OECD – UPDATES ON TEST GUIDELINES FOR GENOTOXICITY ASSESSMENT ASSAYS

Stakeholder Workshop on EFSA's Genotoxicity Guidance Revision
3-4 November 2025

Nathalie Delrue
Environment Health and Safety Division, OECD

BETTER POLICIES FOR BETTER LIVES

MUTUAL ACCEPTANCE OF DATA AND THE TEST GUIDELINE PROGRAMME (TGP)

Mutual Acceptance of Data (MAD)

OECD: 38 member countries
MAD-adhering countries:
Argentina, Brazil, India, Malaysia, Singapore, South Africa and Thailand
(partner countries)

Test Guideline	+	GLP	=	MAD
Internationally harmonised **methods** for evaluating chemical safety		**Principles** and **conditions** under which laboratory studies are conducted, reported and recorded		Studies conducted using OECD TG and according to GLP fall under the **Mutual Acceptance of Data**

MAD is a legal agreement among all member and partner countries that share a common data requirement to accept the data generated by other member countries

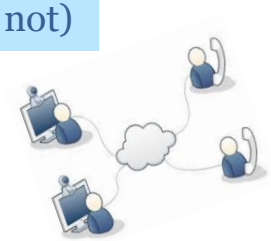
Development of OECD Test Guidelines

- A continuous need for improving testing standards
  - Reflect progress in science,
  - Respond to countries' regulatory needs,
  - Address animal welfare
  - Improve cost-effectiveness of test methods

↳ Set of OECD Test Guidelines is augmented every year with new and updated TG that have undergone a number of stages to demonstrate their **validity** in order to be **accepted by regulatory authorities**

Process for the development of Test Guidelines

- Member country(ies) submit the proposal (SPSF)
- An Expert Group is formed
- Experts help the lead country in developing the method and establishing the validation status
- Member countries adopt the Test Guideline (or not)



If no expert group in place, Secretariat asks WNT to nominate

If approved, project starts

SPSF submitted by lead country(ies) - 15 November

Expert Group (or Validation Management Group) work on technical aspects

Comments from 2 reviews rounds usually addressed by lead and EG

WNT reviews and finally approves TG or guidance document

Committee (CBC) adopt TG, TG is published + all supporting information

Nature of tools developed in the TG Programme

Covered by Mutual Acceptance of Data (MAD)

- Test Guidelines
- Guideline on Defined Approaches
  - Combinations of information sources have been individually evaluated

