EXPERT PANEL FOR COSMETIC INGREDIENT SAFETY

Resource Document

Deleted, Legacy, and Current OECD Genotoxicity Test Guidelines

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This resource document supports the genotoxicity boilerplate used in Cosmetic Ingredient Review (CIR) safety assessments when historical, obsolete, or non-standard genotoxicity assays are identified during the review process. It provides background for excluding such studies from CIR genotoxicity summaries and from the weight-of-evidence (WoE) used in CIR safety assessments. Citations to such studies may be retained, where appropriate, solely to document that the studies were identified and considered.

During the 2014 - 2015 update of the OECD Genetic Toxicology Test Guidelines (TGs), OECD explained that the decision to delete certain TGs was based on rare use across legislative jurisdictions, the availability of robust core tests or newer tests with better performance for the same endpoint, and the preference for mammalian-cell assays over yeast/fungal or insect-based assays because of greater relevance to mammalian biology.[1] In addition to these general rationales, OECD noted an assay-specific concern for the in vitro sister chromatid exchange assay (TG 479): limited understanding of the mechanism(s) underlying the effect detected by the test.

Table 1 summarizes deleted or legacy OECD genotoxicity TGs. OECD states that tests described in deleted TGs should not be used for new testing and are no longer part of the recommended OECD test set.[1] Accordingly, when such assays are identified in published literature, unpublished reports, or data submissions, their results are generally of limited interpretability for current CIR safety assessments and are not summarized or used to support CIR safety conclusions.

Historical and non-standard genotoxicity assays are broader than the OECD TGs listed in Table 1. Assays that have not been incorporated into OECD genotoxicity TGs and are not generally used as validated standard methods in current regulatory genotoxicity assessment should also generally be excluded from CIR genotoxicity summaries and from the WoE evaluation. Examples include the bacteriophage T4D assay, host-mediated assay, SOS response assays such as the SOS chromotest and umu assay, *Bacillus subtilis* Rec assay, polA/DNA repair assays, and other DNA repair or recombination-based assays. These are just some examples, and this is not an exhaustive list. Citations to such studies are retained in CIR reports merely for transparency; the citation in no way implies that the assay result was relied upon in reaching the CIR safety conclusion.

For context, Table 2 summarizes the current OECD genotoxicity TGs commonly used to evaluate gene mutation, chromosomal damage, and DNA strand breaks. This table is intended to orient readers to the current OECD genotoxicity TG set; it is not a required checklist for every CIR safety assessment. The Expert Panel evaluates genotoxicity using a WoE approach, considering the available endpoints, exposure route and relevance, test system, study quality, and whether the data adequately address gene mutation, chromosomal damage, and/or DNA strand breaks, as applicable.

New Approach Methodologies (NAMs), including emerging in vitro, in silico, and integrated approaches, may provide useful mechanistic, human-relevant, or predictive information relevant to genotoxic potential. The Panel recognized that, when appropriately characterized and integrated with established guideline studies and existing toxicological data, NAMs can strengthen a WoE framework and are expected to play an increasingly important role in genotoxicity testing strategies.

OECD has recently adopted one genotoxicity NAM, TG 446A, the ToxTracker gene reporter assay.[2] Published on July 2, 2026, TG 446A describes a stem cell-based reporter assay that uses six fluorescent reporter genes to identify genotoxic hazard and provide information on genotoxic and non-genotoxic mechanisms of action (MoA). Two signaling responses are associated with direct genotoxicity, while the other four reflect stress response pathways, such as oxidative stress or protein damage, that may indirectly contribute to genotoxicity. Several other genotoxicity NAMs are being developed, evaluated, or discussed within the OECD TG Programme. Examples include reconstructed skin micronucleus and reconstructed skin Comet assays for dermal exposure, the *in vitro* γH2AX/pH3 multiplexed biomarker method which combines biomarkers of DNA damage and mitotic disruption, and the *in vitro* alkaline comet assay and enzyme-linked comet assay.[3,4] Because OECD work plans, draft TGs, and regulatory acceptance of specific NAMs continue to change, this resource document does not include a fixed table of NAMs. When NAM data are available for an ingredient, they should be interpreted within the overall WoE and described only when the method, endpoint measured, validation status, and relevance to current regulatory genotoxicity assessment are sufficiently clear.

In summary, CIR safety assessments distinguish historical, obsolete, and non-standard genotoxicity studies from current guideline-based or otherwise well-characterized methods. Results from deleted, legacy, or non-standard assays are generally not summarized or relied upon in CIR genotoxicity evaluations, although the citations are retained for transparency.

