Updates on 3Rs methods for regulatory testing Nov 2022

Final versions of new or revised guidelines that have positive Three Rs implications, published since November 2021

Contents

Organisation for Economic Cooperation and Development (OECD)

Four new Test Guidelines (TG) have been published (TG 251, 467, 470, 492B) and seven TG have been updated or corrected (TG 425, 442C, 442D, 442E, 456, 488).

OECD test guidelines	**T**hree **Rs implications**
Test No. 251: Rapid Androgen Disruption Activity Reporter (RADAR) assay (30 Jun 2022)	NEW GUIDELINE The RADAR assay detects androgen axis signalling in transgenic medaka eleuthroembryos. It is a refinement of in vivo assays that use protected animals to detect androgenic effects.
Test No. 467: Defined Approaches for Serious Eye Damage and Eye Irritation (30 Jun 2022)	NEW GUIDELINE Defined approach bringing together OECD TGs 492 and 437 or 491 and 437, plus physicochemical properties, to evaluate the eye irritation/corrosion potential of chemicals including potency.
Test No. 470: Mammalian Erythrocyte Pig-a Gene Mutation Assay (30 Jun 2022)	NEW GUIDELINE New in vivo gene mutation assay. Though the Pig-a assay uses a similar number of animals as comparable assays, it is conducted with commonly used strains of rodents and does not require the animals to be killed so is easily integrated into other studies (e.g., repeat dose toxicity testing), reducing the overall number of animals required.

Test No. 492B: Reconstructed Human Cornea-like Epithelium (RHCE) Test Method for Eye Hazard Identification (30 Jun 2022)	NEW GUIDELINE In vitro eye irritation test. Similar to OECD TG 492, except uses SkinEthic™ Human Corneal Epithelium (HCE) Time-to-Toxicity (TTT) test instead of EpiOcular.
Test No. 425: Acute Oral Toxicity: Up and Down Procedure (30 Jun 2022)	Correction to paragraph 17 – housing and feeding conditions – to state that animals may be returned to group housing following dosing and that time spent in individual housing should be minimised.
Test No. 442C: In Chemico Skin Sensitisation: Assays addressing the Adverse Outcome Pathway key event on covalent binding to proteins (30 Jun 2022)	Revised to add performance standards for the assessment of proposed similar or modified in vitro skin sensitisation DPRA and ADRA test methods.
Test No. 442D: In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method (30 Jun 2022)	Minor correction to the LuSens test method to address a discrepancy related to the number of wells for the positive control.
Test No. 442E: In Vitro Skin Sensitisation (30 Jun 2022)	Revised to add a new in vitro test method (GARD™skin), to make a total of four assays. The GARDskin method is the first harmonised method that generates and interprets genomic data for a regulatory endpoint.
Test No. 456: H295R Steroidogenesis Assay (30 Jun 2022)	Update to the assay's data analysis procedure to reduce the potential for false positive results, which also potentially reduces the need for follow up tests in animals.
Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (30 Jun 2022)	Revised to permit integration of the TGR with repeat dose toxicity testing and other genotoxicity endpoints, which can reduce the number of animals used.
OECD guidance	**Three Rs implications**
No. 360: Detailed Review Paper on In Vitro Test Addressing Immunotoxicity with a Focus on Immunosuppression (19 Sep 2022)	Paper presenting and discussing the application and interpretation of in vitro immunotoxicity assays and defining an in vitro tiered approach to testing and assessment.

EU Test Methods Regulation (EC/440/2008)

Test Methods Regulation revisions	**T**hree **Rs implications**
9th ATP in progress including a simplification of future TMR adaptations to technical progress	New and amended Three Rs methods will in the future be incorporated in TMR significantly faster than in the past.

European Chemicals Agency (ECHA)

Chapter R.7: Endpoint specific guidance	**T**hree **Rs implications**
None to report	
Other guidance https://echa.europa.eu/support/oecd-eu-test-guidelines	**T**hree **Rs implications**
Advice on dose-level selection for the conduct of reproductive toxicity studies (OECD TGs 414, 421/422 and 443) under REACH (Jan 2022)	Guidance on how to comply with dose-level selection provisions in REACH legal text revised by Commission Regulation (EU) 2021/979, which seeks to ensure that tests conducted are accepted by ECHA as having sufficiently high dosing to be adequate for hazard identification, thus avoiding requests from ECHA for the test to be repeated.
Guidance on the Biocidal Products Regulation, Volume III: Human health, Part A: Information requirements, Version 2 (Mar 2022)	Fully revised to update information requirements in line with amendments to Annexes II and III of Regulation (EU) No 528/2012. Though, overall, the changes increase the testing burden on animals, some 3Rs improvements have been made (e.g. prioritisation of in vitro methods for local toxicity, replacement of the two-generation reproductive toxicity study with an extended one-generation study).
Advice on using read-across for UVCB substances (May 2022)	Guidance on how to read across between UVCB substances according to provisions in REACH legal text revised by Commission Regulation (EU) 2021/979, which supports avoidance of unnecessary animal tests.
How to act in a dossier evaluation (July and Oct 2022)	Updated to reflect that registrants may downgrade the substance's tonnage band after receipt of a draft decision, which supports avoidance of unnecessary animal tests.
Other measures to promote the Three Rs	**T**hree **Rs implications**
Mammalian toxicokinetic database (MamTKDB) 1.0, DOI: 10.2906/101099104097/2	Provides information on elimination half-lives in mammals, including humans, to support prediction and assessment of bioaccumulation.