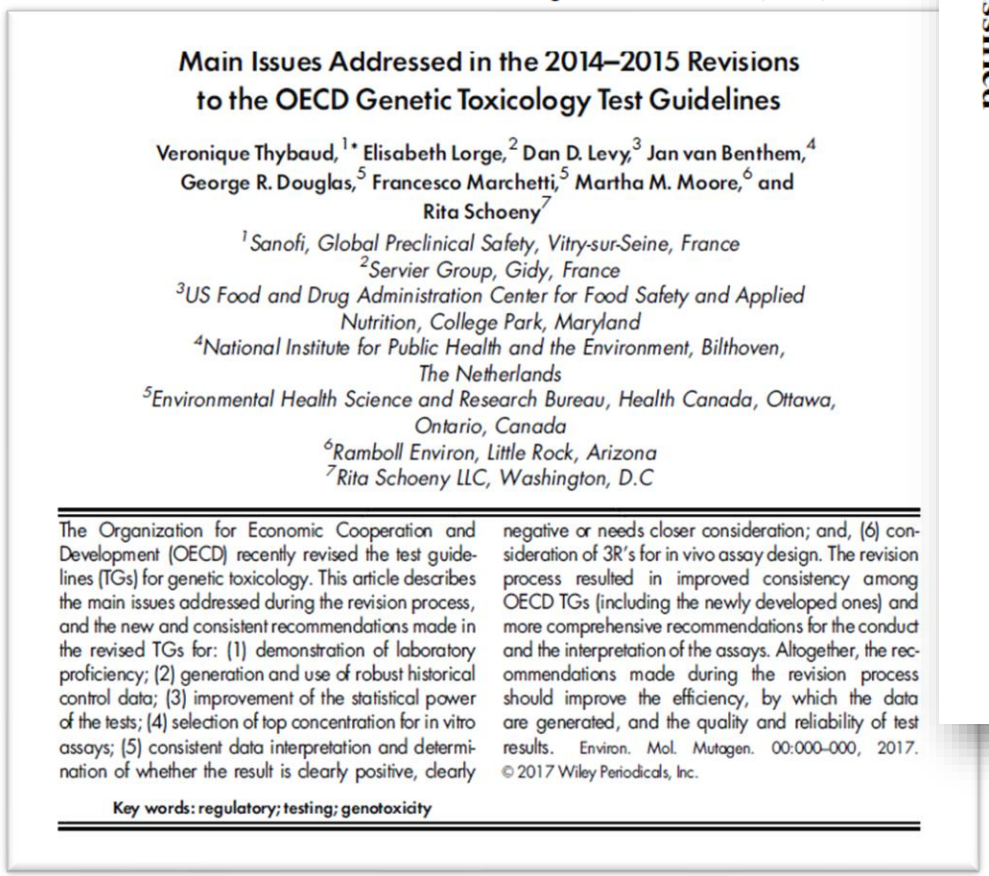
Other important documents

- Guidance Documents in support of testing and assessment
- Detailed Review Papers in new areas of chemical toxicity testing
- Validation reports
- Peer review reports
- Performance Standards
- Case studies
- Feasibility studies
- Various reports e.g. from workshops

- Are developed prior to, or in parallel with, Test Guidelines
- Relate to a specific area of hazard identification
- Are not part of the Council Decision on the Mutual Acceptance of Data
- Development principles from project submission to approval by CBC are the same as for TGs

2011-2015: EXTENDED REVIEW OF THE EXISTING SET OF GENOTOXICITY TESTING GUIDELINES

Publications



Environmental and Molecular Mutagenesis 00:00–00 (2017)

Main Issues Addressed in the 2014–2015 Revisions to the OECD Genetic Toxicology Test Guidelines

