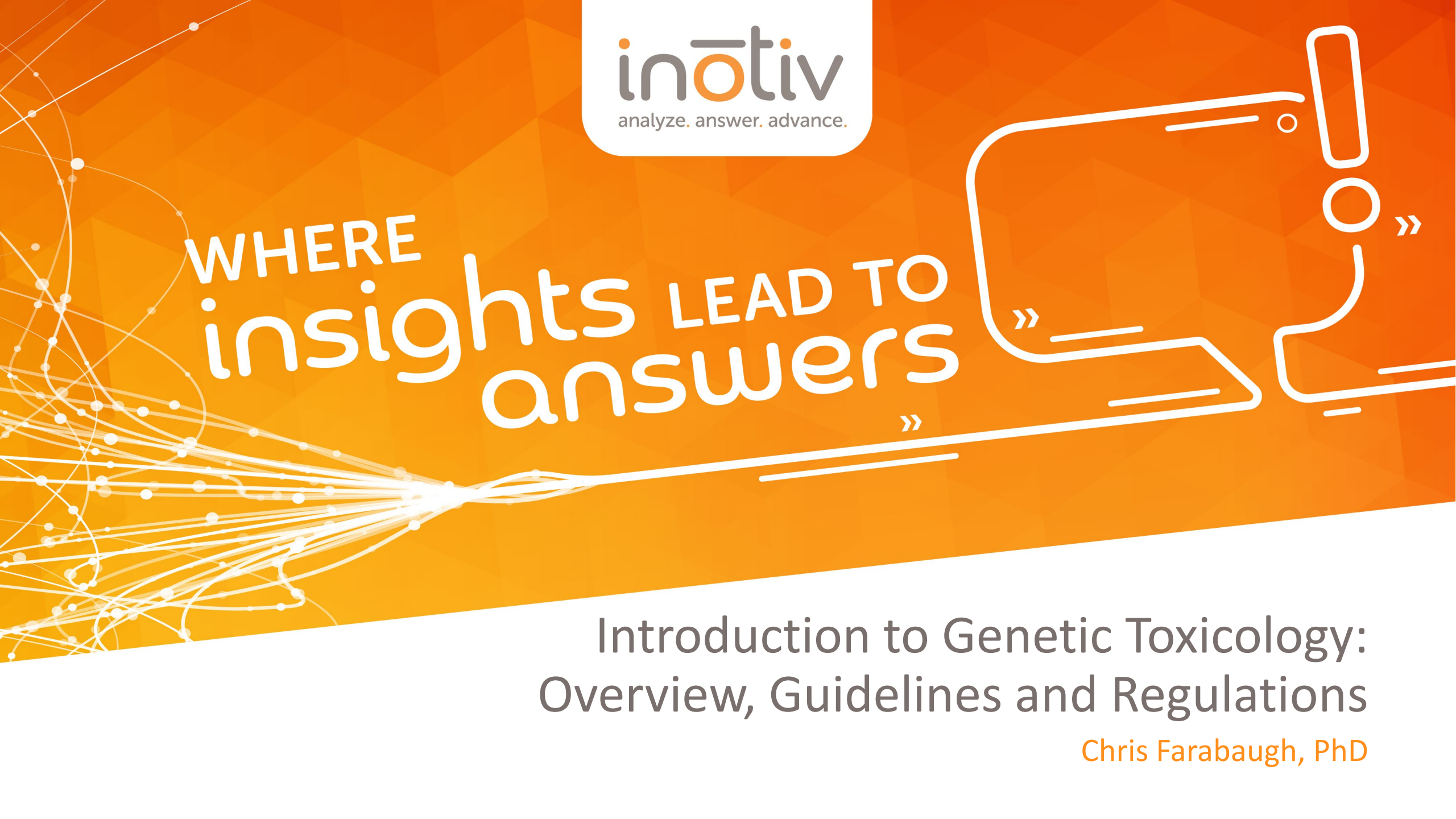




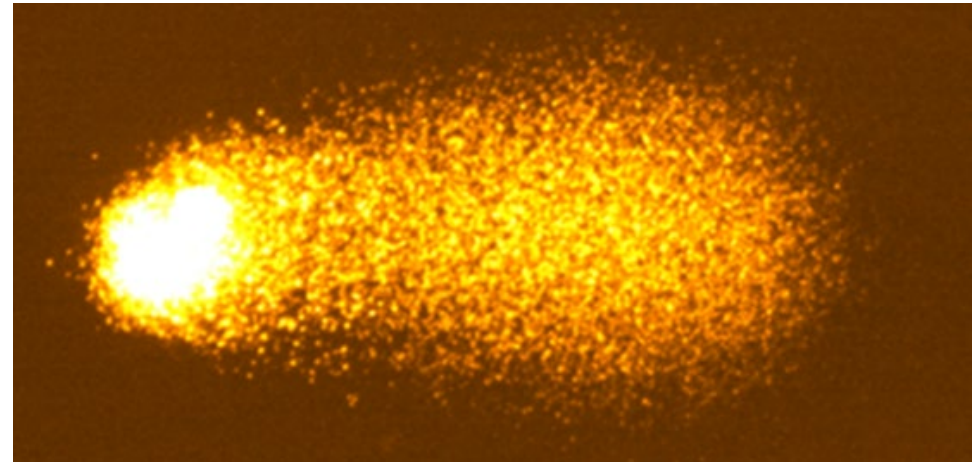
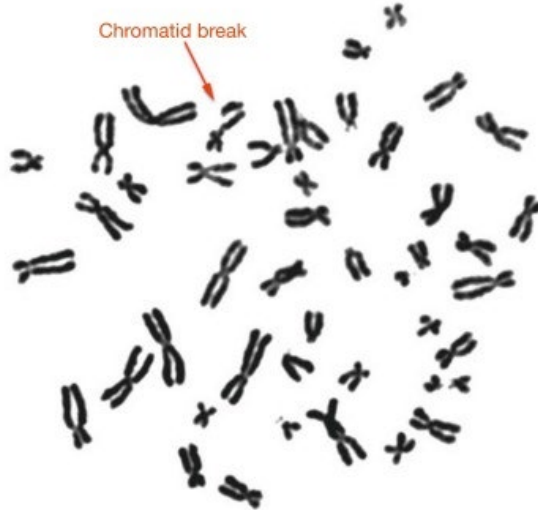
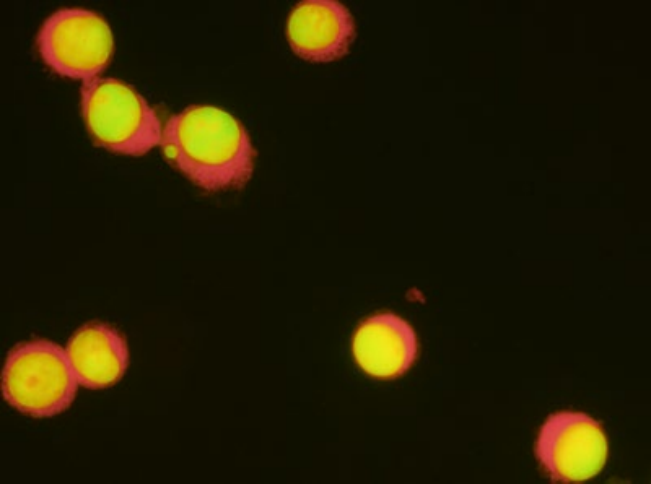
WHERE
insights LEAD TO
answers

Introduction to Genetic Toxicology: Overview, Guidelines and Regulations