Table 1. Deleted or legacy OECD genotoxicity Test Guidelines[1,5]

TG	Guideline	Status
477	Sex-linked recessive lethal test in *Drosophila melanogaster*	Deleted in 2014
479	In vitro sister chromatid exchange (SCE) assay in mammalian cells	Deleted in 2014
480	*Saccharomyces cerevisiae* gene mutation assay	Deleted in 2014
481	*Saccharomyces cerevisiae* mitotic recombination assay	Deleted in 2014
482	DNA damage and repair, unscheduled DNA synthesis (UDS) in mammalian cells in vitro	Deleted in 2014
484	Mouse spot test	Deleted in 2014
485	Mouse heritable translocation assay	Retained, but not revised*
486	Unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo	Retained, but not revised*

*OECD indicated that both tests fulfill the criteria for deletion; however, they were retained but not revised[1]

Table 2. Current OECD Genotoxicity Test Guidelines

Endpoint	TG	Guideline	System	Current version	Refs.
Gene mutation	470	Mammalian erythrocyte Pig-a gene mutation assay	in vivo	2025	[6]
Gene mutation	471	Bacterial reverse mutation test (Ames)	in vitro	2020	[7]
Gene mutation	476	In vitro mammalian cell gene mutation tests (HPRT/XPRT)	in vitro	2016	[8]
Gene mutation	488	Transgenic rodent somatic and germ cell gene mutation assays	in vivo	2025	[9]
Gene mutation	490	In vitro mammalian cell gene mutation tests (TK)	in vitro	2016	[10]
Chromosomal damage	473	In vitro mammalian chromosomal aberration test	in vitro	2016	[11]
Chromosomal damage	474	Mammalian erythrocyte micronucleus test	in vivo	2016	[12]
Chromosomal damage	475	Mammalian bone marrow chromosomal aberration test	in vivo	2016	[13]
Chromosomal damage	478	Rodent dominant lethal test	in vivo	2016	[14]
Chromosomal damage	483	Mammalian spermatogonial chromosomal aberration test	in vivo	2016	[15]
Chromosomal damage	487	In vitro mammalian cell micronucleus test	in vitro	2023	[16]
DNA strand breaks	489	In vivo mammalian alkaline comet assay	in vivo	2016	[17]
Cellular signaling response / MoA information	446A	ToxTracker gene reporter assay	in vitro	2026	[2]

References

1. Organisation for Economic Co-operation and Development (OECD). 2017. Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015. Series on Testing & Assessment No. 238 - 2nd edition. https://www.oecd.org/content/dam/oecd/en/publications/reports/2017/08/overview-of-the-set-of-oecd-genetic-toxicology-test-guidelines-and-updates-performed-in-2014-2015-second-edition_c7a9416d/61eca5cd-en.pdf.

2. Organisation for Economic Co-operation and Development (OECD). 2026. Test No. 446A: ToxTracker gene reporter assay for the identification of genotoxic hazard and genotoxic/non-genotoxic mechanism of action. https://doi.org/10.1787/f2d51b5b-en.

3. Organisation for Economic Co-operation and Development (OECD). 2025. Work plan for the OECD Test Guidelines Programme (TGP). https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/testing-of-chemicals/work-plan-test-guidelines.pdf.

4. Organisation for Economic Co-operation and Development (OECD). 2026. First commenting round on a Detailed Review Paper on in vitro mammalian cell genotoxicity methods based on the phosphorylated histone biomarkers γH2AX and pH3. https://www.oecd.org/en/events/public-consultations/2026/05/first-commenting-round-on-a-detailed-review-paper-on-in-vitro-mammalian-cell-genotoxicity-methods-based-on-the-phosphorylated-histone-biomarkers-h2ax-and-ph3.html.

5. Organisation for Economic Co-operation and Development (OECD). Replaced and deleted Test Guidelines. https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/replaced-and-deleted-test-guidelines.html. Date Accessed: March 05, 2026.

6. Organisation for Economic Co-operation and Development (OECD). 2025. Test No. 470: Mammalian Erythrocyte Pig-a Gene Mutation Assay. https://doi.org/10.1787/4faea90e-en.

7. Organisation for Economic Co-operation and Development (OECD). 2020. Test No. 471: Bacterial Reverse Mutation Test. https://doi.org/10.1787/9789264071247-en.

8. Organisation for Economic Co-operation and Development (OECD). 2016. Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes. https://doi.org/10.1787/9789264264809-en.

9. Organisation for Economic Co-operation and Development (OECD). 2025. Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays. https://doi.org/10.1787/9789264203907-en.

10. Organisation for Economic Co-operation and Development (OECD). 2016. Test No. 490: In Vitro Mammalian Cell Gene Mutation Tests using the Thymidine Kinase Gene. https://doi.org/10.1787/9789264264908-en.

11. Organisation for Economic Co-operation and Development (OECD). 2016. Test No. 473: In Vitro Mammalian Chromosomal Aberration Test. https://doi.org/10.1787/9789264264649-en.

12. Organisation for Economic Co-operation and Development (OECD). 2016. Test No. 474: Mammalian Erythrocyte Micronucleus Test. https://doi.org/10.1787/9789264264762-en.

13. Organisation for Economic Co-operation and Development (OECD). 2016. Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test. https://doi.org/10.1787/9789264264786-en.

14. Organisation for Economic Co-operation and Development (OECD). 2016. Test No. 478: Rodent Dominant Lethal Test. https://doi.org/10.1787/9789264264823-en.

15. Organisation for Economic Co-operation and Development (OECD). 2016. Test No. 483: Mammalian Spermatogonial Chromosomal Aberration Test. https://doi.org/10.1787/9789264264847-en.

16. Organisation for Economic Co-operation and Development (OECD). 2023. Test No. 487: In Vitro Mammalian Cell Micronucleus Test. https://doi.org/10.1787/9789264264861-en.

17. Organisation for Economic Co-operation and Development (OECD). 2016. Test No. 489: In Vivo Mammalian Alkaline Comet Assay. https://doi.org/10.1787/9789264264885-en.