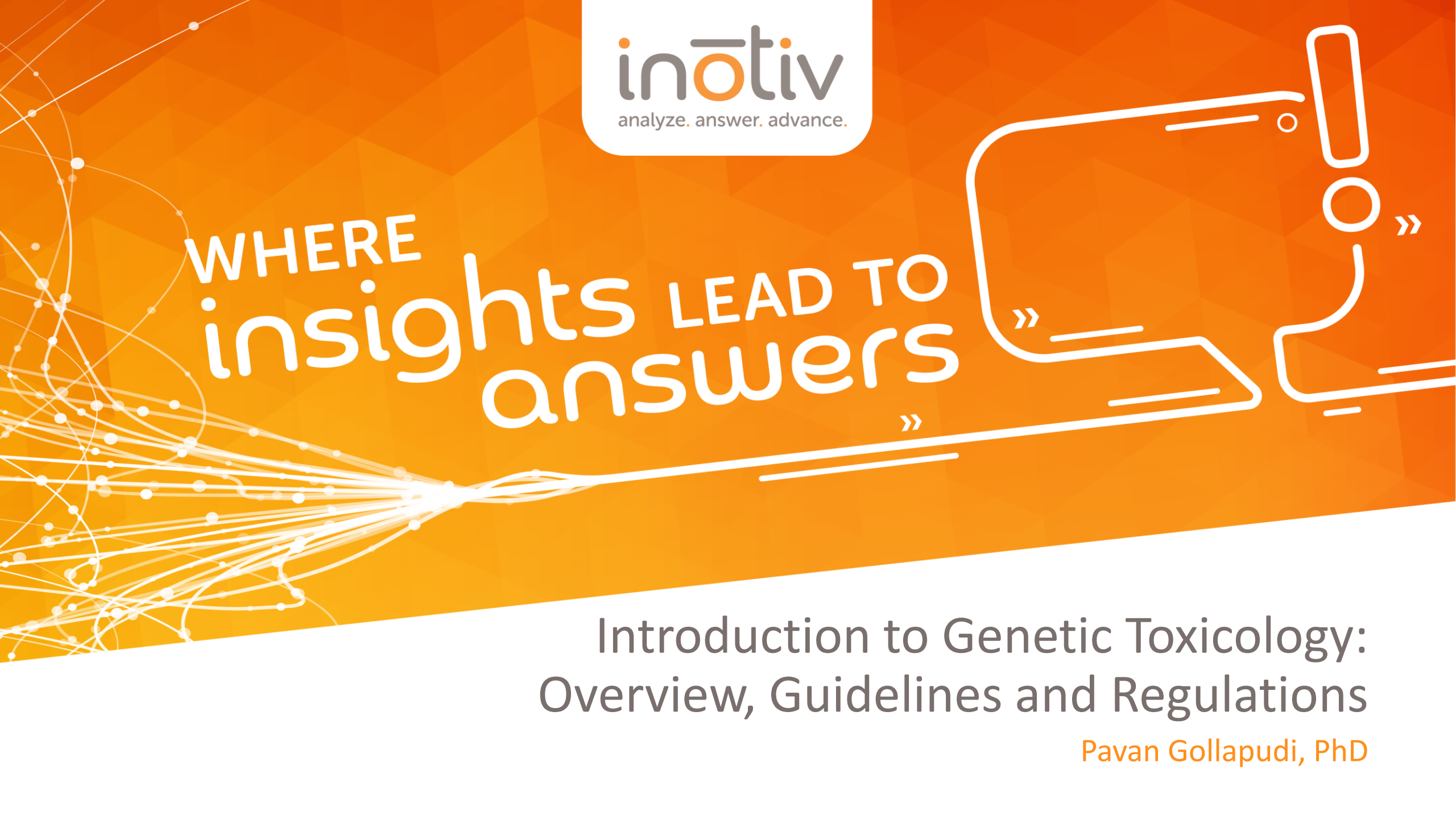




WHERE
insights LEAD TO
answers

Introduction to Genetic Toxicology: Overview, Guidelines and Regulations

Pavan Gollapudi, PhD

Agenda

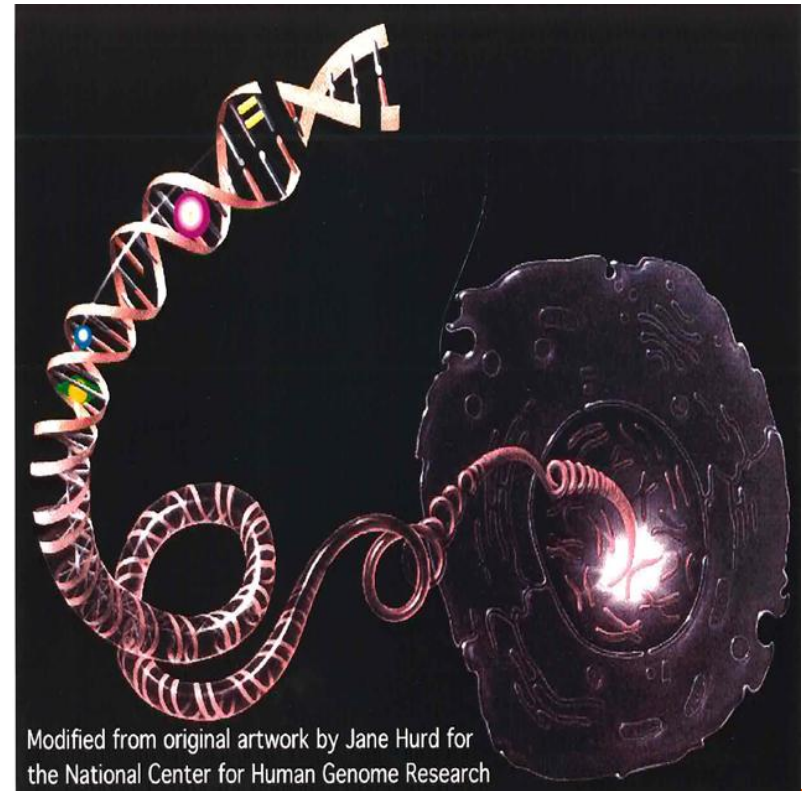
- What is Genetic Toxicology?
- Why do we Evaluate Genetic Toxicology?
- Assay Selection
- Genetic Toxicology Testing Guidelines and Regulations

What is Genetic Toxicology?