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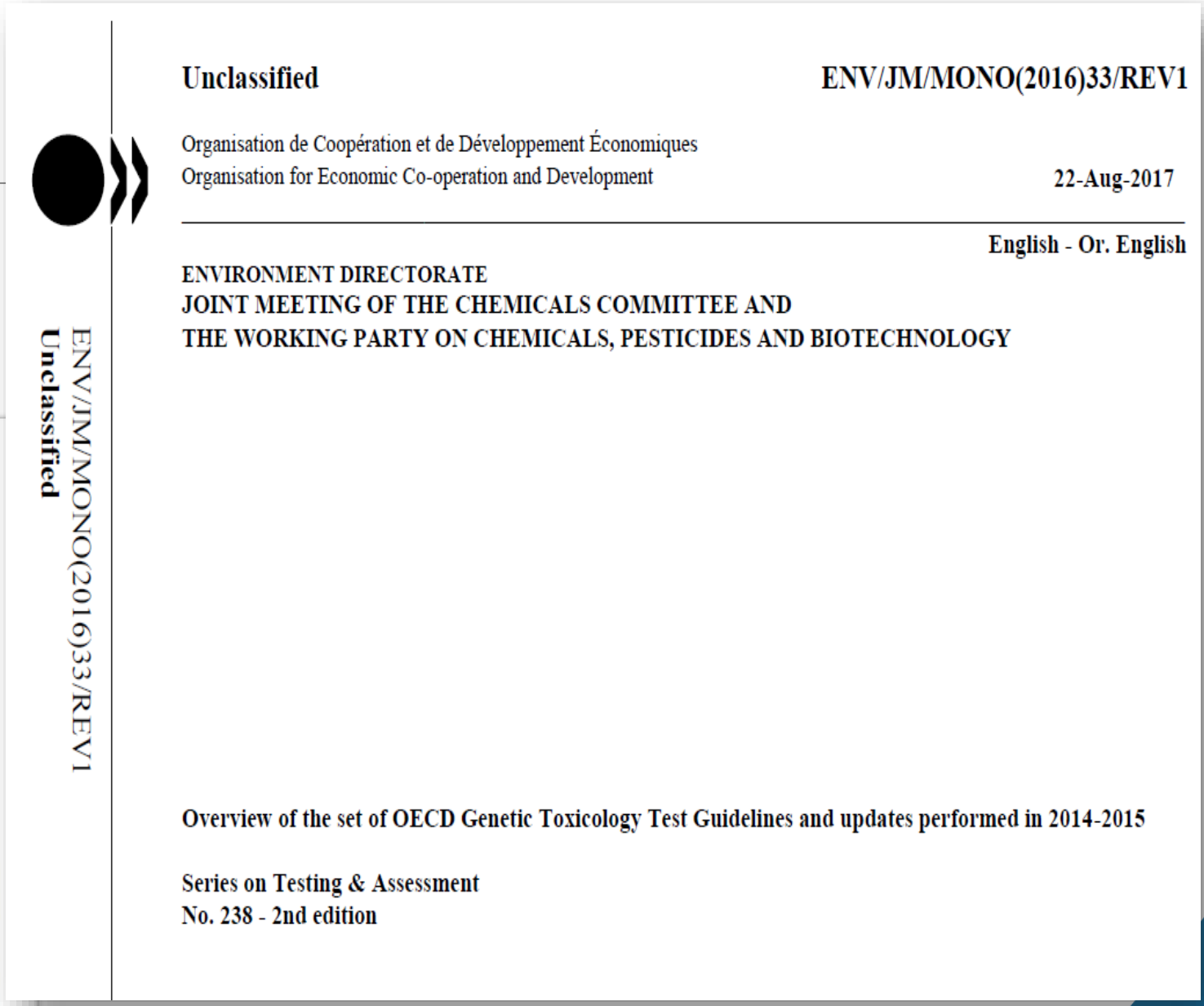
The Organization for Economic Cooperation and Development (OECD) recently revised the test guidelines (TGs) for genetic toxicology. This article describes the main issues addressed during the revision process, and the new and consistent recommendations made in the revised TGs for: (1) demonstration of laboratory proficiency; (2) generation and use of robust historical control data; (3) improvement of the statistical power of the tests; (4) selection of top concentration for in vitro assays; (5) consistent data interpretation and determination of whether the result is clearly positive, clearly negative or needs closer consideration; and, (6) consideration of 3R's for in vivo assay design. The revision process resulted in improved consistency among OECD TGs (including the newly developed ones) and more comprehensive recommendations for the conduct and the interpretation of the assays. Altogether, the recommendations made during the revision process should improve the efficiency, by which the data are generated, and the quality and reliability of test results. Environ. Mol. Mutagen. 00:000–000, 2017.

Key words: regulatory; testing; genotoxicity



Unclassified ENV/JM/MONO(2016)33/REV1

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

22-Aug-2017

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/MONO(2016)33/REV1
Unclassified

Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015

Series on Testing & Assessment
No. 238 - 2nd edition

Set of deleted genotoxicity TG

TG	Title	Adopted	Revised	Deleted
	Archived/Deleted			
472	Genetic toxicology: *Escherichia coli*, Reverse Assay	1983		1997
477	Sex-linked recessive lethal test in *Drosophila melanogaster*	1984		2014
479	*In vitro* sister chromatid exchange assay in mammalian cells	1986		2014
480	*Saccharomyces cerevisiae,* gene mutation assay	1986		2014
481	*Saccharomyces cerevisiae,* mitotic recombination assay	1986		2014
482	DNA damage and repair, unscheduled DNA synthesis in mammalian cells *in vitro*	1986		2014
484	Mouse spot test	1986		2014

OECD test methods for genetic toxicity

Gene mutation	Clastogenicity/aneuploidy	DNA damage/repair
In vitro assays - Bacterial tests: TG 471 - Mammalian test: TG 476, TG 490	In vitro assays - Chromosomal aberration: TG 473 - Micronuclei and aneuploidy: TG 487	In vitro assays -
In vivo assays: - Somatic cells TG 488, TG 470 - Germline cell assays TG 488	In vivo assays - Chromosomal aberration: TG 475 - Micronuclei and aneuploidy: TG 474 - Germ cells assays: TG 483, TG 485, TG 478	In vivo assays - UDS TG 486 - Comet TG 489

Biological relevance and criteria for a positive/negative result

- TGs revised to include 3 equal considerations when assessing whether a response is positive or negative

136. For both *in vitro* and *in vivo* assays (with the exception of the MLA—see below) a response is considered a clear positive in a specific test if it meets all the criteria below in at least one experimental condition:

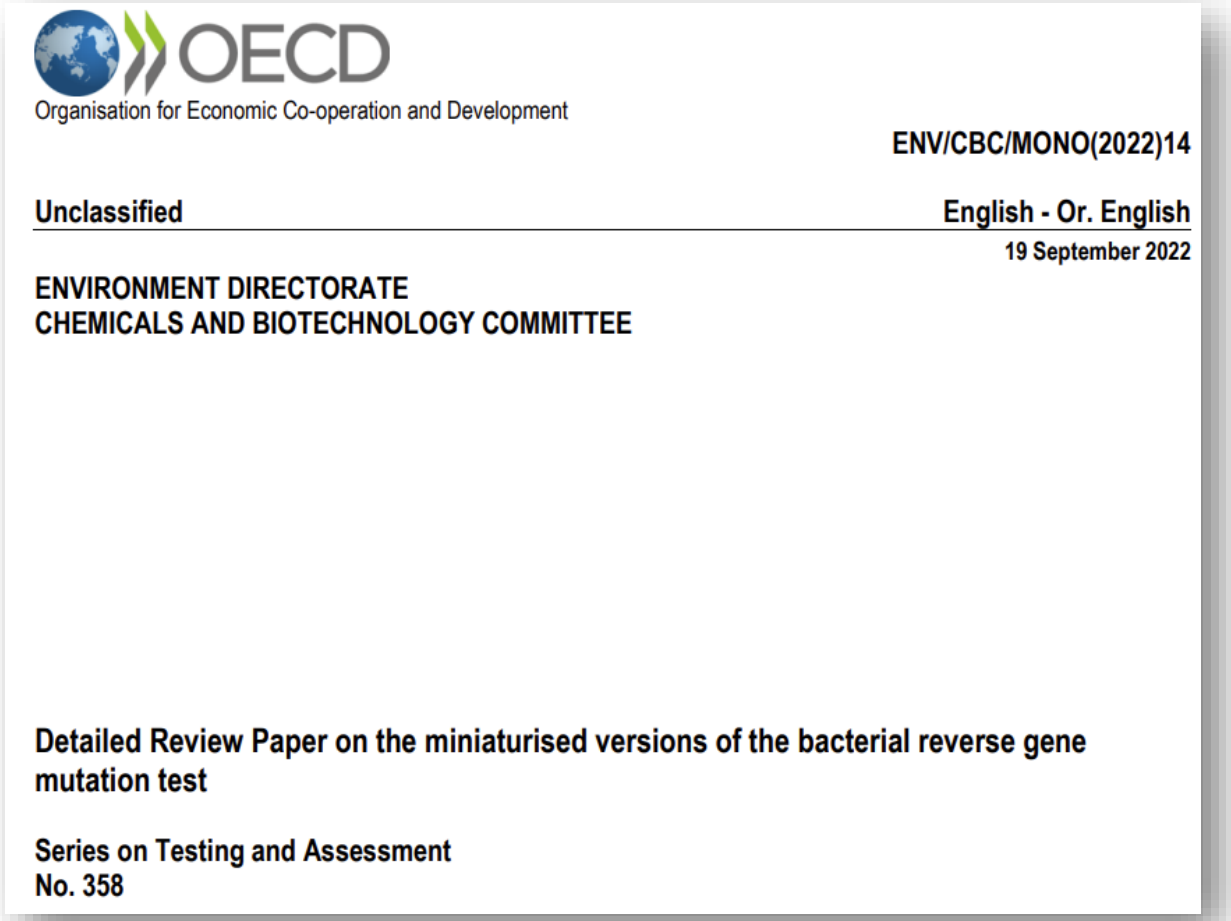
- at least one of the data points exhibits a statistically significant increase compared to the concurrent negative control;
- the increase is concentration- or dose-related at least at one sampling time when evaluated with an appropriate trend test;
- the result is outside the distribution of the historical negative control data (*e.g.* Poisson-based 95% control limits).

137. A test chemical is considered clearly negative if, in all experimental conditions examined, none of the above criteria for a positive result are met.