Chris Farabaugh, PhD

Agenda

- Foundations of Genetic Toxicology
- Assay Toolbox and Testing Strategies
- Genetic Toxicology Testing Guidelines and Regulations

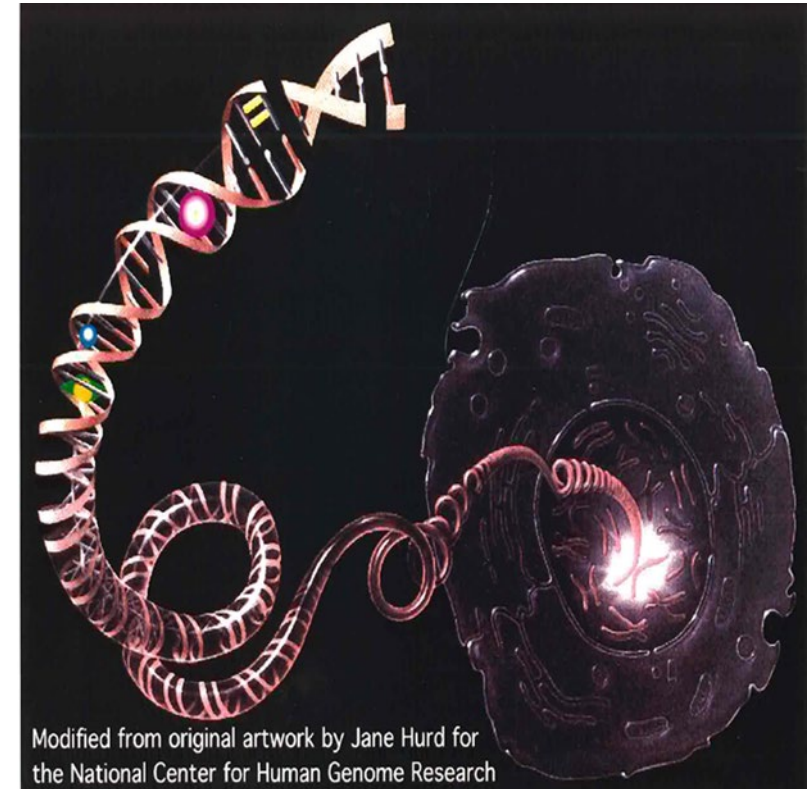


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Foundations of Genetic Toxicology