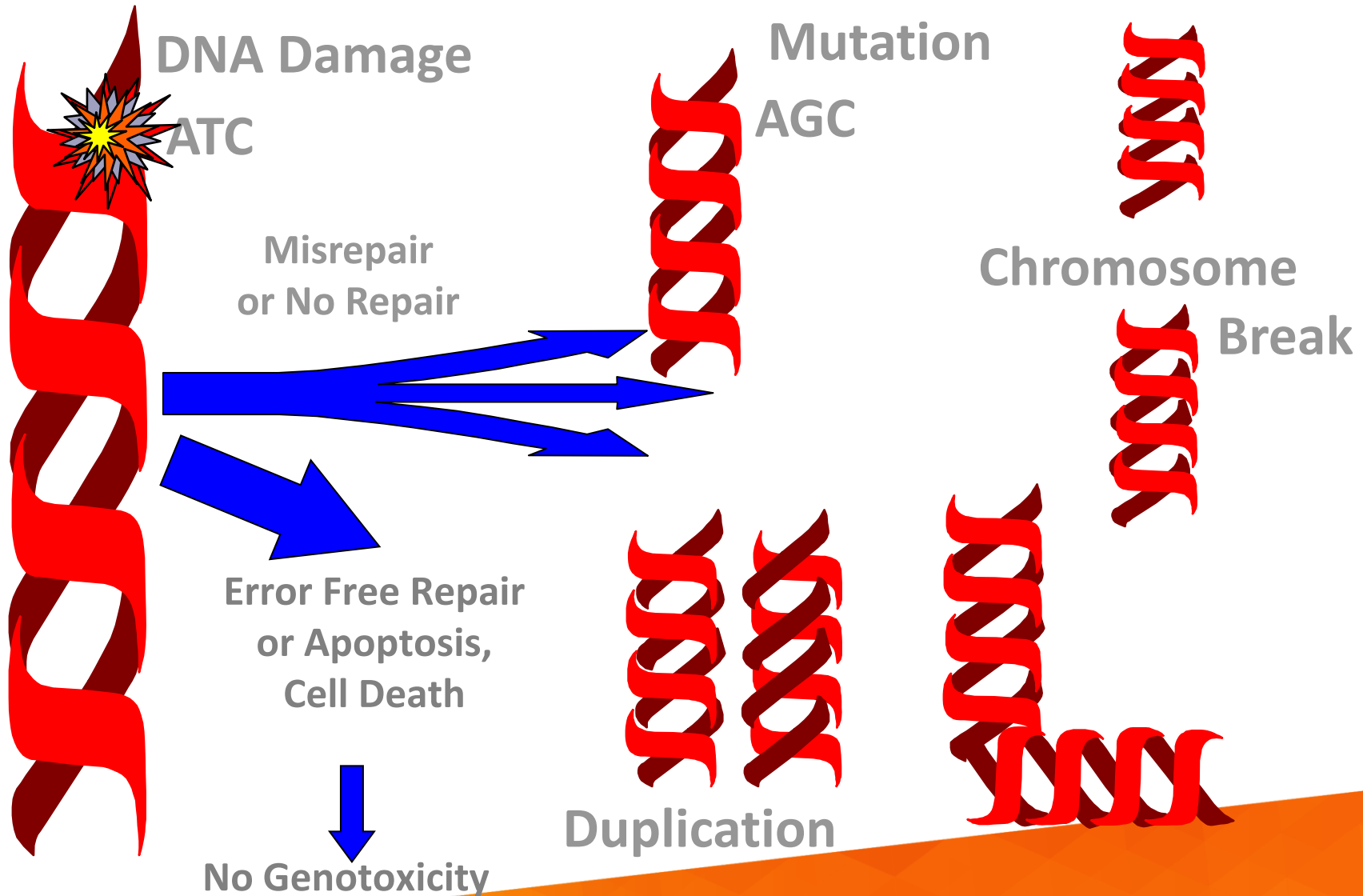
The study of chemical, physical or biological agents that can change the sequence or structure of DNA

DNA damage can be:

- at nucleotide level in DNA, or at the chromosomal level
- induced by direct mechanisms (chemical or metabolite interacts with DNA)
- induced by indirect mechanisms (chemical or metabolite affects other cellular macromolecules, e.g. mitotic spindle fibers)



What is Genetic Toxicology?



Terminology

Mutagen

- agent that induces mutations (change in the DNA sequence)

Clastogen

- agent that induces breaks in chromosome (structural damage)

