

# METHODS FOR PREDICTING GENOTOXICITY

Genotoxicity is the ability of a substance to damage the genetic information within a cell. A testing strategy, for example, using the test methods listed in the table, can be used to assess the genotoxic potential of a substance. Ongoing projects, for example under Partnership for the Assessment of Risks from Chemicals (PARC), focus on the development of AOP networks for genotoxicity and explore how different combinations of *in vitro* assays can be used effectively. Several workflows have been proposed that recommend the use of two OECD test guidelines, the Bacterial Reverse Mutation Test (OECD TG 471) and the *In Vitro* Micronucleus Test (OECD 487), as a first tier (see, for example, the workflow suggested in the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation 12<sup>th</sup> revision). Clear results in OECD TGs 471 and 487 (i.e. both results clearly negative or positive) may define a substance's mutagenic potential without requiring further testing. Equivocal results may necessitate expert judgment to determine if follow up testing is required, and if so, which follow-up *in vitro* tests are needed to reach a conclusion based on a weight of evidence approach. Examples of *in vitro* tests are listed in the table below.

| METHOD                                                                                                                                                   | TEST PRINCIPLE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <a href="#">OECD TG 471</a> : Bacterial Reverse Mutation Test (Ames Test)                                                                                | The Ames Test uses at least five strains of amino acid–requiring <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> to detect point mutations by base substitutions or frameshifts. It detects mutations that revert other mutations present in the test strains, restoring the functional capability of the bacteria to synthesise an essential amino acid. A large database of results for a wide variety of structures is available for bacterial reverse mutation tests, and well-established methodologies have been developed for testing chemicals with different physicochemical properties, including volatile compounds.                                                                                                                                                                       |
| <a href="#">OECD TG 476</a> : <i>In Vitro</i> Mammalian Cell Gene Mutation Tests Using the HPRT and XPRT Genes                                           | This test measures mutation at hypoxanthine-guanine phosphoribosyltransferase (HPRT) and at a transgene of xanthine-guanine phosphoribosyltransferase (XPRT). The HPRT and XPRT mutation tests detect different spectra of genetic events. Mutant frequency is determined by seeding known numbers of cells in a medium containing the selective agent to detect mutant cells and in a medium without the selective agent to determine the cloning efficiency (viability). After a suitable incubation time, colonies are counted.                                                                                                                                                                                                                                                                              |
| <a href="#">OECD TG 487</a> : <i>In Vitro</i> Mammalian Cell Micronucleus Test (MNvit)                                                                   | The MNvit detects micronuclei in the cytoplasm of interphase cells. Micronuclei may originate in acentric chromosome fragments (i.e., those lacking a centromere) or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The assay detects the activity of clastogenic and aneugenic test chemicals in cells that have undergone cell division during or after exposure to the test chemical.                                                                                                                                                                                                                                                                                                                                                                 |
| <a href="#">OECD TG 473</a> : <i>In Vitro</i> Mammalian Chromosomal Aberration Test                                                                      | This method identifies agents that cause structural chromosomal aberrations in cultured mammalian somatic cells. At predetermined intervals after the exposure of cell cultures to the test chemical, the cells are treated with a metaphase-arresting substance, harvested, and stained. Metaphase cells are then analysed microscopically for the presence of chromosomal aberrations.                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <a href="#">OECD TG 490</a> : <i>In Vitro</i> Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene                                         | This TG includes two distinct <i>in vitro</i> mammalian gene mutation assays requiring two specific thymidine kinase (TK) heterozygous cell lines: L5178Y tk+/- 3.7.2C cells for the mouse lymphoma assay and TK6 tk+/- cells for the TK6 assay. Genetic events detected using the TK locus include both gene mutations and chromosomal events. Mutant frequency is determined by seeding known numbers of cells in a medium containing the selective agent in order to detect mutant cells and in a medium without the selective agent in order to determine the cloning efficiency (viability). After a suitable incubation time, colonies are counted.                                                                                                                                                       |
| <a href="#">OECD TG 446A</a> : ToxTracker Gene Reporter Assay for the Identification of Genotoxic Hazard and Genotoxic/Non-Genotoxic Mechanism of Action | The ToxTracker assay uses a panel of six mouse embryonic stem cell lines. It provides mechanistic insights into the mode of action of genotoxic properties of chemicals by combining six fluorescent reporter genes that are selectively activated by different cellular signaling responses. Two of the signaling responses are associated with direct genotoxicity. The other four stress response pathways are associated with cytotoxic effects (e.g. oxidative stress or protein damage) that may lead to genotoxicity indirectly. The six genetically engineered reporter genes, each expressing a different GFP-tagged reporter, are exposed to the test chemical both with and without an exogenous source of metabolic activation. Activation of the GFP reporter genes is measured by flow cytometry. |
| <i>In Vitro</i> Human Reconstructed Skin Comet Assay                                                                                                     | The comet assay detects the migration of DNA in an agarose gel following electrophoresis, which results in a “head” of intact DNA and a “tail” of fragmented DNA. Single cells are isolated from a highly differentiated three-dimensional tissue model consisting of normal, human-derived epidermal keratinocytes cultured on specially prepared tissue culture inserts and evaluated by fluorescence microscopy after staining with a DNA-binding fluorescent agent. The percentage of DNA in the tail of the comet is used as a measure of DNA damage.                                                                                                                                                                                                                                                      |
| <i>In Vitro</i> Human Reconstructed Skin Micronucleus (RSMN) Assay                                                                                       | Like the MNvit, the RSMN test detects micronuclei in the cytoplasm of interphase cells – in this case, a highly differentiated three-dimensional tissue model consisting of normal, human-derived epidermal keratinocytes cultured on specially prepared tissue culture inserts.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| $\gamma$ H2AX/pH3 method                                                                                                                                 | The $\gamma$ H2AX/pH3 method permits the measurement of two biomarkers that can predict genotoxicity while providing insights into the genotoxic mode of action (aneugen or DNA damage (clastogenicity/mutagenicity)). The use of additional biomarkers such as p53 (for example, in the commercially available MultiFlow® assay) may further extend the utility of the method.                                                                                                                                                                                                                                                                                                                                                                                                                                 |