What is Genetic Toxicology

- Study of DNA damage and mutation
 - > Caused by chemicals, metabolites, biologics
 - > Linked to cancer and heritable risk
 - > Direct and indirect mechanisms
 - Direct
 - Chemical or metabolite interacts with DNA
 - Indirect
 - Chemical or metabolite affects other cellular macromolecules, e.g. mitotic spindle fibers



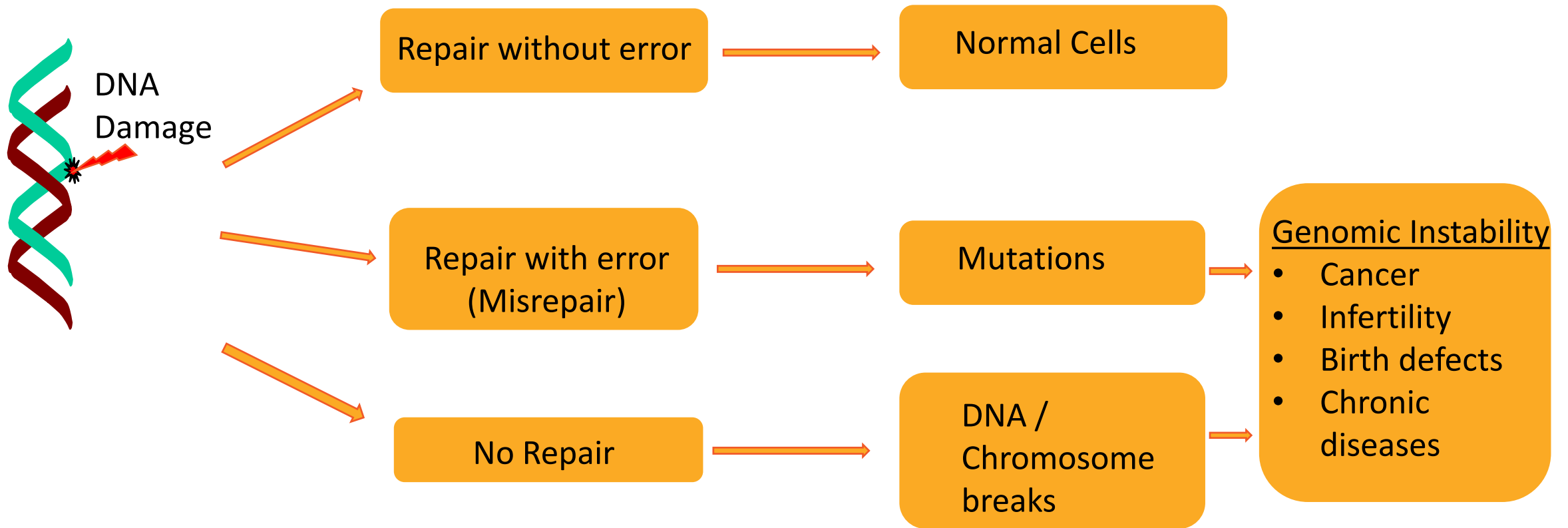
Why Do We Evaluate Genotoxicity?

- Identify DNA-damaging risk early in the drug development process
- Protect patients in clinical trials
- Enable regulatory approval
- Predict carcinogenic potential

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity testing of topotecan has not been performed. Topotecan is known to be genotoxic to mammalian cells and is a probable carcinogen. Topotecan was mutagenic to L5178Y mouse lymphoma cells and clastogenic to cultured human lymphocytes with and without metabolic activation. It was also clastogenic to mouse bone marrow. Topotecan did not cause mutations in bacterial cells.