Aneugen

- agent that induces changes in the number of chromosomes

Genotoxicity

- mutations, chromosome damage, non-specific damage/repair

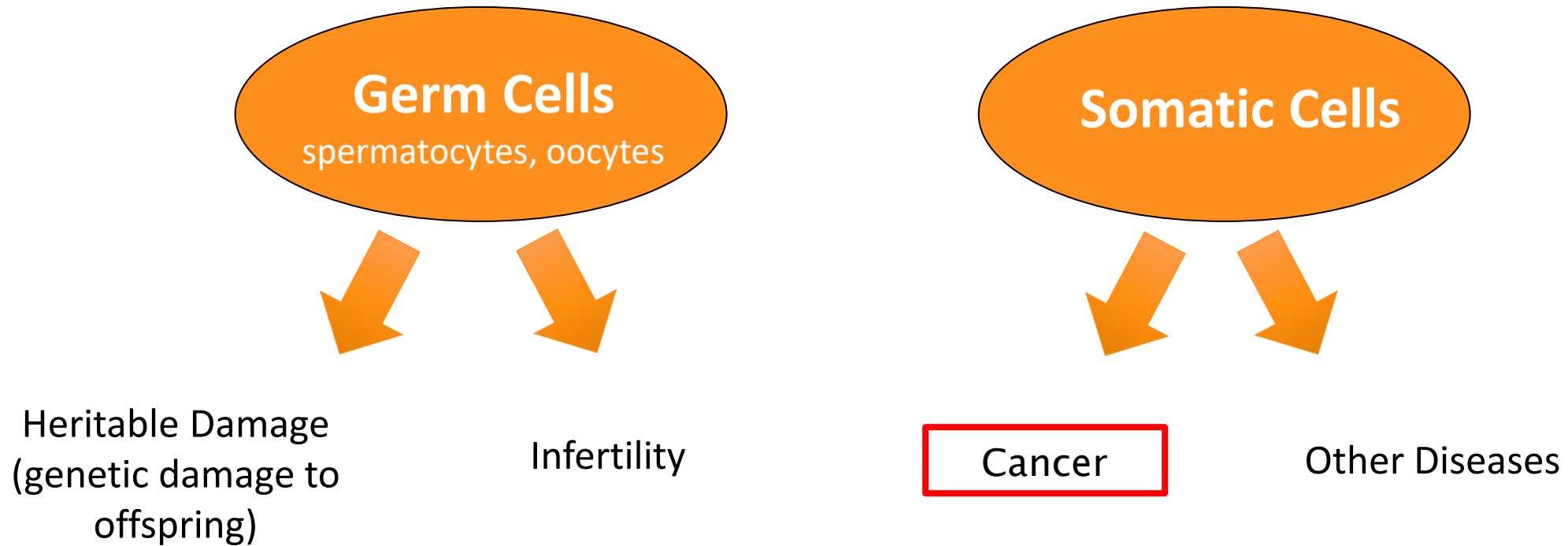
Mutagenicity

- mutations (often used interchangeably with genotoxicity)

“Cytogenetic” Effects

Why Do We Evaluate Genotoxicity?

DNA damage is associated with many human diseases



Objectives of Genetic Toxicology Testing

- Hazard identification/Lead prioritization

- > Companies want products to be safe and to be seen as safe for intended uses and foreseeable misuses
- > Predict whether a chemical is a carcinogen
- > Predict whether a chemical could cause heritable germ cell damage
- > Early testing “screening” allows Companies/Regulators to prioritize chemicals to spend further resources.

- Mechanistic information

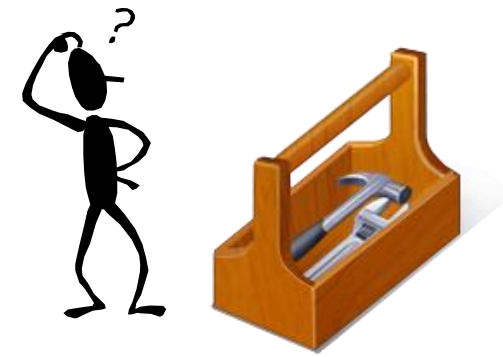
- > Determine mechanism of action for carcinogens (genotoxic vs. non-genotoxic)
- > Basic science: study of DNA damage/repair

Assay Selection: The Genetic Toxicology Toolbox

- Hundreds of assays developed to measure DNA damage
- Assays grouped by endpoint measured
- Types of DNA damage endpoints include:
 - > Mutations
 - > Changes in chromosome structure or number
 - > DNA damage
 - > DNA repair
 - > Biomarkers of DNA damage
- Endpoints can be measured in:
 - > bacteria, yeast, fungi, plants, invertebrates, mammalian cells in culture, animals and humans



A Standard Battery of Tests



Out of hundreds of assays, a standard battery of tests has been defined based on:

- Various analyses on assay performance have identified the most “robust” battery
- Various scientific expert groups develop recommended test batteries which can vary for drugs, cosmetic ingredients, etc.
- Various regulatory agencies and expert groups like OECD (Organisation for Economic Co-operation and Development) guidelines, ICH (International Congress on Harmonization) guidelines, define a minimum acceptable test battery

Measures of Assay Performance

Example: Usefulness of a genotoxicity assay for predicting carcinogenesis

Sensitivity (how well does an assay detect carcinogens?)

carcinogens positive in an assay/# carcinogens tested

59 carcinogens positive in Ames assay/100 carcinogens tested= 59% sensitivity

Specificity (how well does an assay detect non-carcinogens?)

non-carcinogens negative in an assay/# non-carcinogens tested

74 non-carcinogens negative in Ames assay/100 non-carcinogens tested= 74% specificity

Concordance (how well does an assay perform overall?)