| METHOD                                                                    | TEST PRINCIPLE                                                                                                                                                                                                                                                                                                          |
|---------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>In vitro</i> transcriptomics-based biomarkers (e.g., GENOMARK, TGxDDI) | Several methods are currently in development that use gene signatures generated using training sets of genotoxic (and non-genotoxic) substances. The probability that a chemical will cause DNA damage is predicted based on the similarity of the test chemical expression profile to the reference chemical profiles. |

#### Select publications

- Bryce SM, Bernacki DT, Bemis JC, et al. Genotoxic mode of action predictions from a multiplexed flow cytometric assay and a machine learning approach. *Environ Mol Mutagen*. 2016; 57(3):171-189.
- Corvi R, Madia F. *In vitro* genotoxicity testing – can the performance be enhanced? *Food Chem Toxicol*. 2017;106:600-608.
- Demuyneck E, Vanhaecke T, Thienpont A, et al. Advancing genotoxicity assessment by building a global AOP network. *Environ Mol Mutagen*. 2026;66(6-7): 341–366.
- Genotoxicity testing using 3D models. Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment Website. <https://www.gov.uk/government/publications/genotoxicity-testing-using-3d-models/genotoxicity-testing-using-3d-models>. Published 18 July 2024.
- Groff K, Evans SJ, Doak SH, et al. *In vitro* and integrated *in vivo* strategies to reduce animal use in genotoxicity testing. *Mutagenesis*. 2021;36:389-400.
- Pfuhler S, van Benthem J, Curren R, et al. Use of *in vitro* 3D tissue models in genotoxicity testing: strategic fit, validation status and way forward. Report of the working group from the 7th International Workshop on Genotoxicity Testing (IWGT). *Mutat Res Genet Toxicol Environ Mutagen*. 2020;850-851:503135.
- Scientific Committee on Consumer Safety. *The SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation 12th Revision*. European Commission; 2023.
- Thienpont A, Verhulst S, Van Grunsven LA, et al. Novel prediction models for genotoxicity based on biomarker genes in human HepaRG™ cells. *ALTEX*. 2023;40(2):271-286.
- Wang X, Crute CE, Allemang A, et al. TGx-DDI (toxicogenomic DNA damage-inducing) biomarker validation: multi-site ring trial supporting regulatory use. *Toxicol Sci*. 2025;208(2):233-243.

## IN SILICO TOOLS FOR PREDICTING GENOTOXICITY

*In silico* strategies for genotoxicity often rely on two complementary modelling approaches, used in tandem to maximise predictive confidence. **Expert rule-based systems** use "structural alerts" (e.g., specific molecular substructures, such as aromatic amines or nitro groups) known to react with DNA to inform predictions. These systems also provide a mechanistic basis for why a chemical is flagged as potentially mutagenic. Complementing this approach, **statistical models** employ machine learning algorithms trained on vast datasets of experimental results (such as the Ames test) to statistically correlate physicochemical properties with genotoxic activity.