Genetic Toxicology: Study Of Genetic Damage



Key Terminology

Genotoxicant

- > Agent that damages DNA

Mutagen

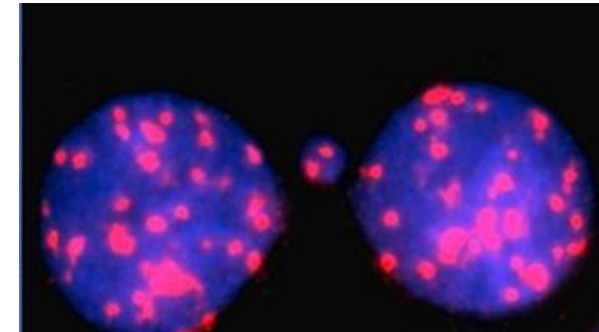
- > Agent that can induce mutations (change in the heritable DNA sequence)
 - > All mutagens are genotoxicants
 - > Not all genotoxicants are mutagens because DNA damage can be repaired

Clastogen

- > Agent that induces breaks in chromosome (structural damage)

Aneugen

- > Agent that induces changes in the number of chromosomes



Albendazole (brand name Albenza) is used to treat various parasitic worm infections, including tapeworms, hookworms, and roundworms. It acts by inhibiting tubulin polymerization, causing energy loss and parasite death.

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Assay Toolbox and Testing Strategies

A Standard Battery of Tests

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

- Various analyses on assay performance identify the most “robust” battery
- Scientific expert groups develop recommended test batteries which can vary for drugs, cosmetic ingredients, etc.
- Regulatory agencies and expert groups define minimum acceptable tests and test batteries
 - OECD (Organisation for Economic Co-operation and Development)
 - ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)

OECD Testing Guidelines

- ***In Vitro* Mutation**
 - > OECD TG 471 Bacterial Reverse Mutation Test – Ames Assay
 - > OECD TG 476 *In Vitro* Mammalian Cell Gene Mutation Tests using the *Hprt* and *xprt* genes – *Hprt* Assay
 - > OECD TG 490 *In Vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene - Mouse Lymphoma Assay
- ***In Vitro* Chromosome Damage**
 - > OECD TG 473 *In Vitro* Mammalian Chromosome Aberration Test – Chrom Abs
 - > OECD TG 487 *In Vitro* Mammalian Cell Micronucleus Test – *In Vitro* Micronucleus
 - > OECD TG 490 *In Vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene – Mouse Lymphoma Assay
- ***In Vivo* Chromosome Damage**
 - > OECD TG 474 Mammalian Erythrocyte Micronucleus Test – *In Vivo* Micronucleus
 - > OECD TG 475 Mammalian Bone Marrow Chromosome Aberration Test – *In Vivo* Chrom Abs
- ***In Vivo* Mutation**
 - > OECD TG 470 Mammalian Erythrocyte *Pig-a* Gene Mutation Assay – *Pig-a*
 - > OECD TG 488 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays – Transgenic Assay
- **DNA Damage**
 - > OECD TG 489 *In Vivo* Mammalian Alkaline Comet Assay – Comet Assay

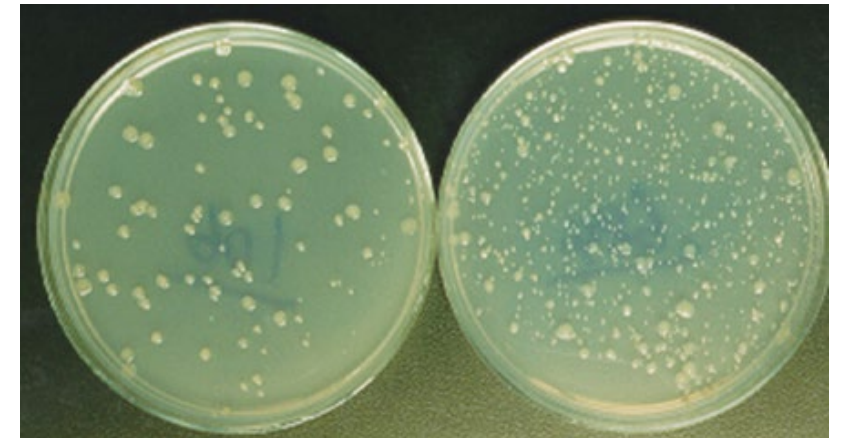
Measures of Assay Performance to Aid in Assay Selection

Example: Power of the Ames assay (TG 471) for predicting carcinogenesis

Metric	Calculation	Result
Sensitivity (Carcinogen Detection)	59/100	59%
Specificity (Non-carcinogen Detection)	74/100	74%
Concordance (Overall Performance)	(59+74)/200	66%

Kirkland *et al.* Mut. Res 584:1-256, 2005

- Ames Assay correctly identifies carcinogens/non-carcinogens $\frac{2}{3}$ ^{rds.} of the time
- Additional assays are essential



Standard Genetic Toxicology Testing Approach

Gene Mutation Assays

- Bacterial
 - Ames (TG 471)
- *In Vitro* Mammalian
 - *Hprt* (TG 476)
 - Mouse Lymphoma (TG 490)
- *In Vivo*
 - *Pig-a* (TG 470)
 - Transgenic (TG 488)