carcinogens positive + # non-carcinogens negative/# chemicals tested

59 + 74 = 133/200 = 66% concordance

Measures of Assay Performance

- **Positive Predictivity** if I have a positive result, how likely is this to be predictive of a carcinogen?
 $\# \text{ carcinogens positive} / \# \text{ chemicals positive}$
- **Negative Predictivity** if I have a negative result, how likely is this to be predictive of a non-carcinogen?
 $\# \text{ non-carcinogens negative} / \# \text{ chemicals negative}$

Standard Genetic Toxicology Testing Approach

In Vitro Genotoxicity Assays



Positive Results

In Vivo Genotoxicity Assays



- Inexpensive
- Relatively quick
- Require addition of metabolic activation system
- Reduce animal use consistent with 3R's

- Mice, rats
- Normal metabolic activation/detoxification
- Normal tissues

- *Replacement - use of non-animal methods*
- *Reduction - methods which reduce the number of animals used*
- *Refinement - methods which improve animal welfare*

Standard Genetic Toxicology Testing Approach



Gene Mutations

In vitro

- Ames assay
- Mouse lymphoma assay TK^{+/-}
- CHO/HGPRT assay

In vivo

- Transgenic Rodent Mutation Assay



Cytogenetic Damage

In vitro

- Chromosome aberrations
- Micronucleus

In vivo

- Micronucleus
- Chromosome aberrations

DNA Damage/Transformation



- DNA damage (e.g. Comet, Greenscreen)
- DNA repair (e.g. UDS assay)
- DNA adducts
- Cell transformation

Most common assays for regulatory submission

When is Genetox Testing Conducted?

Increasing cost, time, other resources

Discovery/Prioritization/Lead I.D.



Lead Optimization

Screening versions of standard assays to predict results of GLP assays

“GLP Gate”