As an example, the International Council for Harmonisation (ICH) [M7](#) guidance on the assessment and control of DNA-reactive (mutagenic) impurities in pharmaceuticals calls for the use of *in silico* models, including an expert rule-based model (e.g., Derek Nexus or Toxtree) and a complementary statistical model (e.g., Sarah Nexus) (See Figure 1).

An important consideration in predicting genotoxicity is bioactivation, the process by which a non-toxic parent chemical (pro-mutagen) is converted into a DNA-reactive metabolite by the body's enzymes. Advanced *in silico* models integrate metabolic simulators with QSAR models to predict the genotoxicity of both the parent compound and its likely metabolites.

A non-exhaustive list of *in silico* tools for predicting genotoxicity is provided below. Please contact Kyle Martin at [kmartin@thepsci.eu](mailto:kmartin@thepsci.eu) to include additional resources on this list or with any questions.

| TOOL                                    | DEVELOPER                                       | METHOD/APPROACH                | AVAILABILITY |
|-----------------------------------------|-------------------------------------------------|--------------------------------|--------------|
| <a href="#">ADMET Predictor</a>         | Simulations Plus                                | Statistical                    | Commercial   |
| <a href="#">CASE Ultra</a>              | MultiCASE Inc.                                  | Statistical                    | Commercial   |
| <a href="#">Derek Nexus</a>             | Lhasa Limited                                   | Expert rule-based              | Commercial   |
| <a href="#">Leadscope Model Applier</a> | Instem                                          | Statistical                    | Commercial   |
| <a href="#">OECD QSAR Toolbox</a>       | OECD/Laboratory of Mathematical Chemistry       | Statistical (read-across/QSAR) | Open-source  |
| <a href="#">Sarah Nexus</a>             | Lhasa Limited                                   | Statistical                    | Commercial   |
| <a href="#">Toxtree</a>                 | IDEAconsult Ltd./European Commission            | Expert rule-based              | Open-source  |
| <a href="#">VEGA Hub</a>                | Istituto di Ricerche Farmacologiche Mario Negri | Consensus/hybrid               | Open-source  |

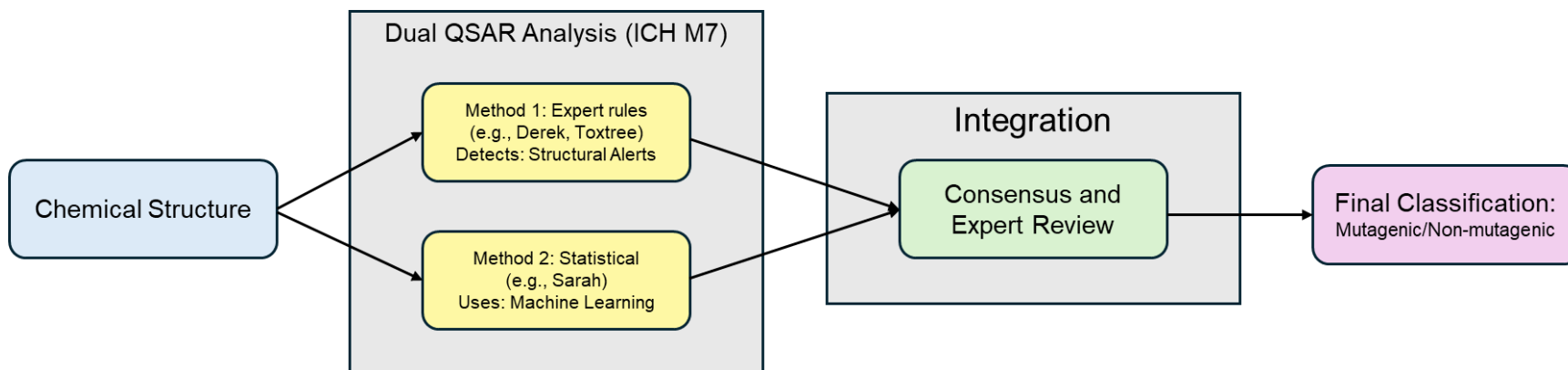


Figure 1. Shows the complementary use of an expert rule-based and a statistical model as described in ICH M7 guidance for predicting the mutagenic potential of pharmaceutical impurities.