Cytogenetic Assays

- *In Vitro*
 - Chromosome Aberration (TG 473)
 - Micronucleus (TG 487)
 - Mouse Lymphoma (TG 490)
- *In Vivo*
 - Micronucleus (TG 474)
 - Chromosome Aberration (TG 475)

DNA Damage

- *In Vivo*
 - Comet (TG 489)

Expanded Genetic Toxicology Toolbox

Gene Mutation Assays

- Bacterial
 - Ames (TG 471)
- *In Vitro* Mammalian
 - *Hprt* (TG 476)
 - Mouse Lymphoma (TG 490)
- *In Vivo*
 - *Pig-a* (TG 470)
 - Transgenic (TG 488)
 - Duplex Sequencing

Cytogenetic Assays

- *In Vitro*
 - Chromosome Aberration (TG 473)
 - Micronucleus (TG 487)
 - Mouse Lymphoma (TG 490)
- *In Vivo*
 - Micronucleus (TG 474)
 - Chromosome Aberration (TG 475)

Follow-up and Tier II

- *In Silico*
 - QSAR
- *In Vitro*
 - Bhas 42 Cell Transformation
 - Neutral Red Uptake (TG 129)
 - Thiazole Blue (MTT) Assay
 - FISH/CREST
- *In Vivo*
 - Comet (TG 489)
 - CREST

When is Genetic Toxicology Testing Conducted in Pharmaceutical Development?

Discovery

- Non-GLP testing, such as *in silico* analysis and screening versions of standard GLP studies
- To identify and eliminate genotoxic compounds early

Pre-IND Enabling

- *In vitro* genotoxicity tests
- Required to support the Investigational New Drug (IND) application

Prior to Phase II

- *In vivo* genotoxicity testing
- Required to further evaluate potential damage before moving to Phase II trials

Late Stage/ Post Marketing

- Testing of drug impurities, metabolites, and degradation products
- Conducted to support New Drug Application (NDA) submissions

Key Takeaways

- Global guidelines (ICH, OECD, FDA, EFSA) define the minimum assays needed to advance a molecule
- Tiered *in vitro* → *in vivo* strategies ensure hazards are identified efficiently and early
- Testing is conducted from discovery screening through IND-enabling studies and into late-stage development
 - > Impurities, degradants, and metabolites require additional evaluation

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Global Guidelines and Regulations

Genetic Toxicology Guidelines and Regulations

- Regulatory Guidelines

- > How to conduct assays
- > Which assays to conduct
- > What tests to conduct for chemicals that are positive in initial testing