From: Overview of the set of OECD genetic toxicology TGs and updates performed in 2014-2015

RECENT PROJECTS COMPLETED AND GENOTOXICITY TG UPDATES

DRP on miniaturised versions of the Ames test - 2022



OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)14

Unclassified

English - Or. English

19 September 2022

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Detailed Review Paper on the miniaturised versions of the bacterial reverse gene mutation test

Series on Testing and Assessment
No. 358

- Overview of the available bacterial reverse gene mutation tests and their applications
- Retrospective data analysis conducted on four models
- Results promising
- More work needed to explore further quantitative aspects
  - related to identification of mutagens which produce weakly positive responses in the test

Mammalian Erythrocyte Pig-a Gene Mutation Assay (TG 470) - 2022

Test No. 470: Mammalian Erythrocyte Pig-a Gene Mutation Assay

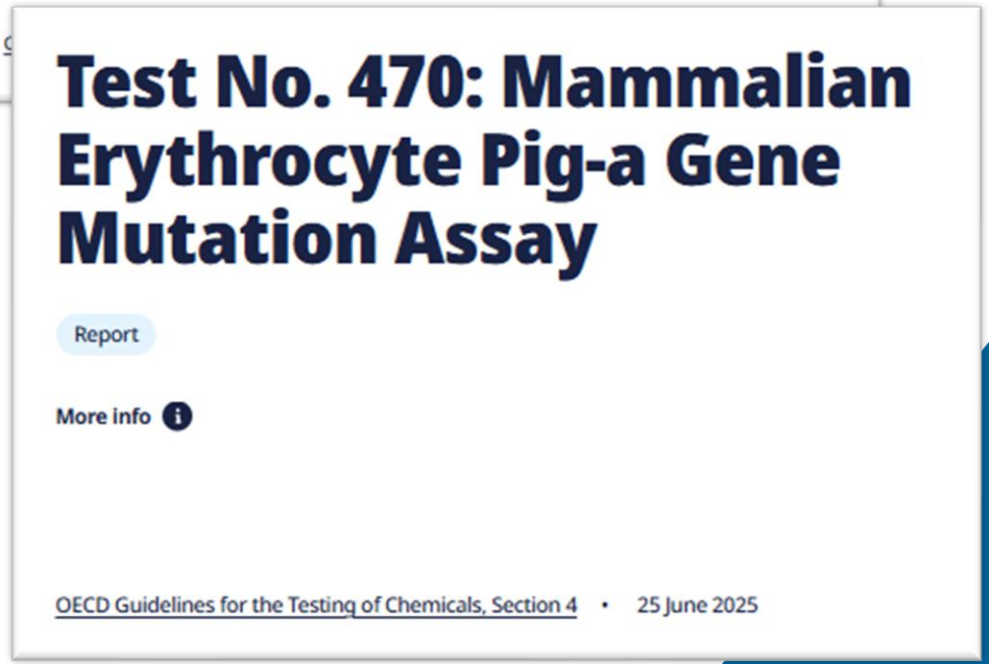
Report

More info

OECD Guidelines for the Testing of Chemicals, Section 4 • 25 June 2025

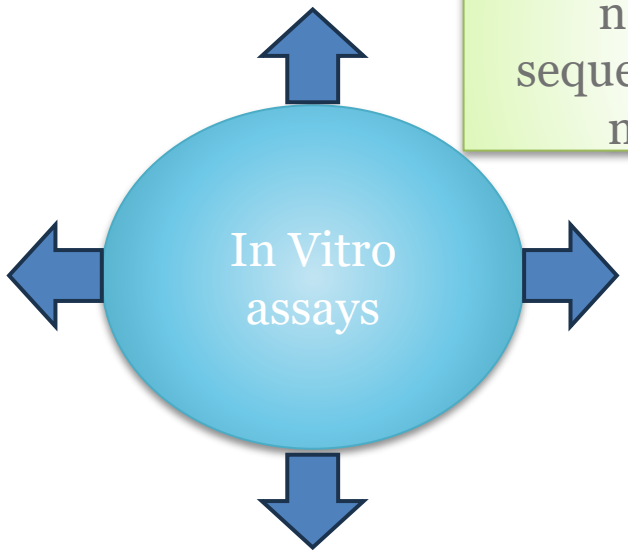
Project on Revision of in vivo genotoxicity Test guidelines - Evaluation and Interpretation of Results - 2025