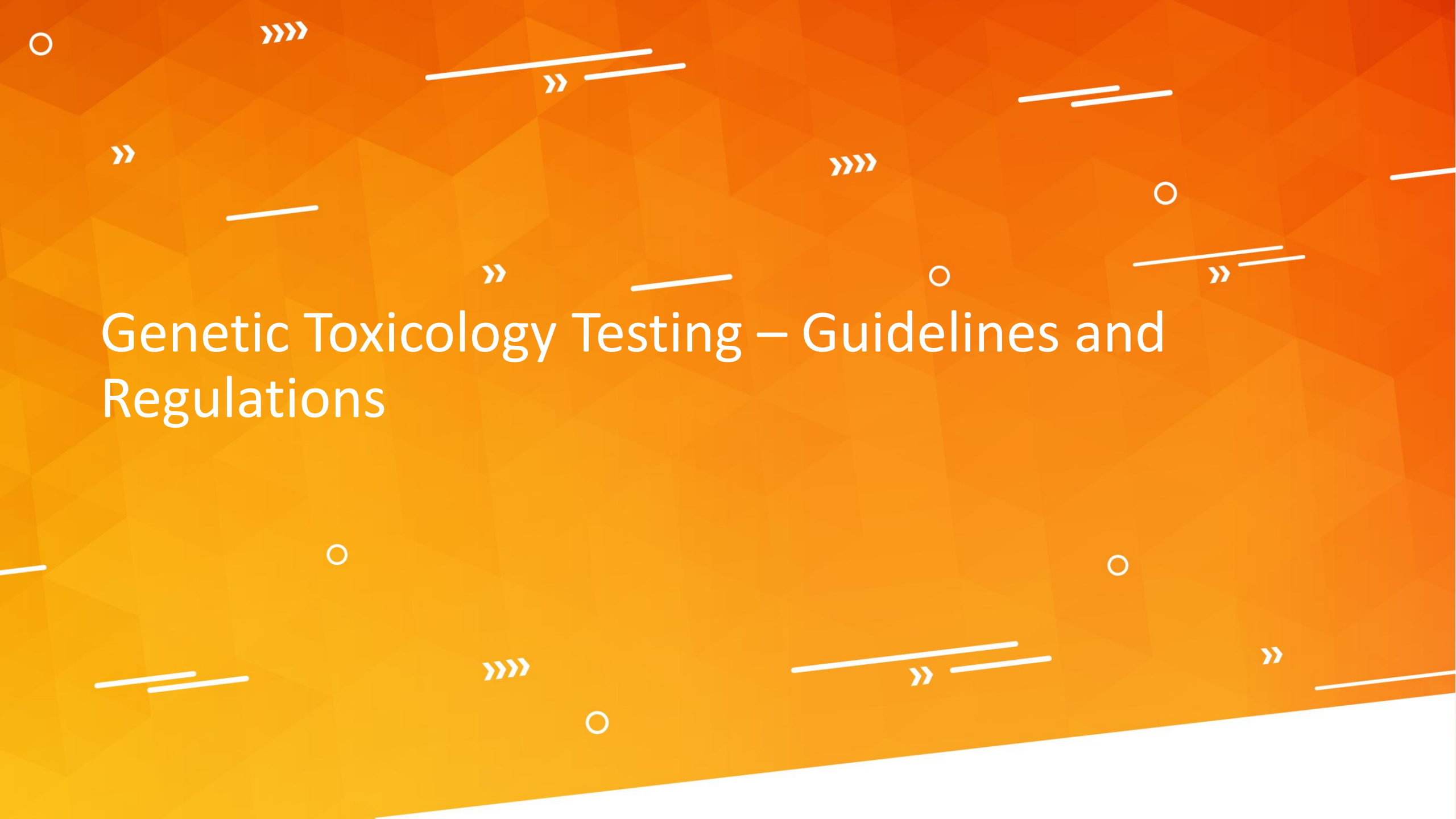
GLP Gene Tox Battery

Perform assays for regulatory submission according to appropriate guidelines



Follow-up assays to solve problems

Additional testing to determine biological relevance of *in vitro* results, investigate mechanisms

The background is a solid orange color with a subtle, repeating geometric pattern of triangles and hexagons. Scattered across the background are various white decorative elements: small circles, double chevrons (»»), and horizontal lines of varying lengths and orientations. Some of these elements are grouped together, while others are isolated.

Genetic Toxicology Testing – Guidelines and Regulations

Genetic Toxicology Guidelines and Regulations

Regulations “Laws”

Regulatory Guidelines

- How to conduct assays
- Which assays to conduct
- What tests to conduct for chemicals that are positive in initial testing, i.e. “follow-up assays”

Expert Guidelines

- Scientific expert groups’ published guidelines/recommendations
- Not officially adopted into a regulatory guideline, but can be used in some situations (e.g. follow-up testing)

Genetic Toxicology “Laws”

Good Laboratory Practice (GLP)

- Controls to ensure studies are planned, conducted, monitored, reported, archived properly
- FDA 21 CFR Part 58
- EPA 40 CFR 160 (FIFRA) and Part 792 (TSCA)
- OECD ENV/MC/CHEM(98)/17
- Japanese MHLW, Ordinance No. 21

US FDA legislation

- Federal Food, Drug and Cosmetic Act (21 CFR Part 9)
- www.fda.gov/opacom/laws/

US EPA legislation

- **New reformed TSCA, FIFRA**