- Expert Guidelines

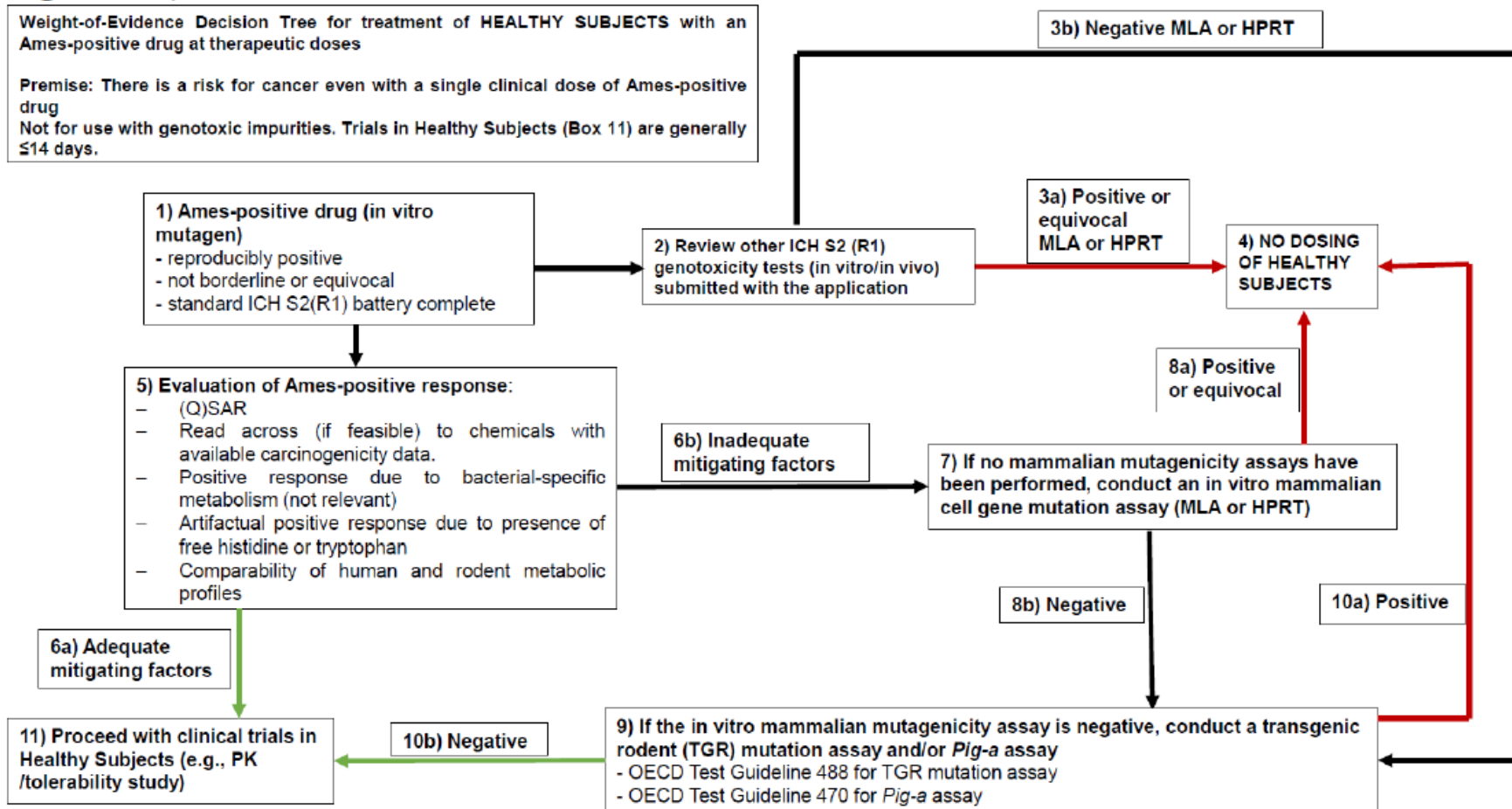
- > Scientific expert groups' published guidelines/recommendations
- > Not officially adopted into a regulatory guideline, but can be used in some situations

- Duplex sequencing for weight of evidence

- Good Laboratory Practice (GLPs)

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

CDER Draft Guidance – Ames Follow-Up



OECD and ICH



- OECD (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, CROs and consultants
 - OECD compliant studies considered acceptable by contributing member countries
 - > Develops guidelines for testing drugs, industrial chemicals, pesticides, food additives
 - > OECD testing guidelines (“protocols”) tell you how to conduct an assay
 - Local regulatory agency testing guidelines tell you which assays to conduct
- ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)
 - > Provides globally accepted approaches – US, Japan, Europe
 - > Builds on OECD guidelines
 - > ICH M7
 - Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk
 - > ICH S2(R1)
 - Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use



ICH S2(R1) Guideline

Option 1*	Option 2
Ames (TG 471) (no repeat if clear positive or negative)	Ames (TG 471) (no repeat if clear positive or negative)
<i>In vitro</i> mammalian cell assay (choose 1): - Chromosome aberrations (TG 473) - Micronucleus (TG 487) <i>tk</i> mutations in mouse lymphoma cells (TG 490) - Gene Mutation Tests using the <i>Hprt</i> and <i>xprt</i> genes (TG 476)	No <i>in vitro</i> mammalian cell assays
<i>In vivo</i> studies (choose 1): - Micronucleus (TG 474) - Chromosome aberrations (TG 475) (rat/mouse, blood/bone marrow)**	<i>In vivo</i> gene tox with two tissues** - Micronucleus (TG 474) - Comet assay (TG 489) 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

Regulatory Battery: EFSA

Assay	Required Tests
Ames (TG 471)	✓
<i>In Vitro</i> Chrom Abs (TG 473)	
<i>In Vitro</i> Micronucleus (TG 487)	✓
Mouse Lymphoma (TG 490)	
<i>Hprt</i> (TG 476)	
<i>In Vivo</i> Micronucleus (TG 474)	Choose one if initial tests are positive
<i>In Vivo</i> Comet (TG 489)	Choose one if initial tests are positive
Transgenic (TG 488)	Choose one if initial tests are positive

adding further *in vitro* mammalian cell tests to the standard two-test base set (Ames test and Mammalian Cell Micronucleus test) would reduce specificity without significant gain in sensitivity

Regulatory Battery: Food Additives (US FDA, CFSAN)



Assay	Required Tests
Ames (TG 471)	√
<i>In Vitro</i> Chrom Abs (TG 473)	Choose one
<i>In Vitro</i> Micronucleus (TG 487)	Choose one
Mouse Lymphoma (TG 490)	Choose one
<i>Hprt</i> (TG 476)	Only used for weight of evidence
<i>In Vivo</i> Micronucleus (TG 474)	Conduct if intake is > 1.5 µg/day (0.5 ppb in diet)
<i>In Vivo</i> Comet (TG 489)	
Transgenic (TG 488)	