- Objective of the project
  - Address issues that came as a result of having three criteria of equal weight for interpreting the data
    - Give less weight to the criteria that compares study data to historical data and
    - Improve the consideration given to the quality of the Historical Control Data (HCD) when interpreting data
- TG 488 and TG 470 updated in 2025 to clarify the use of historical control data in the interpretation of results
- Multi-step project including future revision of TGs 474 and 489
- More discussion and development of a webinar in 2026 providing guidance with regards to the Statistical analysis included in TGs 488 and 470



CURRENT PROJECTS ON THE TGP WORKPLAN

In vitro projects on the TGP workplan



Omics technology

Application of error-corrected next generation DNA sequencing (ecNGS) for gene mutation evaluation

Mechanistic insight

3D tissue technology

Reconstructed skin micronucleus test and reconstructed skin Comet assay

In vitro alkaline comet assay and its enzyme-linked modification

ToxTracker assay

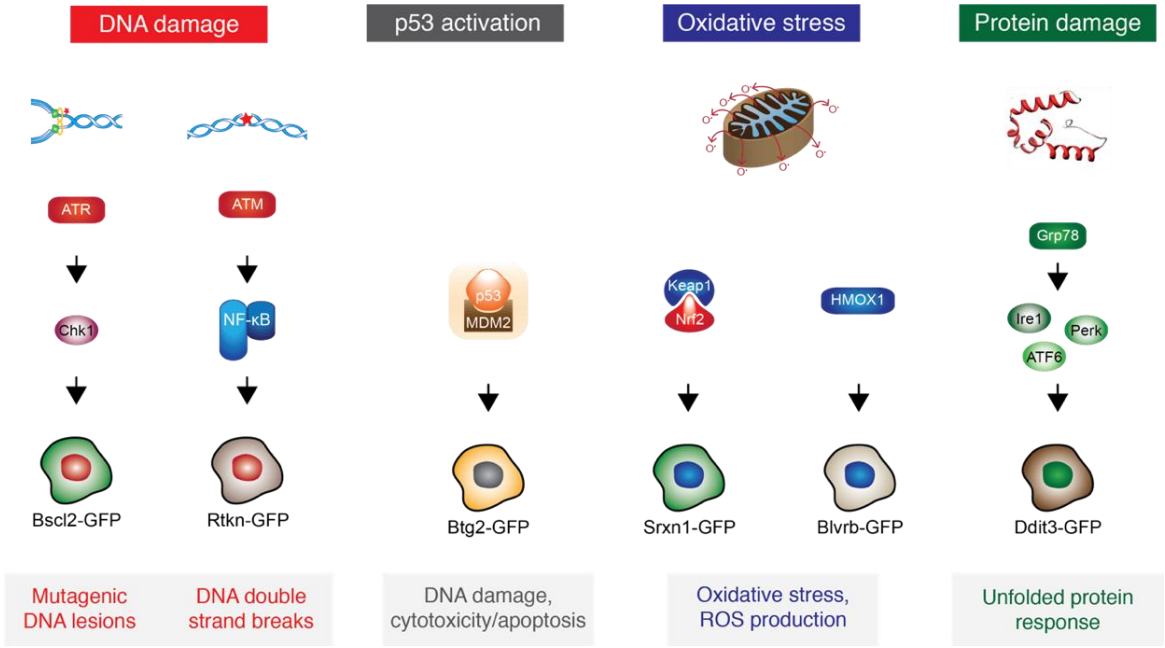
gH2AX/pH3 method

Nanomaterials

Micronucleus assay for Engineered Nanomaterials

Project candidate for approval in 2026: Toxtracker assay

Project	Lead	Inclusion on workplan	Current/Next steps
Project 4.191: Test Guideline on the ToxTracker assay: a stem cell-based reporter assay for mechanistic genotoxicity hazard screening	Netherlands	2017, updated 2025	TG in preparation – Upcoming second WNT commenting round and possible submission for WNT approval in April 2026



Overview of the cellular signaling pathways that activate the different ToxTracker reporter genes
From draft TG – public circulation July 2025