- www.epa.gov/epahome/lawregs.htm



Organisation for Economic Co-operation and Development

- Global organization that develops and coordinates environmental health and safety activities
 - > Multi-stakeholder approach; intergovernmental organization
 - > Guidelines developed by experts from regulatory groups, drug companies, chemical companies, contract research organizations (CROs) and consultants
 - > OECD compliant studies considered acceptable by contributing member countries
- Develops guidelines for testing drug, industrial chemicals, pesticides, food additives
- OECD guidelines (“protocols”) tell you **how** to conduct an assay; Local regulatory agency testing guidelines tell you **which** assays to conduct
- OECD guidelines are used as basis for other agencies’ guidelines (e.g. ICH guidelines for drugs) so most influential globally

Genetic Toxicology Guidelines

- Testing guidelines for drugs, chemicals, pesticides, food additives
- OECD guidelines are used as basis for other agencies' guidelines
 - > 471 Bacterial Reverse Mutation Test
 - > 473 *In Vitro* Mammalian Chromosome Aberration Test
 - > 474 Mammalian Erythrocyte Micronucleus Test
 - > 475 Mammalian Bone Marrow Chromosome Aberration Test
 - > 476 *In Vitro* Mammalian Cell Gene Mutation Tests using the *Hprt* and *xprt* genes
 - > 487 *In Vitro* Mammalian Cell Micronucleus Test
 - > 488 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays
 - > 489 *In Vivo* Mammalian Alkaline Comet Assay
 - > 490 *In Vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene

Guidelines for Pharmaceuticals



ICH

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

- Provides globally accepted approaches – US, Japan, Europe
- ICHS2 guideline finalized Nov 2011- combined and replaced two previous guidelines
 - > ICH S2A: Guidance on specific aspects “How to do assays” (1995)
 - > ICH S2B: Guidance on standard battery “What assays to do” (1997)
- Builds on OECD guidelines “protocols”

www.ich.org

ICH S2R1 Guideline

Former (ICH S2A/S2B)	Revised (ICH S2R1)	
	Option 1*	Option 2
Ames assay (repeat required)	Ames (no repeat if clear positive or negative)	Ames (no repeat if clear positive or negative)
In vitro mammalian cell assay (using 5 mg/ml or 10mM): Chromosome aberrations, OR <i>tk</i> mutations in mouse lymphoma cells	In vitro mammalian cell assay: (1 mM top concentration) Chromosome aberrations, OR: <i>tk</i> mutations in mouse lymphoma cells OR: in vitro micronucleus assay	No in vitro mammalian cell assays
In vivo cytogenetic assay	In vivo micronucleus or chromosome aberration assay (rat/mouse, blood/bone marrow)**	In vivo gene tox with two tissues** 1) Micronuclei 2) Typically DNA breakage in liver 3) Prefer both endpoints in 1 study

* Required for chemicals toxic to bacteria (e.g. antibiotics)

** Can be integrated into repeat dose studies.

Micronuclei analysis can be evaluated by flow cytometry

European Medicines Agency

► Home ► Human regulatory ► Scientific guidelines ► Non-clinical ► Toxicology

ICH S2 (R1) Genotoxicity testing and data interpretation for pharmaceuticals intended for human use

 Email  Print  Help  Share

Current effective version	 Revision 1 - Adopted guideline
Reference number	CHMP/ICH/126642/08
Published	01/12/2011
Effective from	01/06/2012
Keywords	Genotoxicity, standard test battery, in vitro test, in vivo test, results interpretation
Description	This document aims to optimize the standard genetic toxicology battery for prediction of potential human risks. It describes internationally agreed upon standards for follow-up testing and interpretation of positive results in vitro and in vivo in the standard genetic toxicology battery, including assessment of non relevant findings. This guidance does not apply to biologics.

Document history

Revision 1 <i>Current version</i>	 Adopted guideline	In operation: 01/06/2012–present
Superseded documents	 Adopted guideline (ICH S2A)	In operation: 01/04/1996–31/05/2012
	 Adopted guideline (ICH S2B)	In operation: 01/03/1998–31/05/2012



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

ICH M7 Guideline on Mutagenic Impurities

- Compounds demonstrated to induce genetic mutations, chromosomal breaks, and/or chromosomal rearrangements
 - > are considered genotoxic
 - > have the potential to cause cancer in humans
- Focus on DNA-reactive impurities, i.e. mutagenic impurities typically positive in the bacterial mutagenicity assay
- Threshold of Toxicological Concern (TTC) concept applies – $1.5 \mu\text{g/day} = <1 \times 10^{-5}$ lifetime risk
- Less than Lifetime (LTL) principle applies
 - > Clinical development and marketed products with shorter treatment durations have higher acceptable levels
- Evaluate actual impurities plus risk-based subset of potential impurities
- When an impurity is also a metabolite, safety evaluation is primarily focused on the risk assessment of the metabolite

European Food Safety Authority (EFSA)

- **EFSA** is the European Union risk assessment body for food and feed safety
 - > independent scientific advice to risk managers
- Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment
 - > EFSA Journal 2011;9(9):2379



Initial Tests for EFSA



- Bacterial reverse mutation assay (OECD TG 471), and
- *In vitro* micronucleus test (OECD TG 487)
 - > “Addition of any further *in vitro* mammalian cell tests in the basic battery would significantly reduce specificity with no substantial gain in sensitivity”
- Positive results in initial tests are evaluated in *in vivo* tests