Regulations listed in Redbook 2000 (2007)

<https://www.fda.gov/media/79074/download>

Regulatory Battery: ECHA (Industrial Chemicals)



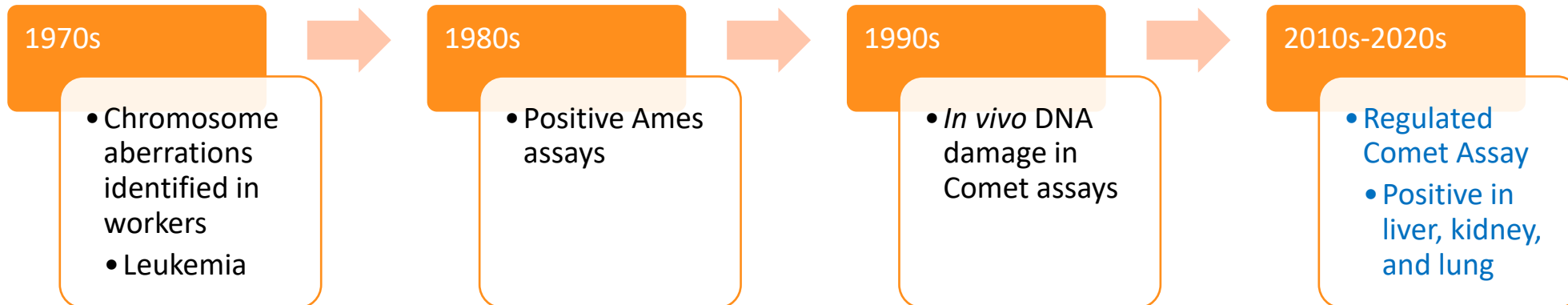
Assay	Required Tests
Ames (TG 471)	≥1-10 Tonnage
<i>In Vitro</i> Chrom Abs (TG 473)	Choose one for ≥10 Tonnage
<i>In Vitro</i> Micronucleus (TG 487)	Choose one for ≥10 Tonnage
Mouse Lymphoma (TG 490)	Choose one if TG 473 or TG 487 are positive
<i>Hprt</i> (TG 476)	Choose one if TG 473 or TG 487 are positive
<i>In Vivo</i> Micronucleus (TG 474)	Choose one if <i>in vitro</i> tests are positive
<i>In Vivo</i> Chrom Abs (TG 475)	Choose one if <i>in vitro</i> tests are positive
<i>In Vivo</i> Comet (TG 489)	Case by case for any <i>in vitro</i> positive
Transgenic (TG 488)	Case by case for any <i>in vitro</i> positive

REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) <https://echa.europa.eu/guidance-documents/guidance-on-reach>

REACH Example

- Epichlorohydrin

- > Primarily used in the production of epoxy resins
- > Consistent genotoxicity across every major test system
- > Modern REACH-era data confirmed *in vivo* DNA damage, reinforcing its strict regulatory control
- > Restricted from being used in substances or mixtures intended for the general public



Regulatory Battery: US EPA



Assay	Required Tests
Ames (TG 471)	√
<i>In Vitro</i> Chrom Abs (TG 473)	Choose one
<i>In Vitro</i> Micronucleus (TG 487)	Choose one
Mouse Lymphoma (TG 490)	Choose one if Ames is positive or there are structural alerts
<i>Hprt</i> (TG 476)	Choose one if Ames is positive or there are structural alerts
<i>In Vivo</i> Micronucleus (TG 474)	Choose one if <i>in vitro</i> tests are positive
<i>In Vivo</i> Chrom Abs (TG 475)	Choose one if <i>in vitro</i> tests are positive
<i>In Vivo</i> Comet (TG 489)	Case by case for any <i>in vitro</i> positive
Transgenic (TG 488)	Case by case for any <i>in vitro</i> positive

<https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines>

Regulations and Guidelines for Cosmetics

- EU 7th Amendment to Cosmetics Directive
 - > Prohibits animal testing for cosmetics in EU
 - Ban on *in vivo* genotoxicity assays 2009
 - Ban on complex studies such as repeat dose studies implemented 2013
- 3R's: Replacement, Reduction, Refinement
- Global companies comply with global regulations and new assays/approaches
 - *In vitro* genotoxicity assays
 - > Ames (TG 471)
 - > *In vitro* micronucleus (TG 487)
 - Follow-up approaches for positive results
 - > 3D Skin Micronucleus Assay
 - > 3D Skin Comet
 - > Cell Transformation Assay
 - Bhas 42

ICH M7 Guideline on Mutagenic Impurities



For 95%+ of impurities, QSAR + Ames test are sufficient, and no additional studies are needed.