European Directorate for the Quality of Medicines & HealthCare (EDQM)

European Pharmacopeia Monographs	**T**hree **Rs implications**
Rabies vaccine (inactivated) for veterinary use (0451), published in Ph. Eur. Supplement 11.1 and effective on 1 Apr 2023	As a 3Rs commitment, the NIH test is reserved for development and qualification of standards/reference preparations only and not used anymore as a routine batch potency test. Deletion of requirement to perform residual live virus test at final product stage; if test must still be conducted, it must be performed in vitro.
Clostridium botulinum vaccine for veterinary use (0360), published in Ph. Eur. Supplement 11.2 and effective on 1 July 2023	Potency test: addition of humane endpoints; cell-based assays specifically mentioned as a possible alternative for the batch potency test
Radiopharmaceutical preparations (0125), published in Ph. Eur. Supplement 11.1 and effective on 1 Apr 2023	The section on the performance of the physiological distribution test has been removed as, in line with the 3R principles, the test should no longer be used.
Other measures to promote the Three Rs	**T**hree **Rs implications**
Strategy for removing or replacing the rabbit pyrogen test: New pyrogenicity strategy of the European Pharmacopoeia Commission (Sep 2022)	Published document presenting a strategy that will lead to complete elimination of the rabbit pyrogen test from the Ph. Eur.

European Medicines Agency (EMA)

EMA Guidelines (human medicines)	**T**hree **Rs implications**
None to report	
EMA Guidelines (veterinary medicines)	**Three Rs implications**
Guideline on data requirements for veterinary medicinal products intended to reduce the risk of transmission of vector-borne pathogens in dogs and cats (adopted 15 Jun 2022, coming into effect 1 Jan 2023).	New guideline to be read in conjunction with Regulation (EU) 2019/6. Includes statements on the 3Rs and animal welfare. Describes the use of “rescue protocols” to reduce harms experienced by animals in the untreated (negative) control groups e.g. clear-cut withdrawal criteria, intensive monitoring and rescue medications.
Guideline on safety and residue data requirements for applications for non-immunological veterinary medicinal products intended for limited markets submitted under Article 23 of Regulation (EU) 2019/6 (adopted in July 2021, implemented Jan 2022).	New guideline to be read in conjunction with Regulation (EU) 2019/6. Outlines circumstances under which data requirements for limited market veterinary products can be reduced. Promotes the use of New Approach Methodologies (NAMs) and includes several examples.

Guideline on determination of withdrawal periods for edible tissues (adopted Mar 2022, implemented Aug 2022).	Slight revision of the guideline made in context of Regulation (EU) 2019/6. Statement on compliance with 3Rs principles was added.
Guideline on determination of withdrawal periods for milk (adopted Mar 2022, implemented Aug 2022).	Slight revision of the guideline made in context of Regulation (EU) 2019/6. Statement on compliance with 3Rs principles was added.
Guideline on injection site residues(adopted Mar 2022, implemented Aug 2022).	Slight revision of the guideline made in context of Regulation (EU) 2019/6. Statement on compliance with 3Rs principles was added.
Guideline on clinical trials with immunological veterinary medicinal products (IVMPs) (adopted Jan 2022, implemented Jan 2022).	New guideline to be read in conjunction with Regulation (EU) 2019/6. Outlines circumstances under which clinical efficacy trials could be omitted from marketing authorisation applications of IVMPs, thus reducing animals used in such studies.
Guideline on data requirements for adjuvants in vaccines for veterinary use (adopted Jul 2021, implemented Jan 2022).	New guideline containing a statement on compliance with 3Rs principles.

ICH (human medicines)

ICH Guidelines	**T**hree **Rs implications**
ICH guideline S1B(R1) on testing for carcinogenicity of pharmaceuticals (adopted 16 Sep 2022, coming into effect 16 Mar 2023)	Addendum expands the evaluation process for assessing human carcinogenic risk of pharmaceuticals by introducing an additional approach that is not described in the original S1B Guideline. This is an integrative approach that provides specific weight of evidence (WoE) criteria that inform whether or not a 2-year rat study is likely to add value to a human carcinogenicity risk assessment. The Addendum also adds a plasma exposure ratio-based approach for setting the high dose in the rasH2-Tg mouse model. Application of this integrative approach reduces the use of animals in accordance with the 3Rs principles and shifts resources to focus on generating more scientific mechanism-based carcinogenicity assessments, while continuing to promote safe and ethical development of new pharmaceuticals.
ICH guideline E14/S7B: clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential - questions and answers (adopted 27 Jan 2022, coming into effect 27 Aug 2022)	Guidance regarding best practices for the design, conduct, analysis, interpretation and reporting of in vitro, in silico and in vivo nonclinical assays in order for these assays to influence nonclinical and clinical evaluations.

VICH (veterinary medicines)

VICH Guidelines	**T**hree **Rs implications**
None to report	