Suitable *In Vivo* Tests

- Mammalian erythrocyte micronucleus test (OECD TG 474)
 - > Broad experience with the micronucleus test shows feasibility of integrating both blood and bone marrow micronucleus analysis into RDT studies
- Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488)
- *In vivo* Comet assay
 - > Due to its flexibility, the *in vivo* Comet assay could easily be incorporated into repeat dose studies

Food Additives: US FDA, CFSAN

Conduct ICH battery if intake is $> 1.5 \mu\text{g/day}$ (0.5 ppb in diet)

If $< 1.5 \mu\text{g/day}$ then only *in vitro* assays (a and b) needed

- a. Gene mutation in bacteria (Ames test)
- b. *In vitro* cytogenetic test using mammalian cells

OR

In vitro mouse lymphoma gene mutation assay

- c. *In vivo* test for chromosome damage in bone marrow
(Micronucleus assay)

Regulations listed in Redbook 2000 (2007)

<http://www.cfsan.fda.gov/~redbook/red-ivb2.html>

Regulatory Battery: Industrial Chemicals

1. *Salmonella/E. coli*, "Ames" Assay

1-10T REACH

US EPA PMN exposure, pesticide, high production volume (HPV)

2. *In vitro* chromosome aberration or micronucleus assay

10-100T REACH

EPA HPV (*or in vivo*)

3. *In vitro* mammalian mutation

10-100T REACH (if above cytogenetic assay negative)

US EPA (pesticides, MLA)

4. *In vivo* chromosome aberration or micronucleus assay

Canada Schedule III and VIII

US EPA PMN exposure, pesticides, HPV (*or in vitro*)

Follow-up test for *in vitro* positives

5. Other *in vivo* assays

Case by case as follow-up test for any *in vitro* positive

REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) echa.europa.eu/web/guest/regulations/reach

EPA guidelines: Cimino, Env. Mole. Mutagen. 47: 362-30, 2006

http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm

REACH Testing

European Chemical REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals)
echa.europa.eu/web/guest/regulations/reach; (ECHA) administers REACH



Annual Tonnage	Registration Year	Annex	Genetic Toxicology Testing
>1000	2010	X	• Ames • In vitro cytogenetic • If above negative, in vitro mammalian mutation
100-1000	2013	IX	• Ames • In vitro cytogenetic • If above negative, in vitro mammalian mutation
10-100	2018	VIII	• Ames • In vitro cytogenetic • If above negative, in vitro mammalian mutation
1-10	2018	VII	• Ames

Regulations and Guidelines for Cosmetics

- EU 7th Amendment to Cosmetics Directive
 - > Prohibits animal testing for cosmetics in EU
 - > Ban on *in vivo* genotoxicity assays March 2009
 - > Ban on repeat dose studies implemented 3/11/13
- 3R's Replacement, Reduction, Refinement
- Global companies – need to comply with global regulations-new assays/approaches

Tier 1 *In vitro* genotoxicity assays

- Bacterial mutation – Ames
- Mammalian mutation
- Chromosomal damage



Tier 2 *In vivo* genotoxicity tests

- *In vivo* micronucleus (bone marrow)
- *In vivo* Comet
- Other

Regulations and Guidelines for Cosmetics

Scientific Committee on Consumer Safety (SCCS)

Notes of Guidance for the Testing of Cosmetic Substances and Their Safety Evaluation 12th Revision Dec 2023

- *Salmonella/E. coli* Ames Assay
- ~~*In vitro* mammalian mutation assay~~
- *In vitro* micronucleus assay ~~or *in vitro* chromosome aberration assay~~

Follow-up approaches for positive results

3D Skin Micronucleus Assay

3D Skin Comet

Cell Transformation Assay

Pfuhler *et al.* Reg. Tox Pharm. 57:351-324, 2010

Regulations and Guidelines for Other Products

- Medical Devices
 - > ISO 10993-3 (2014) – Tests for genotoxicity, carcinogenicity and reproductive toxicity
 - > ISO 10993-33 (2015)- Guidance on tests to evaluate genotoxicity – supplement to ISO 10993-3
- Tobacco products
 - > Regulated under FDA Center for Tobacco Products (CTP)
 - > Includes traditional tobacco products (smoked and smokeless) along with ENDS products

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Thank you for listening!

Explore our Genetic Toxicology Services