ICH M7 Guideline on Mutagenic Impurities

- Threshold of Toxicological Concern (TTC) concept applies
 - 1.5 µg/day which corresponds to <1 in 100,000 lifetime cancer risk
 - Even if an impurity is a mutagenic carcinogen, if human exposure is below the TTC, the cancer risk is considered negligible
- Less than Lifetime (LTL) principle applies
 - Clinical and marketed products with shorter treatments have higher acceptable levels

Duration of treatment	≤1 month	>1 - 12 months	>1 - 10 Years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

Ranitidine (Zantac) developed by GSK, was recalled in 2020 due to contamination with N-nitrosodimethylamine (NDMA), a probable human carcinogen.

- NDMA was present due to the unstable nature of the ranitidine molecule, which breaks down into NDMA.
- NDMA levels increased in ranitidine over time, or when stored at higher temperatures.
- The FDA approved reformulated, stable ranitidine in 2025.

Regulatory Battery: Tobacco Products



Assay	Required Tests
Ames (TG 471)	√
<i>In Vitro</i> Chrom Abs (TG 473)	Choose one
<i>In Vitro</i> Micronucleus (TG 487)	Choose one
Mouse Lymphoma (TG 490)	Choose one
<i>Hprt</i> (TG 476)	
<i>In Vivo</i> Micronucleus (TG 474)	Choose one
<i>In Vivo</i> Comet (TG 489)	Choose one
Neutral Red Uptake Assay (TG 129)	Optional
Thiazole Blue (MTT) Assay	Optional
Bhas 42 Cell Transformation Assay	Optional



Regulatory Battery: Medical Devices

Assay	Required Tests
Ames (TG 471)	√
<i>In Vitro</i> Chrom Abs (TG 473)	Choose one
<i>In Vitro</i> Micronucleus (TG 487)	Choose one
Mouse Lymphoma (TG 490)	Choose one
<i>Hprt</i> (TG 476)	Choose one
<i>In Vivo</i> Micronucleus (TG 474)	Choose one if necessary to resolve results from <i>in vitro</i> tests
<i>In Vivo</i> Comet (TG 489)	Choose one if necessary to resolve results from <i>in vitro</i> tests
Transgenic (TG 488)	Choose one if necessary to resolve results from <i>in vitro</i> tests

- > ISO 10993-3 (2014) Tests for genotoxicity, carcinogenicity and reproductive toxicity
- > ISO 10993-12 (2012) Sample preparation
- > ISO 10993-33 (2015) Guidance on tests to evaluate genotoxicity – supplement to ISO 10993-3



Regulatory Battery: Medical Devices

- Two extracts – polar and non-polar
- Assays are run in parallel
- Standard extraction is $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 72 ± 2 hours
 - Default for most medical devices to simulate physiological conditions
- Materials shall be cut into small pieces before extraction to enhance submersion in the extract media, except when otherwise inappropriate

Table 1 — Standard surface areas and extract liquid volumes

Thickness mm	Extraction ratio (surface area or mass/volume) ±10 %	Examples of forms of materials
<0,5	6 cm ² /ml	Film, sheet, tubing wall
0,5 to 1,0	3 cm ² /ml	Tubing wall, slab, small moulded items
>1,0	3 cm ² /ml	Larger moulded items
>1,0	1,25 cm ² /ml	Elastomeric closures
Irregularly shaped solid devices	0,2 g/ml	Powder, pellets, foam, non-absorbent moulded items
Irregularly shaped porous devices (low-density materials)	0,1 g/ml	Membranes, textiles
NOTE While there are no standardized methods available at present for testing absorbents and hydrocolloids, a suggested protocol is as follows: — determine the volume of extraction vehicle that each 0,1 g or 1,0 cm ² of material absorbs; — then, in performing the material extraction, add this additional volume to each 0,1 g or 1,0 cm ² in an extraction mixture.		

Key Takeaways

- Global regulatory frameworks converge on a tiered, risk-based genotoxicity strategy
- Typically Ames + mammalian cell assays + targeted *in vivo* follow-up
- Variation with preferred assays



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Summary

Summary

- Genetic toxicology is essential for assessing risk across product lifecycles
- Tiered *in vitro* → *in vivo* testing ensures efficient hazard identification
- Regulatory frameworks guide assay selection
- Supporting assays (duplex sequencing, FISH, CREST) strengthen decision-making
- Continuous evaluation of impurities, degradants, and metabolites is required



» Thank you for listening!
Explore our Genetic Toxicology Services