Other in vitro projects on the TGP workplan

Project	Lead /inclusion on workplan	Current status
Project 4.139: In vitro genotoxicity testing for dermal exposure using 3D skin models: reconstructed skin micronucleus test and reconstructed skin Comet assay	Germany/France 2019	Discussion ongoing at the lead level, following ESAC peer review
Project 4.168: DRP and a Retrospective Performance Analysis for the in vitro gH2AX/pH3 method: a multiplexed biomarker approach that provides information on genotoxic mode of action	France/Germany 2023	Initial draft shared for expert review – addressing comments
Project 4.174: Validation of the In Vitro Micronucleus assay for Engineered Nanomaterials	UK/DE/FR/NO and partners: US/LU 2024	Work ongoing at the lead level
Project 4.175: DRP on the application of error-corrected next generation DNA sequencing (ecNGS) for gene mutation evaluation	United States 2024	Work ongoing at the lead level
Project 4.190: In vitro alkaline comet assay and its enzyme-linked modification	Austria/ Croatia 2025	Under consideration at the lead level

Other projects of the TGP workplan

Projects	Lead /inclusion on workplan	Current status
In vivo assay updates		
Project 4.156: Update of TG 489 Comet Assay for gonadal cells to study germ cell specific genotoxic effects	Norway/Denmark 2022	Work ongoing at the lead level – feasibility report under preparation
Project 4.177: Revision of in vivo genotoxicity test guidelines' "Evaluation and Interpretation of Results" and "Test Report" language	United States/Canada 2024	First 2 TGs adopted. Webinar on Statistical analysis to be developed
Overarching projects		
Project 4.157: Support to UN GHS for modification of germ cell mutagenicity criteria in Chapter 3.5	EC-JRC / Secretariat 2022	UN GHS project
Project 4.182: Adverse Outcome Pathway (AOP) network leading to genotoxicity	Belgium/France 2024	Work ongoing at the lead level – PARC project

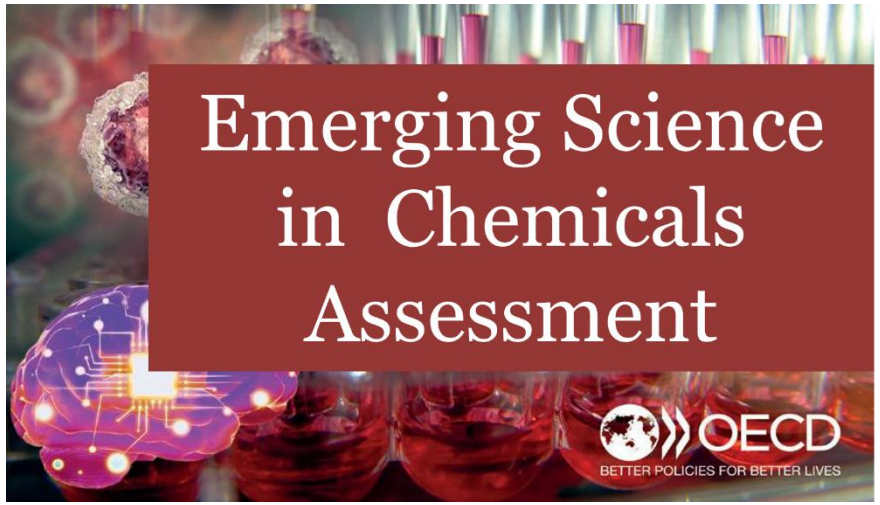
MORE PROJECTS – NOT INCLUDED IN THE OECD TGP WORKPLAN

Other projects of relevance – not on the OECD TGP workplan

- Joint session of the WNT and ESCA in April 2025

How omics and artificial intelligence could modernise and improve genotoxicity assessment – experience gained and potential application to more endpoints

  - Presentation of the TGx-DDi and GenoMark biomarkers for genotoxicity
    - Potential project submission to the OECD in the future



- Other assays under development – not on OECD workplan
  - In vivo micronucleus test in liver and GI tract
  - And more amenable to standardisation…

THANK YOU FOR LISTENING!

Stay connected and learn more about OECD:

https://www.oecd.org/chemicalsafety/

OECD TGP workplan (publicly available and updated every year)

Guidelines for the Testing of Chemicals | OECD

Draft work plan of the Test Guidelines Programme

Newsletter sign up: http://bit.ly/newsletter-chemical-safety

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